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A Molecular-Cytogenetic Method for Locating Genes to Pericentromeric Regions Facilitates a Genomewide Comparison of Synteny Between the Centromeric Regions of Wheat and Rice

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ABSTRACT

Centromeres, because of their repeat structure and lack of sequence conservation, are difficult to assemble and compare across organisms. It was recently discovered that rice centromeres often contain genes. This suggested a method for studying centromere homologies between wheat and rice chromosomes by mapping rice centromeric genes onto wheat aneuploid stocks. Three of the seven cDNA clones of centromeric genes from rice centromere 8 (*Cen8*), 6729.t09, 6729.t10, and 6730.t11 which lie in the *Cen8* kinetochore region, and three wheat ESTs, BJ301191, BJ305475, and BJ280500, with similarity to sequences of rice centromeric genes, were mapped to the centromeric regions of the wheat group-7 (W7) chromosomes. A possible pericentric inversion in chromosome 7D was detected. Genomewide comparison of wheat ESTs that mapped to centromeric regions against rice genome sequences revealed high conservation and a one-to-one correspondence of centromeric regions between wheat and rice chromosome pairs W1-R5, W2-R7, W3-R1, W5-R12, W6-R2, and W7-R8. The W4 centromere may share homology with R3 only or with R3 + R11. Wheat ESTs that mapped to the pericentromeric region of the group-5 long arm anchored to the rice BACs located in the recently duplicated region at the distal ends of the short arms of rice chromosomes 11 and 12. A pericentric inversion specific to the rice lineage was detected. The depicted framework provides a working model for further studies on the structure and evolution of cereal chromosome centromeres.

CENTROMERES and their associated kinetochores are protein–DNA complexes that mediate spindle microtubule attachment during mitosis and meiosis and are necessary for the accurate segregation of the chromosomes into daughter nuclei. Despite the conservation of centromere function, centromere sequence composition consisting of highly repetitive satellite DNA and retrotransposons varies widely among different organisms (Henikoff *et al.* 2001; Sullivan *et al.* 2001; Jiang *et al.* 2003; Lee *et al.* 2005; Ma *et al.* 2007; Kanizay and Dawe 2009). The most abundant sequences in plant centromeres are the 180-bp satellite repeat pAL1 in Arabidopsis, CentO satellite repeats in rice, CentC repeats in maize, and the B-specific repeats in the centromere of maize B chromosome (Round *et al.* 1997; Ananiev *et al.* 1998; Dong *et al.* 1998; Copenhaver *et al.* 1999; Cheng *et al.* 2002; Jin *et al.* 2004, 2005; Birchler *et al.* 2009). Centromeric satellites serve as the core of the centromere, which is flanked by pericentric heterochromatin rich in middle repetitive elements, including retroelements and transposons. Because of the abundance of various repeats, centromeres of most eukaryotic chromosomes are upward of 1 Mb in size, mostly devoid of genes, and their sequencing and assembly pose a big challenge (Su *et al.* 1997; Hosouchi *et al.* 2002). Among the sequenced genomes of many multicellular eukaryotes, including *Drosophila melanogaster*, human, mouse, *Arabidopsis thaliana*, and rice, only the centromeres of rice chromosomes 3, 4, 5, and 8 have been fully assembled (Nagaki *et al.* 2004; Wu *et al.* 2004; Y. Zhang *et al.* 2004; International Rice Genome Sequencing Project 2005; Yan *et al.* 2006).

Unlike centromere DNA sequences, a group of proteins specific to the centromere/kinetochore complex is highly conserved among diverse organisms, including fungi, animals, human, and plants. The centromere-specific histone H3 variants (CENH3s) were found in fungi (Cse4), insect (Cid), nematodes (HCP-3), mammals (CENP-A), Arabidopsis (HTR-12), rice, and maize (Palmer *et al.* 1991; Dawe *et al.* 1999; Henikoff *et al.* 2001; Talbert *et al.* 2002; Zhong *et al.*

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2002; Jiang *et al.* 2003). Previous studies have reported expressed genes and transcripts in the flanking regions of some centromeres (Copenhaver *et al.* 1999; Schueler *et al.* 2001) and a human neocentromere (Saffery *et al.* 2003). Nagaki *et al.* (2004) reported active genes in the sequenced centromere of rice chromosome 8 (*Cen8*); at least 16 active genes reside within a ~750-kb core domain associated with CENH3 of *Cen8* (Yan *et al.* 2005). In the centromere of rice chromosome 3, 19 transcribed genes have been localized to the ~1881-kb CENH3 domain (Yan *et al.* 2006). The genes present in the conserved domains open possibilities for the comparative mapping of centromeric regions among groups of organisms where gene synteny is conserved.

The cereal crops wheat ($1x = 7$), maize ($1x = 10$), sorghum ($1x = 10$), and rice ($1x = 12$) share 65 million years of evolutionary history, differ in basic chromosome number and genome size (40-fold), and yet maintain large syntenic blocks and in some cases whole chromosome homologies (Ahn *et al.* 1993; Gale and Devos 1998; Huang *et al.* 2002; Sorrells *et al.* 2003; Paterson *et al.* 2004, 2009; Singh *et al.* 2007; Wei *et al.* 2007; Salse *et al.* 2008). However, the information about centromere synteny between rice, wheat, and other species is still limited because most of the rice centromere cores are in sequencing gaps. The observation that active genes were found in the centromeres of rice chromosomes, combined with the fact that wheat telosomic chromosomes can be used to precisely map genes to centromeric and pericentromeric regions (Sandhu *et al.* 2001; Francki *et al.* 2002; Qi *et al.* 2006) provides an excellent opportunity to study syntenic relationships between centromeres of wheat and rice. In the present study, we report the mapping of centromeric genes of rice chromosome 8 to wheat chromosome centromeric regions using wheat aneuploid stocks. These data, together with bioinformatics analysis of previous data (Sandhu *et al.* 2001; Francki *et al.* 2002; Qi *et al.* 2006), provide a framework for genomewide comparisons of homology among the centromeric regions of wheat, rice, and other cereal species and novel insights into their karyotypic evolution.

MATERIALS AND METHODS

Wheat aneuploid stocks: Twenty-one wheat nullitetrasomic (NT) lines in *Triticum aestivum* L. cv Chinese Spring (CS) background were used to assign rice genes to specific wheat chromosomes. Sixteen CS ditelosomic (Dt) lines and 20 wheat-alien ditelosomic addition (DtA) lines involving homologous group 3, 5, and 7 chromosomes were used to locate genes to a specific arm or centromeric or pericentromeric region of wheat and alien chromosomes (supporting information, Table S1). In addition, 64 chromosome deletion (del) lines with the distal segment deleted for a specific chromosome were used to assign genes to specific chromosome segments with respect to the centromere (pericentromeric or not) (Table S2, Endo and Gill 1996). The del5DL-7 line was reselected from a cross of the original del5DL-7 + monosmic 5D with N5DT5B to remove chromosome 5D from this line. In an alien DtA line, the first number designates the homologous group, followed by the genome symbol; the # sign is used to distinguish between chromosomes belonging to the same homologous group but derived from different accessions, and last, the arm location. The genetic stocks and Triticeae species are maintained at the Wheat Genetic and Genomic Resources Center at Kansas State University (http://www.k-state.edu/wgrc/).

RFLP analysis: Procedures for genomic DNA isolation, restriction endonucleases digestion, gel electrophoresis, and DNA gel blot hybridization were as described in Qi *et al.* (2003). The genomic DNAs of the selected genetic stocks were digested with four enzymes of *Eco*RI, *Hin*dIII, *Dra*I, and *Bam*HI. The rice centromere clones and wheat ESTs were provided by J. Jiang, University of Wisconsin, Madison and Y. Ogihara, Kyoto Prefectural University, Shimogamo, Sakyo-ku, Japan.

Nucleic acid sequence alignments: The sequences of three rice centromeric clones, 6729.t09, 6729.t10, and 6730.t11, and *Cen8* BAC B1052H09, were subjected to BLASTN searches of the National Center for Biotechnology and Information (NCBI) dbEST database (http://www.ncbi.nlm.nih.gov/dbEST/) to identify corresponding wheat ESTs and/or tentative consensus (TC) sequences. The sequences of selected wheat bin-mapped ESTs and one RFLP clone were anchored to the 12 rice pseudomolecules composed of ordered BACs/PACs to compare microcolinearity in the centromeric regions between wheat and rice (http://rice.plantbiology.msu.edu/pseudomolecules/info.shtml).

BAC library screening: The filters of BAC libraries of *Aegilops tauschii* Coss. and *Ae. speltoides* were provided by J. Dvorak, University of California, Davis. Each high-density colony filter contains 18,432 clones. Library screening was performed using four filters that contain 73,728 clones for each EST marker. The procedure for colony filter hybridization was similar to the one used for Southern blot hybridization.

BAC-FISH analysis: In addition to centromere assignment based on aneuploid stocks, BAC-fluorescence *in situ* hybridization (FISH) was used to map genes to the centromeres. Positive BAC clones for specific genes digested with *Hin*dIII were first screened with three centromere-specific clones pAet6-09, Hi10, and pRCS1 to reveal their potential centromeric locations (Abbo *et al.* 1995; Dong *et al.* 1998; P. Zhang *et al.* 2004). BAC clones with strong hybridization signals with the centromere-specific repeats were selected as probes for FISH experiments. BAC-FISH was as described by P. Zhang *et al.* (2004). Slides were analyzed with an epifluorescence Zeiss Axioplan 2 microscope. Images were captured using a SPOT 2.1 CCD (charge-coupled device) camera (Diagnostic Instruments; http://www.diaginc.com) and processed with Photoshop v5.5 software (Adobe Systems; http://www.adobe.com).

RESULTS

The methodology for centromere mapping of RFLP fragments in wheat and the Triticeae is illustrated in Figure 1 (see also Qi *et al.* 2006). The Dt stocks of wheat and those of other Triticeae species often arise from breaks in the centromere (Sears and Steinitz-Sears 1978; Zhang *et al.* 2001). Briefly, if an RFLP fragment is assigned to a specific chromosome but is not missing in either of the telosomic stocks for that chromosome then

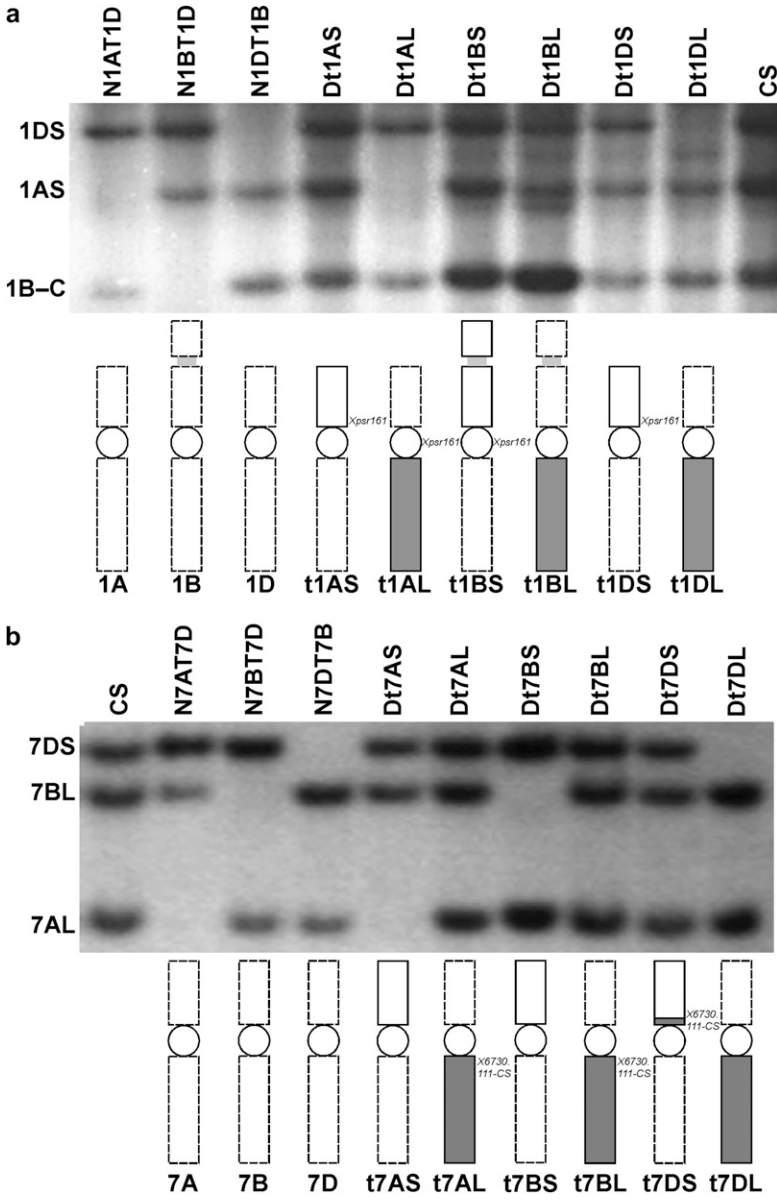

FIGURE 1.—An example of the localization of RFLP loci to the centromere and pericentromeric regions of wheat homologous chromosomes. Chromosome ideograms in a and b indicate the specific chromosome constitution in the corresponding genetic stocks. The dashed line indicates a missing chromosome or chromosome arm. The open and shaded bars represent the short and long arms, respectively. (a) An autoradiograph of a Southern hybridization of genomic DNA of standard Chinese Spring (CS) and aneuploid stocks for group-1 chromosomes. Genomic DNA of nullisomic-tetrasomic (NT) and ditelosomic (Dt) lines was digested with *Eco*RI and hybridized with clone PSR161. The top fragment detected by this clone was missing in N1DT1B and Dt1DL, respectively, and was mapped to the short arm of chromosome 1D (1DS). Similarly, the second fragment was mapped to the short arm of chromosome 1A (1AS). However, the third fragment was missing in N1BT1D, but present in both telosomics of the short and long arms of chromosome 1B, indicating the centromere location of PSR161 on chromosome 1B. The position of wheat cDNA clone PSR161 is shown in italics on the right of each chromosome. (b) An autoradiograph of a Southern hybridization of genomic DNA of standard CS and aneuploid stocks for group-7 chromosomes. Genomic DNA of the nullisomic-tetrasomic and ditelosomic lines was digested with *Hin*dIII and hybridized with the PCR product 6730.t11-CS. The top fragment detected by this clone was missing in N7DT7B and Dt7DL, respectively, and was mapped to the short arm of chromosome 7D (7DS). However, the second fragment was missing in N7BT7D and Dt7BS, respectively, and was mapped to the long arm of chromosome 7B (7BL). Similarly, the third fragment was mapped to the long arm of chromosome 7A (7AL). It revealed a pericentric inversion in chromosome 7D in comparison to the standard arrangement in homologous chromosomes 7A and 7B. The position of clone 6730.t11-CS is shown in italics on the right of each chromosome.

it is assigned to the centromeric region (Figure 1a). More often, a fragment maps to the short arm in some telosomic stocks and to the long arm in other telosomic stocks, and then it is assigned to the pericentromeric region (Figure 1b). All DtA lines, in which a pair of alien chromosome arms is added to the wheat complement, were developed in CS wheat background. Physical mapping of loci to an alien chromosome arm using DtA lines is based on intergenomic polymorphism. If a polymorphic fragment is observed in a specific DtA line when compared to the hybridization pattern of CS, this fragment can be mapped to a specific arm of an alien chromosome. In wheat, these stocks can be used to assign RFLP loci to centromeres or centromeric bins in the specific Triticeae species.

Mapping of rice R8 centromeric genes in wheat and the Triticeae: Rice chromosome 8 centromere (*Cen8*) genes were selected for a test of centromeric region homology between rice and wheat. The seven rice centromeric clones selected in the present study are expressed genes located in the centromere of rice chromosome 8, and three of them lie in the *Cen8* kinetochore region (NAGAKI *et al.* 2004, Figure S1). These clones were mapped by Southern hybridization to determine their chromosome, arm, and deletion bin location in wheat using a set of wheat aneuploid stocks (Table S1 and Table S2). Three clones, 3507.t05, 6731.t10, and 6731.t12, failed to yield clear hybridization signals and could not be mapped. Of the remaining four clones, 6733.t09 was mapped to the distal regions of the short arms of 3A, 3B, and 3D of the wheat group-3 chromosomes (Table 1, Figure 2). Three clones, 6729.t09, 6729.t10, and 6730.t11, which were previously located on the kinetochore region of the rice chromo-

TABLE 1

Clone/enzyme combination and chromosome location of clones

Clone/enzyme	Chromosome location
6733.t09/*Eco*RI	3AS, 3BS, 3DS
6733.t09/*Hin*dIII	3SS, 3HS
6729.t10/*Eco*RI	7AS, 7BS, 7DS, 7SS, 7HS
6729.t10/*Bam*HI	7RL
6729.t10/*Hin*dIII	5AL, 5BL, 5DL
6729.t10/*Dra*I	5HL
6729.t09/*Eco*RI	7AS
6729.t09/*Hin*dIII	7BS, 7DS, 7SS
6730.t11/*Hin*dIII	7AL, 7BL, 7DS
6730.t11/*Dra*I	7RS
6730.t11-CS/*Hin*dIII	7AL, 7BL, 7DS
6730.t11-CS/*Dra*I	7RS
BJ280500/*Hin*dIII	7AL, 7BL, 7DS, 7SL, 7RL, 7HL
BJ305475/*Bam*HI	7AS, 7BS, 7DS, 7SS, 7RL, 7HS
BJ301191/*Eco*RI	7AS, 7BS, 7DS, 7RL, 7HS

some 8 centromere, mapped in the centromeric bins of wheat chromosomes 7A, 7B, and 7D. Within the centromeric bin, 6729.t09 and 6729.t10 mapped to the short arms of chromosomes 7A and 7B, clone 6730.t11 mapped to the centromeric bin of the long arms of chromosomes 7A and 7B, and all three mapped to the short arm centromeric bin in chromosome 7D. A paralogous locus for 6729.t09 was detected at a proximal position in the long arm of all three group-5 chromosomes, as well as in the long arm of the barley chromosome 5H, indicating a sequence duplication event in the Triticeae (Figure 2).

A PCR-amplified fragment from CS wheat using RT–PCR primer of clone 6730.t11 (Nagaki *et al.* 2004), named 6730.t11-CS, gave an RFLP pattern similar to that of 6730.t11, but the signal intensity was much stronger (Figure 1b). 6730.t11-CS was mapped to the same centromeric bins as 6730.t11 (Figure 2).

A collection of ditelosomic addition stocks of *Ae. speltoides*, barley, and rye was used to determine the location of rice centromeric genes in these species. Digested genomic DNA of CS and six CS-alien DtA lines of DtA3S#3S, DtA3S^{l}#2L, DtA3RS, DtA3EL, DtA3HS, and DtA3HL were probed with clone 6733.t09. The polymorphic fragments were only detected in DtA3S#3S and Dt3HS, mapping them to the short arms of chromosome 3 of *Ae. speltoides* and barley, respectively (Table 1, Figure 2). It confirmed that the rice *Cen8* clone 6733.t09 is located on the short arm of homologous group-3 chromosomes in the Triticeae.

Southern hybridization with four clones, 6729.t10, 6729.t09, 6730.t11, and 6730.t11-CS, to DtA lines of homologous group-7 revealed that clone 6729.t10 mapped to the short arms of chromosome 7S of *Ae. speltoides* and chromosome 7H of barley but to the long arm of rye chromosome 7R. Clone 6729.t09 was only mapped to the short arm of chromosome 7S of *Ae. speltoides*. Two other clones, 6730.t11 and 6730.t11-CS, were mapped to the short arm of rye chromosome 7 (Table 1, Figure 2). The mapping of these clones to the group-7 chromosomes in *Ae. speltoides*, barley, and rye again indicated that similar to wheat, these clones are located in the pericentromeric region of all the Triticeae chromosomes.

Wheat ESTs homologous to the rice *Cen8* clones and their physical mapping: To find homologous centromeric sequences of three rice centromeric clones, 6729.t09, 6729.t10, and 6730.t11, in wheat, we aligned these rice clone sequences against all the sequences present in the wheat EST database using BLASTN searches at a higher stringency level ($E > e^{-20}$) (Altschul *et al.* 1997). The clones 6729.t09 and 6729.t10 had similarity to the same wheat contig TC249611, coding a poly (A)-binding protein. The clone 6730.t11 had significant similarity to the contig TC255802, which is predicted to encode a CBS domain-containing protein (Table 2). In addition, a *Cen8* BAC B1052H09 (http://rice.plantbiology.msu.edu/pseudomolecules/centromere.shtml) was also used to search the wheat EST database and gave hits to wheat EST contigs TC265287, TC265289, TC265290, and TC255432. These contigs were again subjected to BLAST searches of the rice pseudomolecule database. Only two contigs, TC265290 and TC255432, matched against the centromeric BAC B1052H09. TC265290 encodes a TGF-β receptor-interacting protein-like protein and TC255432 encodes a putative Rer1 protein (Table 2).

Searching all ESTs in the four contigs that matched rice sequences to the mapped wheat EST database revealed that none of them has been mapped (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). Six wheat ESTs from the four contigs were selected and hybridized to a set of wheat NT, Dt, DtA, and del lines to compare their mapping positions with corresponding rice clones in wheat (Table 2). Of the six ESTs, BJ244076, BJ222044, and BJ219066 did not provide useful information because of high copy number. Two ESTs, BJ301191 and BJ305475, which matched the rice clones 6729.t09 and 6729.t10, mapped to the short arm of group-7 chromosomes, similar to the previous mapping of these two rice clones in wheat. In addition, these two wheat ESTs mapped to the short arms of *Ae. speltoides* 7S and barley 7H, but to the long arm of rye 7R (Table 1, Figure 2). The EST BJ280500, which aligned to the rice *Cen8* BAC, mapped to the long arms of chromosomes 7A and 7B but to the short arm of 7D, similar to rice clone 6730.t11. However, this EST mapped to only the long arms of *Ae. speltoides* 7S, barley 7H, and rye 7R, indicating a long arm origin.

Genomewide comparison of wheat ESTs mapped to centromeric regions with rice genomic sequences: In previous studies, a total of 24 ESTs and one RFLP clone were physically mapped in the centromeric regions of

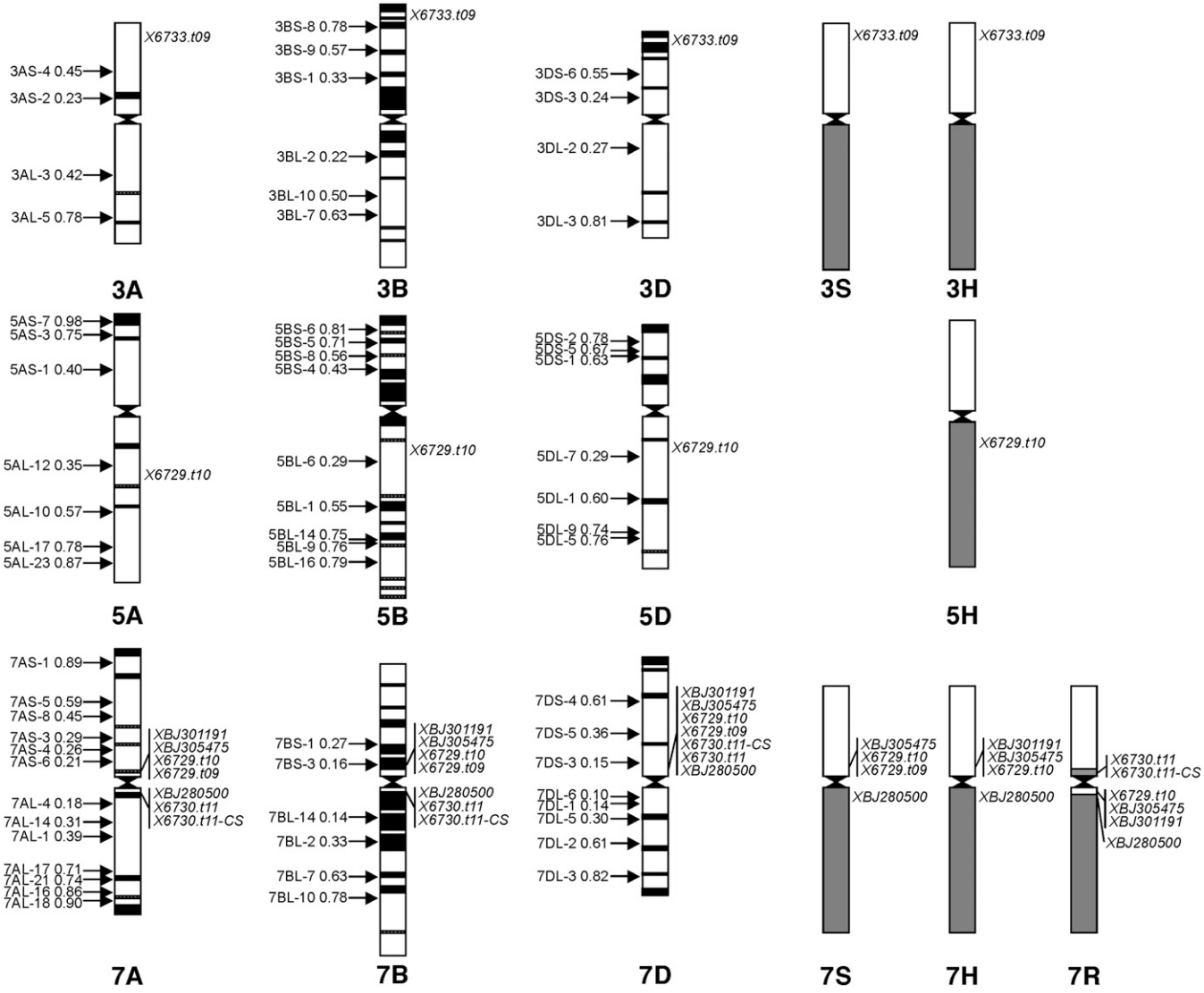

Figure 2.—Physical mapping of rice centromeric genes and wheat ESTs in individual chromosome bins of wheat and the Triticeae chromosomes. The deletion names and breakpoints (indicated as fraction length from the centromere) are on the left of each chromosome. The rice cDNA clones and wheat ESTs mapped to the bins are in italics on the right of each chromosome. The ideogram of C-banded chromosomes of groups 3, 5, and 7 is after Gill *et al.* (1991).

wheat chromosomes. These ESTs are diagnostic markers detecting pericentric inversions in homologous groups 2, 3, 4, 5, and 6 in the Triticeae and are markers for the pericentromeric regions of wheat chromosomes (Conley *et al.* 2004; Linkiewicz *et al.* 2004; Miftahudin *et al.* 2004; Qi *et al.* 2004, 2006). Two cDNA clones, PSR161 and BCD1072, were previously mapped to the centromere of chromosome 1B (Sandhu *et al.* 2001; Francki *et al.* 2002). Searches against the rice genomic DNA database revealed that 22 of the 24 mapped wheat ESTs and PSR161 matched rice expressed genes (http://rice.plantbiology.msu.edu/pseudomolecules/info.shtml, Table S3). The positions of the anchored rice BACs were compared with that of the rice centromeric BACs in each rice chromosome to discover the conservation of colinearity of the centromere regions between wheat and rice (Figure 3). The rice centromeric BACs were selected from "Information about the Centromeres in the Rice Genome Annotation Project Pseudomolecules Release 6" (http://rice.plantbiology.msu.edu/pseudomolecules/centromere.shtml).

Wheat cDNA PSR161 is the only centromeric clone in chromosome 1B identified so far (Sandhu *et al.* 2001; Francki *et al.* 2002), and it had similarity to a sequence on rice BAC OJ1234_D05. This BAC clone was located at position 13.5 Mb in the pseudomolecule of rice chromosome 5 (R5) and was only 1 Mb away from the centromeric BAC P0697B04 (Figure 3). Of the two group-2 pericentromeric ESTs, BE404630 anchored to a BAC on the interstitial region of the R7 short arm. The EST BE500625 did not match any rice sequence (Figure 3). All four wheat group-3 (W3) pericentromeric ESTs gave hits on the short or long arm of rice chromosome

TABLE 2

Blast search results of rice centromeric clones and BAC against wheat EST database

Rice clone or BAC	Wheat EST	*E*-value	Description	Selected EST from TC[a] for mapping
B1052H09	TC265290	2.40*E*-96	TGF-beta receptor-interacting protein-like protein	BJ244076, BJ222044
	TC255432	3.30*E*-59	Rer1 protein, putative, expressed	BJ280500
6730.t11	TC255802	2.70*E*-43	CBS domain-containing protein	BJ219066
6729.t09, 6729.t10	TC249611	2.60*E*-253	Polyadenylate-binding protein, putative, expressed	BJ301191 BJ305475

[a] Wheat EST tentative consensus sequence.

R1 encompassing the centromeric BAC B1061G08 (Figure 3). The closest rice BAC OsJNBa0086A10 was at a distance of 2.7 Mb from the *Cen1* BAC.

The pericentromeric region of the group-4 chromosomes of wheat and the Triticeae is highly dynamic involving multiple and independent inversion events, and eight EST clones mark the region (Qi *et al.* 2006). Seven of the eight clones (seven ESTs and one RFLP clone) gave hits on rice chromosomes R3, R4, and R11. Four gave hits on rice chromosome R3 and EST BE497635 aligned to the *Cen3* BAC OsJNBb0047D08 (Figure 3). Two gave hits on rice chromosome R11, and the rice BAC OSJNBb0018P20 anchored by EST BE637507 was adjacent to the *Cen11* BAC OSJNBa0046A04 within 0.2 Mb distance (Figure 3). However, the second hit on R11 BAC OSJNBa0042J05 by EST BF202706, which was physically mapped to the centromeric region of wheat chromosomes by BAC-FISH (see below), was located toward the terminal end in the long arm. EST BE494281, a multicopy clone detecting 7–13 restriction fragments with different probe/enzyme combinations, gave a hit in the long arm of R4.

All W5 ESTs except one first hit on R12 and second on R11, which shares a duplicated segment with R12 (Table S3). The EST BE403618 of the W5 short arm gave a hit on the R12 BAC at 4.4 Mb from the *Cen12* BAC and a second hit on the *Cen11* BAC OsJNBa0046A04 (Figure 3). Further sequence search of the W5 short arm ESTs mapped to the centromeric bin against rice genome sequences (see below) discovered two rice BACs, OJ1060_G11 anchored by EST BG314119 and OSJNBb115B15 anchored by EST BE604729, were located at distances of 0.7 Mb and 1.6 Mb from the *Cen12* BAC OsJNBa0088J04, respectively (Table S4, Figure 3). The other five pericentromeric ESTs of the W5 long arm gave hits on the distal ends of the short arms of both R12 and R11, representing a 3.5- to 3.9-Mb region in R12 pseudomolecules and a 3.4- to 4.1-Mb region in R11, a part of the duplicated regions on R11S and R12S (Figure 3, Wu *et al.* 1998; Rice Chromosomes 11 and 12 Sequencing Consortia 2005; Yu *et al.* 2005).

Four pericentromeric ESTs of group-6 gave hits on rice chromosome R2, two in each arm. EST BE405809 aligned to rice BAC P0705A04 1.2 Mb from the *Cen2* BAC B1120G10d (Figure 3). Our study provided direct evidence that the rice *Cen8* is related to the centromeres of the W7 chromosomes. Three rice *Cen8* clones were mapped to the pericentromeric region of W7. Three wheat ESTs, with matches to rice *Cen8* active genes, along with the *Cen8* BAC, also mapped to the pericentromeric regions of the W7 chromosomes (Figure 3).

Syntenic block between W5 and the distal region of rice chromosome R12: As noted above, W5 pericentromeric ESTs were aligned to the distal end of rice chromosome 12S. To investigate this discrepancy further in the context of chromosome homology in the proximal region of the centromere, we searched 179 wheat bin-mapped ESTs against rice genome sequences. Of those, 22 were mapped to the region proximal to the centromere in the short arm and 157 in the long arm of W5. W5 short-arm ESTs had the most hits in rice chromosome 12 (36%), three in the long arm and five in the short arm (Table S4). Of 157 W5 long-arm ESTs, 32 had no hit to any rice pseudomolecules. The highest percentage (50%) of W5 long-arm ESTs were mapped to rice chromosome 9 and about 23% (29 ESTs) were mapped to rice chromosome 12 (Table S4). Most W5 long-arm ESTs aligned to rice chromosome 12 are located in the region close to the W5 centromere, including 9 ESTs, which detected the pericentromeric inversion in chromosome 5A. Twenty-nine W5 long-arm ESTs mapped to the short arm of rice chromosome 12, covering a region from 0.1 Mb to 5.7 Mb with an opposite gene order compared to the position of these ESTs in wheat (Table S4). Most of them mapped to the distal end of the short arm of rice chromosome 12 known as a recently duplicated region in R11S and R12S.

Wheat ESTs assigned to the centromere by BAC-FISH: In wheat, with its complex and large genome, single-copy ESTs cannot be mapped by FISH (P. Zhang *et al.* 2004). We used BAC-FISH to further confirm the possible centromeric location of the ESTs mentioned above. A total of 10 wheat ESTs previously mapping to the pericentromeric regions of wheat group-1–group-6 chromosomes and three rice centromeric clones were used to screen high-density BAC filters from *Ae. tauschii* or *Ae. speltoides* (Table 3). Positive BAC clones were detected for all except rice clones 6729.t09 and 6730.t11

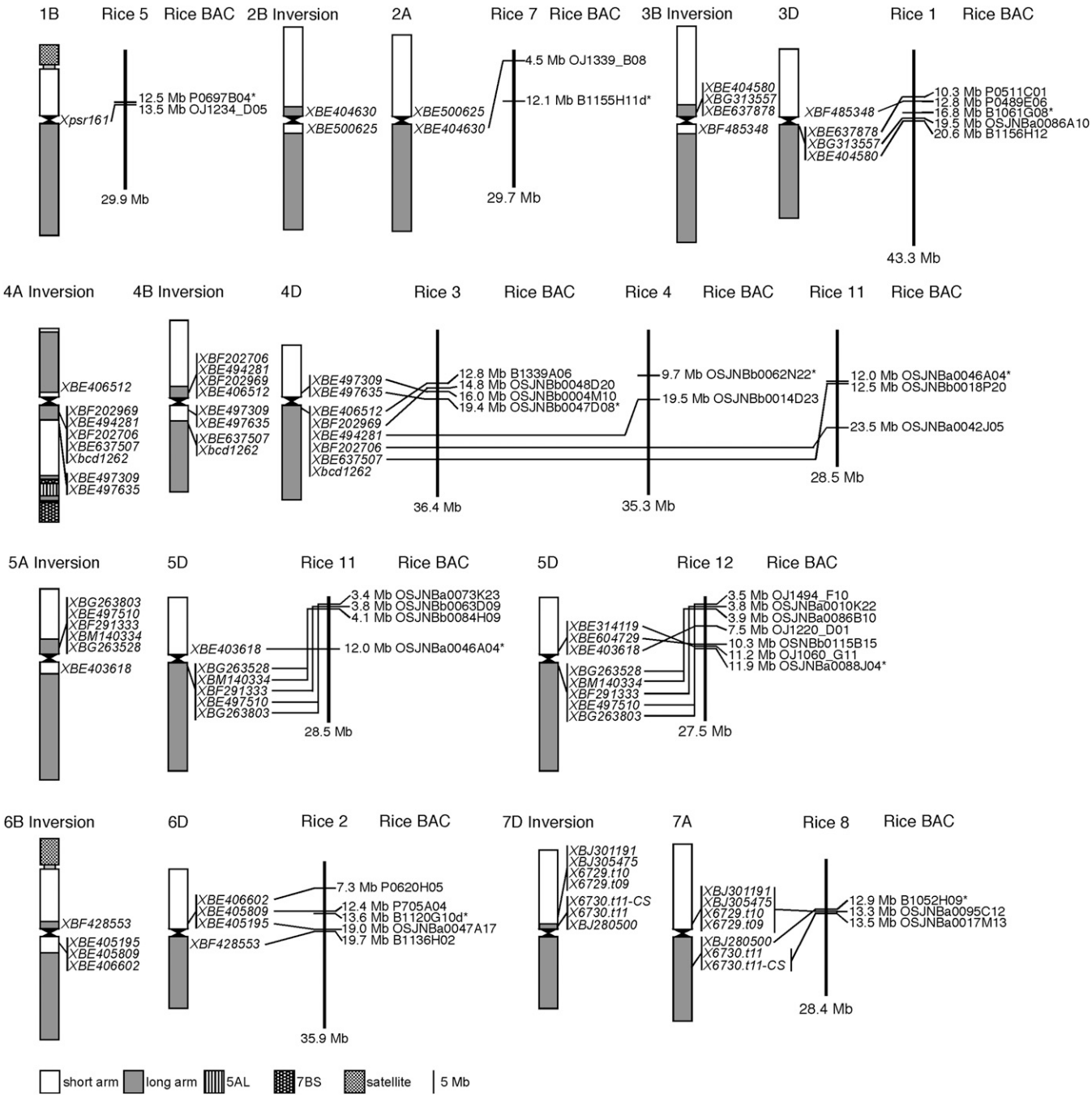

FIGURE 3.—Blast search results of wheat EST sequences against rice pseudomolecules. The wheat ESTs previously mapped to the centromeric regions showed pericentromeric inversions in chromosomes of homologous groups 2–7. The ESTs mapped in the D-genome chromosomes were selected as ancestral except for chromosomes 1B and 7A. The rice BAC positions in the maps are based on megabase distances in the pseudomolecule and were taken from the Rice Genome Annotation Project–MSU Rice Genome Annotation (Osa1) Release 6 (http://rice.plantbiology.msu.edu/cgi-bin/gbrowse/rice/). *Rice centromeric BAC.

(Table 3). *Hin*dIII-digested positive BAC clones were probed with the centromere-specific clones pAet6-09, Hi10, and pRCS1 to identify BAC clones containing centromere-specific repeats. More than half of the positive BAC clones did not hybridize with centromere-specific repeats. However, most BAC clones of *Ae. tauschii* or *Ae. speltoides* that were anchored with W4 ESTs strongly hybridized with centromeric clones and showed a tandem repeat pattern with a range of 6–17 fragments (Table 3).

To further confirm their physical location, the BAC clones anchored by W4 ESTs were mapped by BAC-FISH to mitotic metaphase chromosomes of CS wheat. Wheat EST BE497635 mapped to the R3 centromere. Of the *Ae. tauschii* BACs harboring this locus, HD073F23 preferentially hybridized to the centromeres of CS

TABLE 3

Hybridization results of BACs with centromeric-specific clones and BAC-FISH results with the selected BACs

Group	Marker	Associated BAC	Contig[b]	BAC library	No. fragments of BAC hybridizing to: pAet6-09	Hi10	PRCS1	BAC-FISH signal
1	PSR161	13K16[a]	NA	*Ae. speltoides*	0	0	0	P/+++
		27O24	NA	*Ae. speltoides*	0	0	0	
		49E02	NA	*Ae. speltoides*	0	0	0	
		83M17	NA	*Ae. speltoides*	0	0	0	
		144A17	NA	*Ae. speltoides*	0	0	0	
2	BE404630	RI017L168[a]	ctg3127	*Ae. tauschii*	1	1	1	−
		RI018G22	ctg3127	*Ae. tauschii*	1	1	1	
		RI032A4	Singleton	*Ae. tauschii*	1	1	1	
3	BE485348	HD90D18	ctg734	*Ae. tauschii*	0	0	0	
		RI031J15[a]	ctg734	*Ae. tauschii*	1	1	1	P/+++
		HD082G15	Singleton	*Ae. tauschii*	1	1	1	
		HI068F4	Singleton	*Ae. tauschii*	0	0	0	
	BE404580	HD012G15	ctg1754	*Ae. tauschii*	0	0	0	
4	BE497309	HD008H1[a]	Singleton	*Ae. tauschii*	17	13	6	C/++
	BE497635	RI003E6[a]	ctg2862	*Ae. tauschii*	2	1	0	P/+++
		HD073F23[a]	Singleton	*Ae. tauschii*	2	1	0	C/+++; P/++
	BF202706	RI004I21	ctg8723	*Ae. tauschii*	0	0	0	
		HD024H2[a]	Singleton	*Ae. tauschii*	3	2	1	C/++; P/++
		HD015P19[a]	Singleton	*Ae. tauschii*	3	1	1	C/++; P/++
		HD003D13	Singleton	*Ae. tauschii*	3	2	1	
		21E12[a]	NA	*Ae. speltoides*	11	5	3	C/+++
		256K19[a]	NA	*Ae. speltoides*	11	4	6	C/+++
5	BE403618	HD67C12	ctg5061	*Ae. tauschii*	0	0	0	
		HI004K2[a]	ctg3651	*Ae. tauschii*	1	1	1	−
		HI074N14[a]	Singleton	*Ae. tauschii*	1	1	1	−
		HD90B04	Singleton	*Ae. tauschii*	0	0	0	
6	BF428533	HD062L14	ctg3048	*Ae. tauschii*	0	0	0	
		HD80B22	ctg3048	*Ae. tauschii*	0	0	0	
		HI80I03[a]	ctg3048	*Ae. tauschii*	1	1	1	P/+++
		HD14P08	Singleton	*Ae. tauschii*	0	0	0	
	BE405809	RI31A22	ctg3817	*Ae. tauschii*	1	1	1	
7	6729.t10	HD28P20[a]	ctg6291	*Ae. tauschii*	0	0	1	P/+++
		HD57F11[a]	ctg6291	*Ae. tauschii*	0	0	0	P/++
	6730.t11-CS	HD32N11[a]	Singleton	*Ae. tauschii*	1	1	1	P/+++

Localization of FISH signals: C, centromere; P, paint along entire chromosomes. − and + represent, respectively, the presence and absence of hybridization signals: +++, strong signal; ++, intermediate signal. NA, contig information is not available for *Ae. speltoides* BACs.

[a] Selected BACs for BAC-FISH.

[b] Contig information is taken from http://wheatdb.ucdavis.edu:8080/wheatdb. FPC assembly is 1.1 version.

chromosomes, but also hybridized weakly over their entire length. The second BAC RI003E6 gave a dispersed signal (Table 3). The *Ae. tauschii* BAC clone HD008H01 harboring BE497309 (mapped to the short arm of R3 at a distance of 3.4 Mb from the centromere) was exclusively localized at the primary constriction of CS chromosomes (Figure 4). Of the *Ae. tauschii* and *Ae. speltoides* BAC clones harboring BE202706, the *Ae. speltoides* BACs gave a strong signal exclusively at the centromeres of CS chromosomes, whereas *Ae. tauschii* BACs gave a signal at the centromeres as well as along their entire length (Table 3, Figure 4). The apparently centromeric BE202706 clone in wheat was mapped in the terminal end of the long arm of R11 at 11.5 Mb from the centromere in rice.

Nine BAC clones that showed only one fragment with the centromere-specific repeats harboring pericentromeric ESTs from chromosomes W1, W2, W3, W5, W6, and W7 were also analyzed by BAC-FISH. Six gave a FISH signal over the entire length of all CS chromosomes due to their high content of noncentromere-specific repetitive DNA, and three did not hybridize to any of the wheat chromosomes (Table 3).

DISCUSSION

The impetus for the present study came from the discovery of transcribed genes in the CENH3 core domains of the centromeres of rice chromosomes 3 and 8 (Nagaki *et al.* 2004; Yan *et al.* 2005, 2006). In

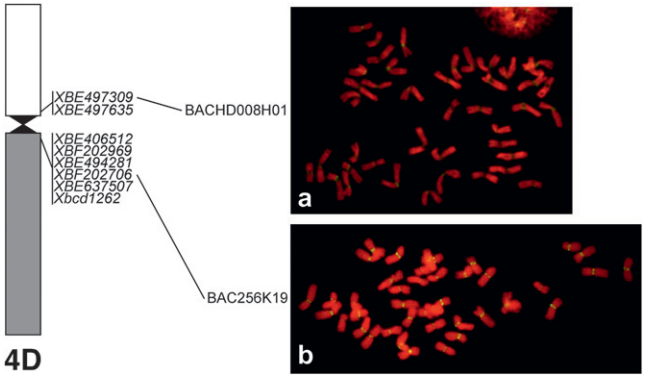

Figure 4.—FISH patterns of (a) *Ae. tauschii* BAC HD008H01 and (b) *Ae. speltoides* BAC 256K19. Both BACs anchored by group-4 ESTs are exclusively located in the wheat centromeres visualized by yellow-green FITC florescence.

wheat, a collection of telosomic chromosomes with breaks at the centromere are available for all 42 arms of the 21 chromosomes and for many of the related Triticeae species added to wheat as telosomic additions (Sears and Steinitz-Sears 1978; Islam *et al.* 1981; Mukai *et al.* 1992; Friebe *et al.* 1993, 2000). These stocks allow mapping of any probe to the short arm, the long arm, or the centromere (see Figure 1). Because gene synteny is conserved between wheat and rice, comparative mapping of centromeric genes of rice on wheat aneuploid stocks provides an elegant system for testing centromere homology between wheat and rice. For *Cen8*, contiguous rice genes 6729.t09 and 6729.t10 located in the CENH3 core mapped to the centromeric bin in the short arm of wheat chromosomes 7A, 7B, and 7D. Another CENH3-domain rice gene 6730.t11, located at 200 kb from 6729.t09/10, mapped to the long arm of wheat chromosomes 7A and 7B but to the short arm of 7D. Thus, these two genes that are 200 kb apart in the rice CENH3 domain span the large centromere in wheat group-7 chromosomes and are subjected to frequent inversions due to the dynamic nature of pericentromeric regions. Gene 6733.t09 located outside the CENH3 domain in rice mapped to the distal ends of group-3 chromosomes and provided evidence for the breakdown of synteny. The data presented here provide a method for determining centromere homology between wheat and rice. These and other aspects in the structure and evolution of the wheat and rice centromeric regions are discussed below.

Recurrent origin of pericentromeric inversions: Previously Qi *et al.* (2006) reported that pericentric regions in the Triticeae, especially those of group-4 chromosomes, have undergone rapid and recurrent rearrangements. Pericentromeric inversions close to the centromere regions were detected in wheat chromosomes 2B, 3B, 4A, 4B, 5A, and 6B, as well as in other Triticeae species (Conley *et al.* 2004; Linkiewicz *et al.* 2004, Miftahudin *et al.* 2004; Qi *et al.* 2004, 2006). Analyzing W7-R8 centromere homology, we observed two independent pericentromeric inversions involving chromosome 7R of rye and 7D of wheat (Figures 1b and 2). This is the first report on a pericentromeric inversion in a D-genome chromosome of wheat, a relatively conserved genome compared to other genomes of hexaploid wheat (Qi *et al.* 2004, 2006).

Pericentromeric inversions were also reported between chimpanzee and human chromosomes on the basis of comparative karyotyping and on chromosome 4 of *A. thaliana* specific to several ecotypes (Yunis and Prakash 1982; Nickerson and Nelson 1998; Fransz *et al.* 2000; Goidts *et al.* 2005; Kehrer-Sawatzki *et al.* 2005). It is not clear why pericentromeric regions are prone to inversions. One possibility could be the occurrence of more frequent ectopic recombination, because the centromeres are enriched in tandem satellite repeat units. Another reason could be the stress imposed on the centromeres especially during movement to the spindle poles. Kinetochores trap the microtubules and act as "arm joints" that bear the chromosome load during their movement to the poles and may be damaged. The repair of damaged centromeres in the subsequent interphase may lead to structural changes including pericentromeric inversions. Nevertheless, as a result of pericentric inversions, the CENH3 domains and associated genes are moving targets changing position, moving in, out, and around the centromeric regions, and there may be an adaptive value to these perturbations for specific chromosome services rendered. The practical value and useful outcome of the identification of the genes and clones involved in pericentromeric inversions is that they must be close to the centromere and thus provide useful markers in analyzing centromere homology in taxa where gene synteny is conserved, as is the case between wheat and rice.

Centromere synteny between wheat and rice: Wheat and the Triticeae with $1x = 7$ have 7 centromeres and rice with $1x = 12$ has 12 centromeres. If $1x = 12$ was the ancestral chromosome number of the common progenitor of wheat and rice (Salse *et al.* 2008), then what was the fate of the 5 centromeres during the evolutionary reduction of the basic chromosome number from $1x = 12$ to $1x = 7$ in wheat and the Triticeae? During this reduction in basic chromosome number, some centromeres may have been conserved, whereas others were inactivated or eliminated, and 2 may have fused to form 1, or others may have arisen *de novo*. One of the important findings of the present study is that most wheat centromeres showed one-to-one correspondence to rice centromeres. We detected homology, with one possible exception, between 7 wheat and 7 rice centromeres.

Large-scale EST sequence comparisons using bin-mapped wheat ESTs and rice pseudomolecules had previously indicated colinearity between W3 and R1 and between W6 and R2 chromosomes (Sorrells *et al.* 2003;

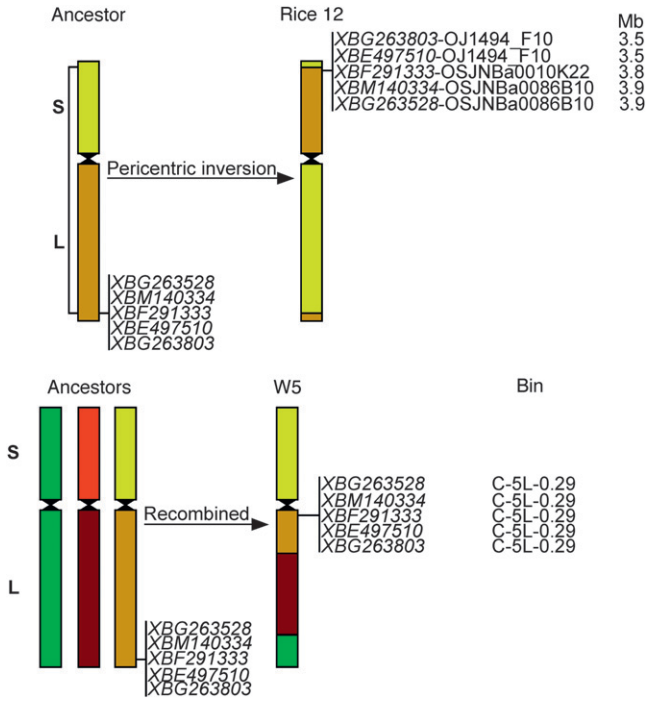

FIGURE 5.—Chromosome evolution of rice chromosome 12 and wheat group-5 chromosome. Different ancestral chromosomes are color coded. Five wheat ESTs that mapped to the pericentromeric region of the long arm of group-5 chromosomes were selected and represent homologous sequences in the ancestor, wheat, and rice. S, short arm; L, long arm.

La Rota and Sorrells 2004; Munkvold *et al.* 2004; Randhawa *et al.* 2004). Their centromere homology was confirmed in this study. W3-R1 and W6-R2 chromosomes and their centromeres have maintained perfect synteny (Figure 3). The remaining wheat and Triticeae chromosomes are associated with linkage blocks corresponding to two or three rice chromosomes (Ahn *et al.* 1993; Gale and Devos 1998; Sorrells *et al.* 2003; Conley *et al.* 2004; Hossain *et al.* 2004; La Rota and Sorrells 2004; Linkiewicz *et al.* 2004; Miftahudin *et al.* 2004; Peng *et al.* 2004; Randhawa *et al.* 2004; Salse *et al.* 2008). Chromosome W1 arose from the fusion of chromosomes with homology to R5 and R10 but its centromere was derived from R5 (Figure 3). Chromosome W2 was derived from the fusion of ancestral chromosomes sharing homology with R4 and R7, and the W2 centromere is homologous to R7. Chromosome W5 arose from the fusion of ancestral chromosomes sharing homology with R3, R9, and R12, but its centromere was derived from R12. Chromosome W7 arose from the fusion of a chromosome sharing homology with R6 and R8 and, as deduced previously, we confirmed that its centromere was derived from R8.

The centromere region of W4 appears to be more complex and may be an exception to the single-centromere origin in wheat (Figure 3). Chromosome W4 arose mainly from the fusion of chromosomes sharing homology with R3 and R11 and its centromere may be derived from R3, but a hybrid origin containing parts of R3 and R11 centromeres cannot be ruled out (Figure 6). The EST BF202706 was mapped to the pericentromeric region of W4 by both deletion mapping and BAC-FISH analysis (Figure 4). However, rice BAC OsNBa0042J05 aligned by this EST is located in the distal region of rice chromosome 11 (Figure 3). A possible paracentric inversion could explain the difference in location of this EST between wheat and rice.

We did not detect centromere homology of any of the wheat chromosomes to the centromeres of R4, R6, R9, and R10. On the basis of these results, the most likely hypothesis is that centromeres sharing homologies with R4, R6, R9, and R10 were either eliminated or inactivated (Birchler *et al.* 2009). However, a rigorous test of this hypothesis must await the complete assembly of the centromeres of these chromosomes (similar to R3 and R8; Nagaki *et al.* 2004; Wu *et al.* 2004; Yan *et al.* 2006) followed by comparative mapping in wheat aneuploid stocks using the approach outlined in this article.

Surprisingly, our present study indicated that most of the pericentromeric ESTs of the W5 long arm

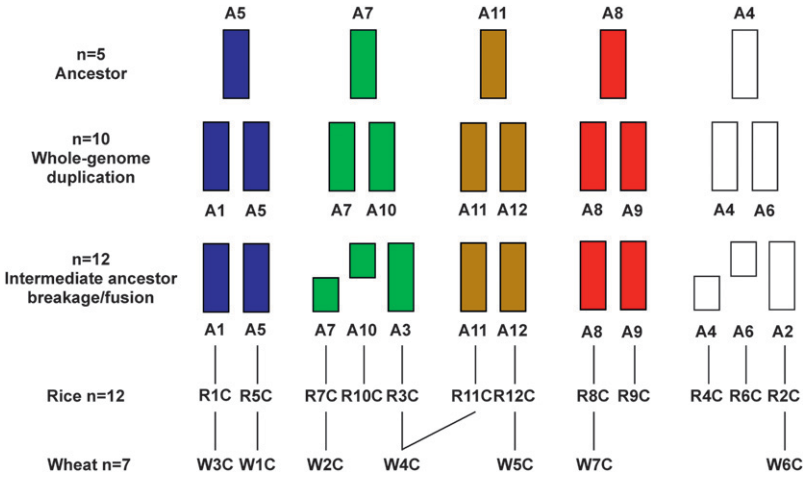

FIGURE 6.—Model for centromere evolution of rice and wheat from a common ancestor with $n = 5$ chromosomes, modified from Salse *et al.* (2008). C, centromere.

matched the short arm BACs located on the duplicated block of rice R11S and R12S (Table S4, Figure 3). Sequence alignment of the same W5 ESTs against the Brachypodium 8× release database and BAC-FISH using Brachypodium BAC clones anchored by W5 ESTs as probes indicated that the Brachypodium homologous sequences to W5 ESTs are located in the distal end of the long arm of *Brachypodium distachyon* chromosome 4 with similar gene order to that in wheat (L. L. Qi, B. Friebe, Y. Q. Gu, Q. Chen, B. S. Gill, unpublished data, http://www.brachybase.org/blast/) A hypothesis of evolutionarily independent inversion events is illustrated in Figure 5 to explain the difference in location of these homologous sequences in wheat and rice. In an ancestral chromosome, homologous sequences represented by selected wheat ESTs reside in the distal end of the long arm. A pericentric inversion occurred to form rice chromosome 12. In wheat, an insertion or translocation moved the sequences from the distal end of the ancestral chromosome to the region proximal to the centromere of the long arm of W5 chromosomes with the same gene order as that in the ancestor. It is evident that the long arm of wheat group-5 chromosomes was known as a recombined chromosome arm, with sequence similarity to rice chromosomes R12, R9, and R3 (Figure 5, La Rota and Sorrells 2004; Linkiewicz *et al.* 2004; Rice Chromosomes 11 and 12 Sequencing Consortia 2005). The pericentric inversion is specific to the rice lineage. It is supported by data that more than 90% of wheat ESTs that mapped to the short arms of group-5 chromosomes aligned to the long arm of rice chromosome 12, and most of wheat ESTs that mapped to the long arms of group-5 chromosomes aligned to the short arm of rice chromosome 12 (Sorrells *et al.* 2003; Linkiewicz *et al.* 2004; Rice Chromosomes 11 and 12 Sequencing Consortia 2005). In barley, most ESTs in the distal part of the short arm of chromosome 5H mapped to the distal region of the long arm of rice chromosome 12 (Stein *et al.* 2007).

In the model of cereal karyotype evolution proposed by Salse *et al.* (2008), a common ancestor with five chromosomes, A5, A7, A11, A8, and A4, underwent whole-genome duplication to produce an intermediate ancestor with $n = 10$ chromosomes that, following breakage and fusion events, produced the $n = 12$ karyotype of rice (Figure 6). The centromeres of wheat chromosomes can be traced to the ancestral chromosomes as follows: W1 and W3 trace their centromere to A5 through duplicated chromosomes A1 and A5; W2 and W4 trace their lineages to ancestral chromosome A7. In addition, W4 may contain a part of a centromere tracing to the A11 lineage (Figure 6). W5, W6, and W7 trace their lineages directly to ancestral chromosomes A11, A8, and A4, respectively. The depicted framework provides a working model for further studies on the structure and evolution of cereal chromosome centromeres.

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A Molecular-Cytogenetic Method for Locating Genes to Pericentromeric Regions Facilitates a Genomewide Comparison of Synteny Between the Centromeric Regions of Wheat and Rice

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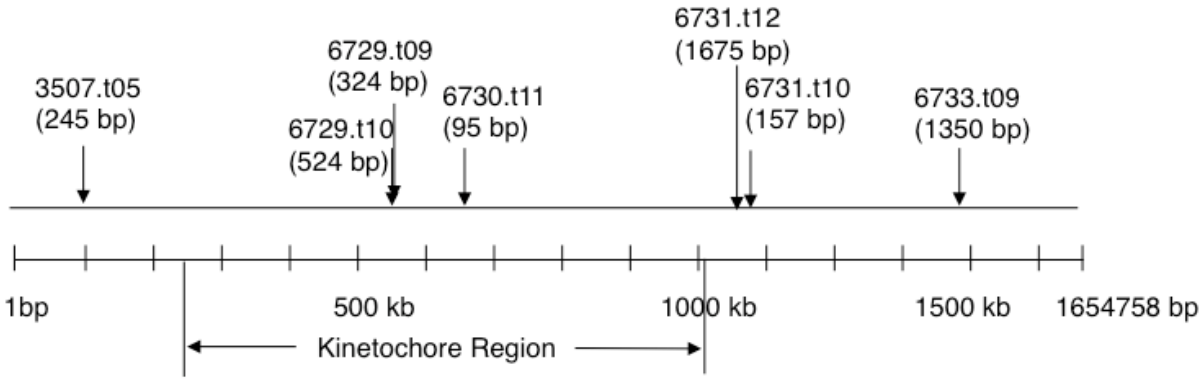

FIGURE S1.—Location of rice RT-PCR clones selected for mapping in the centromere virtual contig of chromosome 8. The number in parentheses indicates the clone size.

TABLE S1

List of genetic stocks used in the study. All lines are in the Chinese Spring background unless otherwise indicated (TA Triticeae accession)

TA no.	Genetic Stocks	Description	Reference
TA3258	N1AT1D	Nullisomic 1A tetrasomic 1D	SEARS, 1954
TA3260	N1BT1D	Nullisomic 1B tetrasomic 1D	SEARS, 1954
TA3262	N1DT1B	Nullisomic 1D tetrasomic 1B	SEARS, 1954
TA3263	M2AT2B*	Monosomic 2A tetrasomic 2B	SEARS, 1954
TA3266	N2BT2D	Nullisomic 2B tetrasomic 2D	SEARS, 1954
TA3267	N2DT2A	Nullisomic 2D tetrasomic 2A	SEARS, 1954
TA3270	N3AT3D	Nullisomic 3A tetrasomic 3D	SEARS, 1954
TA3272	N3BT3D	Nullisomic 3B tetrasomic 3D	SEARS, 1954
TA3274	N3DT3B	Nullisomic 3D tetrasomic 3B	SEARS, 1954
TA3278	N4AT4D	Nullisomic 4A tetrasomic 4D	SEARS, 1954
TA3276	M4BT4D*	Monosomic 4B tetrasomic 4D	SEARS, 1954
TA3279	N4DT4B	Nullisomic 4D tetrasomic 4B	SEARS, 1954
TA3063	N5AT5D	Nullisomic 5A tetrasomic 5D	SEARS, 1954
TA3065	N5BT5D	Nullisomic 5B tetrasomic 5D	SEARS, 1954
TA3067	N5DT5B	Nullisomic 5D tetrasomic 5B	SEARS, 1954
TA3152	N6AT6B	Nullisomic 6A tetrasomic 6B	SEARS, 1954
TA3154	N6BT6A	Nullisomic 6B tetrasomic 6A	SEARS, 1954
TA3157	N6DT6B	Nullisomic 6D tetrasomic 6B	SEARS, 1954
TA3281	N7AT7D	Nullisomic 7A tetrasomic 7D	SEARS, 1954
TA3284	N7BT7D	Nullisomic 7B tetrasomic 7D	SEARS, 1954
TA3286	N7DT7B	Nullisomic 7D tetrasomic 7B	SEARS, 1954
TA3104	Dt3AS	Ditelosomic 3AS	SEARS and STEINITZ-SEARS, 1978
TA3105	Dt3AL	Ditelosomic 3AL	SEARS and STEINITZ-SEARS, 1978
TA3115	Dt3BS	Ditelosomic 3BS	SEARS and STEINITZ-SEARS, 1978
TA3116	Dt3BL	Ditelosomic 3BL	SEARS and STEINITZ-SEARS, 1978
TA3193	Dt3DS	Ditelosomic 3DS	SEARS and STEINITZ-SEARS, 1978

TA3192	Dt3DL	Ditelosomic 3DL	SEARS and STEINITZ-SEARS, 1978
TA7739	CS-AESP DtA3S#3S	CS-*Aegilops speltoides* ditelosomic addition 3S#3S	FRIEBE *et al.* 2000
TA7520	CS-AELON DtA3S^{l}#2L	CS-*Aegilops longissima* ditelosomic addition 3S^{l}#2L	FRIEBE *et al*. 1993
TA3566	CS-I DtA 3RS	CS-*Secale cereale* cv imperial ditelosomic addition 3RS	MUKAI *et al*. 1992
TA3674	CS-AGEL DtA3EL	CS-*Agopyron elongatum* ditelosomic addition 3EL	DVORAK and KNOTT 1974
TA3591	CS-HVUL DtA3HS	CS-*Hordeum vulgare* cv Betzes ditelosomic addition 3HS	ISLAM *et al*. 1981
TA3592	CS-HVUL DtA3HL	CS-*Hordeum vulgare* cv Betzes ditelosomic addition 3HL	ISLAM *et al*. 1981
	Mt5AS*	Monotelosomic 5AS	QI unpublished data
TA3107	Dt5AL	Ditelosomic 5AL	SEARS and STEINITZ-SEARS, 1978
TA3118	Dt5BL	Ditelosomic 5BL	SEARS and STEINITZ-SEARS, 1978
TA3127	Dt5DL	Ditelosomic 5DL	SEARS and STEINITZ-SEARS, 1978
TA7523	CS-AELON DtA5S^{l}#2S	CS-*Aegilops longissima* ditelosomic addition 5S^{l}#2S	FRIEBE *et al*. 1993
TA7704	CS-AESP DtA5S#3L	CS-*Aegilops speltoides* ditelosomic addition 5S#3L	FRIEBE *et al*. 2000
TA3569	CS-I DtA 5RS	CS-*Secale cereale* cv imperial ditelosomic addition 5RS	MUKAI *et al*. 1992
TA3597	CS-HVUL DtA5HS	CS-*Hordeum vulgare* cv Betzes ditelosomic addition 5HS	ISLAM *et al*. 1981
TA3598	CS-HVUL DtA5HL	CS-*Hordeum vulgare* cv Betzes ditelosomic addition 5HL	ISLAM *et al*. 1981
TA3108	Dt7AS	Ditelosomic 7AS	SEARS and STEINITZ-SEARS, 1978
TA3109	Dt7AL	Ditelosomic 7AL	SEARS and STEINITZ-SEARS, 1978
TA3119	Dt7BS	Ditelosomic 7BS	SEARS and STEINITZ-SEARS, 1978
TA3120	Dt7BL	Ditelosomic 7BL	SEARS and STEINITZ-SEARS, 1978
TA3130	Dt7DS	Ditelosomic 7DS	SEARS and STEINITZ-SEARS, 1978
TA3069	Dt7DL	Ditelosomic 7DL	SEARS and STEINITZ-SEARS, 1978
TA7698	CS-AESP DtA7S#3S	CS-*Aegilops speltoides* ditelosomic addition 7S#3S	FRIEBE *et al*. 2000
TA7699	CS-AESP DtA7S#3L	CS-*Aegilops speltoides* ditelosomic addition 7S#3L	FRIEBE *et al*. 2000
TA3571	CS-I DtA7RS	CS-*Secale cereale* cv imperial ditelosomic addition 7RS	MUKAI *et al*. 1992
TA3572	CS-I DtA7RL	CS-*Secale cereale* cv imperial ditelosomic addition 7RL	MUKAI *et al*. 1992
TA3587	CS-HVUL DtA7H	CS-*Hordeum vulgare* cv Betzes ditelosomic addition 7HS	ISLAM *et al*. 1981
TA3588	CS-HVUL DtA7H	CS-*Hordeum vulgare* cv Betzes ditelosomic addition 7HL	ISLAM *et al*. 1981

* N2AT2B and N4BT4D plants were selected from progenies of M2AT2B and M4BT4D; Mt5AS was selected from a cross between ditelo 5AS monotelo 5AL and N5AT5D.

TABLE S2

Deletion lines and fraction length (FL) values

TA no.	Deletion	FL value	TA no.	Deletion	FL value
4522, 4	3AS-4	0.45	4538, 1	5DS-1	0.63
4522, 2	3AS-2	0.23	4539, 7	5DL-7	0.29
4523, 3	3AL-3	0.42	4539, 1	5DL-1	0.60
4523, 5	3AL-5	0.78	4539, 9	5DL-9	0.74
4524, 8	3BS-8	0.78	4539, 5	5DL-5	0.76
4524, 9	3BS-9	0.57	4519, 2	7AS-1	0.89
4524, 1	3BS-1	0.33	4511, 5	7AS-5	0.59
4525, 2	3BL-2	0.22	4546, 8	7AS-8	0.45
4525, 10	3BL-10	0.50	4546, 3	7AS-3	0.29
4525, 7	3BL-7	0.63	4546, 4	7AS-4	0.26
4526, 6	3DS-6	0.55	4546, 6	7AS-6	0.21
4526, 3	3DS-3	0.24	4547, 4	7AL-4	0.18
4518, 4	3DL-2	0.27	4547, 14	7AL-14	0.31
4527, 3	3DL-3	0.81	4547, 1	7AL-1	0.39
4534, 7	5AS-7	0.98	4546, 8	7AL-17	0.71
4534, 3	5AS-3	0.75	4529, 13	7AL-21	0.74
4539, 7	5AS-1	0.40	4526, 3	7AL-16	0.86
4535, 12	5AL-12	0.35	4526, 6	7AL-18	0.90
4535, 10	5AL-10	0.57	4548, 1	7BS-1	0.27
4535, 17	5Al-17	0.78	4548, 3	7BS-3	0.16
4535, 23	5AL-23	0.87	4549, 14	7BL-14	0.14
4536, 6	5BS-6	0.81	4551, 6	7BL-2	0.33
4536, 5	5BS-5	0.71	4549, 7	7BL-7	0.63
4536, 8	5BS-8	0.56	4524, 8	7BL-10	0.78
4536, 4	5BS-4	0.43	4551, 4	7DS-4	0.61
4537, 6	5BL-6	0.29	4551, 5	7DS-5	0.36
4537, 1	5BL-1	0.55	4551, 3	7DS-3	0.15

4537, 14	5BL-14	0.75	4550, 6	7DL-6	0.10
4537, 9	5BL-9	0.76	4550, 1	7DL-1	0.14
4537, 16	5BL-16	0.79	4550, 5	7DL-5	0.30
4538, 2	5DS-2	0.78	4550, 2	7DL-2	0.61
4538, 5	5DS-5	0.67	4550, 3	7DL-3	0.82

TABLE S3

Blast search results of wheat ESTs against rice genomic DNA and ordered BACs and PACs

		Rice					
Wheat group	Wheat clone	Chromosome	Hit score	E-value	Genomic clone	BAC clone	Description
Cen1B	PSR161	5	628	1.90E-38	OS05g23740	OJ1234_D05	DnaK family protein, putative, expressed
2S	BE500625	N/A	N/A	N/A	N/A	N/A	N/A
2L	BE404630	7	457	3.10E-39	OS07g08840	OJ1339_B08	thioredoxin, putative, expressed
3S	BF485348	1	1281	6.40E-52	OS01g22900	P0489E06	neutral/alkaline invertase, putative, expressed
3L	BE637878	1	320	7.70E-19	OS01g18390	P0511C01	OSRCI2-1 - Putative low temperature and salt responsive protein
3L	BG313557	1	300	4.60E-16	OS01g35184	OSJNBa0086A10	CAMK_KIN1/SNF1/Nim1_like.10 - CAMK includes calcium/calmodulin
3L	BE404580	1	564	1.70E-81	OS01g36930	B1156H12	ubiquitin carboxyl-terminal hydrolase 6, putative, expressed
4S	BE497309	3	816	5.60E-41	OS03g27840	OSJNBb0004M10	splicing factor, arginine/serine-rich 16, putative, expressed
4S	BE497635*	3	1102	9.30E-44	OS03g34040	OSJNBb0047D08**	ribOSomal protein, putative, expressed
4L	BE406512	3	578	5.30E-20	OS03g22350	B1339A06	Brix domain containing protein, putative, expressed
4L	BF202969	3	453	1.20E-28	OS03g25940	OSJNBb0048D20	cystathionine gamma-synthase, putative, expressed
4L	BE494281	4	797	2.70E-50	OS04g32710	OSJNBb0014D23	40S ribOSomal protein S27, putative, expressed
4L	BF202706	11	405	2.70E-32	OS11g40140	OSJNBa0042J05	peptidase, T1 family, putative, expressed
4L	BE637507	11	1070	3.60E-42	OS11g21990	OSJNBb0018P20	Expressed protein
4L	BCD1262	N/A	N/A	N/A	N/A	N/A	N/A
5S	BE403618*	12	615	5.80E-86	OS12g13380	OJ1220_D01	adenylate kinase, putative, expressed
		11	564	9.50E-72	OS11g20790	OSJNBa0046A04**	adenylate kinase, putative, expressed
5L	BG263528	12	864	2.90E-33	OS12g07700	OSJNBa0086B10	NifU, putative, expressed
		11	801	3.00E-30	OS11g07916	OSJNBb0084H09	NifU, putative, expressed
5L	BM140334	12	718	9.90E-64	OS12g07670	OSJNBa0086B10	transmembrane 9 superfamily member, putative, expressed

		11	598	9.80E-59	OS11g07910	OSJNBb0084H09	transmembrane 9 superfamily member, putative, expressed
5L	BF291333	12	520	4.10E-28	OS12g07540	OSJNBa0010K22	growth regulator related protein, putative, expressed
		11	401	2.40E-19	OS11g07510	OSJNBb0063D09	growth regulator related protein, putative, expressed
5L	BG263803	12	612	2.10E-45	OS12g07190	OJ1494_F10	CBS domain-containing protein, putative, expressed
		11	557	7.80E-40	OS11g06930	OSJNBa0073K23	CBS domain-containing protein, putative, expressed
5L	BE497510	11	1120	3.20E-62	OS11g06890	OSJNBa0073K23	vacuolar ATP synthase, putative, expressed
		12	1121	3.10E-60	OS12g07140	OJ1494_F10	expressed protein
6S	BE406602	2	577	3.90E-27	OS02g13530	P0620H05	40S ribOSomal protein S24, putative, expressed
6S	BE405809	2	509	1.50E-53	OS02g20930	P0705A04	apoptOSis inhibitor 5, putative, expressed
6S	BE405195	2	486	1.00E-15	OS02g32160	OSJNBa0047A17	copine, putative, expressed
6L	BF428553	2	477	2.10E-15	OS02g33149	B1136H02	OR, putative, expressed
6L	BE604879	N/A	N/A	N/A	N/A	N/A	N/A
7S	BJ301191	8	933	9.30E-58	OS08g22354	OSJNBa0095C12	polyadenylate-binding protein, putative, expressed
7S	BJ305475	8	884	2.20E-70	OS08g22354	OSJNBa0095C12	polyadenylate-binding protein, putative, expressed
7L	BJ280500*	8	426	5.20E-13	OS08g21760	B1052H09**	Rer1 protein, putative, expressed

* Wheat EST aligns to rice centromeric BAC. ** Rice centromeric BAC. N/A not available.

TABLE S4

Syntenic block between wheat ESTs that mapped to the proximal region of the centromere in the group 5 with rice chromosome 12 and rice 11S and 12S duplications

		Rice					
Wheat EST	Bin location in W5	Chromosome	Position (Mb)	Score bit	E-value	Genomic clone	BAC clone
BE352603	5S-0.40-0.56	12L	25	482	2.60E-40	OS12g40510	OSJNBa0056D07
BE424034	C-5AS1-0.40	12L	22.7	940	1.30E-36	OS12g37060	OSJNBb0092G12
BE443466	C-5S-0.40	12L	20	654	1.70E-23	OS12g33180	OJ1126_F07
BG314119	C-5S-0.40	12S	11.2	894	1.00E-74	OS12g19304	OJ1060_G11
BE604729	C-5S-0.40	12S	10.3	1671	1.70E-69	OS12g17900	OSJNBb0115B15
BE495184	C-5S-0.40	12S	7.5	798	2.90E-44	OS12g13460	OJ1003_E07
BE403618*	C-5S-0.40, C-5AL12-0.35	12S	7.5	615	5.80E-86	OS12g13380	OJ1220_D01
		11	12	564	9.50E-72	OS11g20790	OSJNBa0046A04**
BE606654	C-5S-0.40	12S	7.1	1444	7.00E-68	OS12g12850	OSJNBb0069I24
BE444353	C-5DL1-0.60	12S	5.7	824	5.80E-82	OS12g10650	OSJNBb0071I17
BF291857*	C-5AS1-0.40, C-5L-0.60	12S	5	848	1.90E-60	OS12g09580	OJ1561_A05
BM140458	C-5DL1-0.60	12S	5	703	6.40E-48	OS12g09580	OJ1561_A05
BE403518*	C-5AS1-0.40, C-5L-0.29	12S	4.2	704	8.40E-33	OS12g08280	OSJNBb0089D09
BE403761*	C-5AS1-0.40, C-5L-0.29	12S	4.2	719	1.70E-63	OS12g08260	OSJNBb0089D09
BG263504	C-5AS1-0.40	12S	3.9	508	1.80E-35	OS12g07720	OSJNBa0086B10
BG263528*	C-5AS1-0.40, C-5L-0.60	12S	3.9	864	2.90E-33	OS12g07700	OSJNBa0086B10
		11S	4.1	801	3.00E-30	OS11g07916	OSJNBb0084H09
BM140334*	C-5AS1-0.40, C-5L-0.29	12S	3.9	718	9.90E-64	OS12g07670	OSJNBa0086B10
		11S	4.1	598	9.80E-59	OS11g07910	OSJNBb0084H09
BF291333*	C-5AS1-0.40, C-5L-0.29	12S	3.8	520	4.10E-28	OS12g07540	OSJNBa0010K22
		11S	3.8	401	2.40E-19	OS11g07510	OSJNBb0063D09
BE425161*	C-5AS1-0.40, C-5DL1-0.60	12S	3.5	1165	4.70E-66	OS12g07140	OJ1494_F10
		11S	3.4	1216	6.10E-66	OS11g06890	OSJNBa0073K23
BE497510*	C-5AS1-0.40, C-5L-0.29	12S	3.5	1121	3.10E-60	OS12g07140	OJ1494_F10
		11S	3.4	1120	3.20E-62	OS11g06890	OSJNBa0073K23
BG263803*	C-5AS1-0.40, C-5L-0.29	12S	3.4	612	2.10E-45	OS12g07190	OJ1494_F10
		11S	3.5	557	7.80E-40	OS11g06930	OSJNBa0073K23
BF200949	C-5L-0.60	12S	3.3	941	3.00E-70	OS12g06800	OJ1057_G11
		11S	3.3	585	9.90E-38	OS11g06690	OSJNBa0011J22

BG262756	C-5L-0.60	12S	3.2	2017	8.80E-86	OS12g06640	OJ1057_G11
		11S	3.1	1617	1.40E-69	OS11g06410	OSJNBa0081F16
BM138619	C-5L-0.60	12S	3.2	1243	4.30E-50	OS12g06630	OJ1587_D05
		11S	3.1	844	5.00E-32	OS11g06420	OSJNBa0081F16
BF292055	C-5L-0.29	12S	3.2	837	1.10E-31	OS12g06560	OJ1587_D05
BE406545	5L-0.29-0.35	12S	3.2	434	5.50E-18	OS12g06660	OJ1057_G11
BE500582	5L-0.35-0.55	12S	2.2	374	5.50E-13	OS12g05000	OSJNBb0077O07
		11S	2.1	359	4.90E-10	OS11g04990	OSJNBa0039D03
BE496864	C-5L-0.60	12S	1.8	1174	2.00E-50	OS12g04290	OSJNBb0041E01
		11S	1.9	1161	1.60E-55	OS11g04520	OSJNBa0068G15
BE605032	C-5L-0.60	12S	1.8	662	8.60E-24	OS12g04270	OSJNBb0041E01
		11S	1.9	705	9.60E-26	OS11g04500	OSJNBa0068G15
BE490079	C-5AL12-0.35	12S	1.3	699	7.50E-46	OS12g03360	OJ1003_C01
		11S	1.4	709	9.80E-77	OS11g03590	OSJNBb0035B18
BE498305	C-5L-0.60	12S	1	1270	5.70E-63	OS12g02810	OJ1126_F08
		11S	1	1261	1.10E-62	OS11g02830	OSJNBa0048P17
BG604847	C-5L-0.60	12S	1.1	723	4.50E-49	OS12g02910	OJ1311_G04
		11S	1.1	723	4.30E-49	OS11g03160	OSJNBa0017B18
BE405060	5L-0.29-0.35	12S	0.7	1967	3.00E-84	OS12g02200	OJ1136_E08
		11S	0.6	1958	7.90E-84	OS11g02240	OSJNBa0025K19
BE585743	5L-0.29-0.35	12S	0.6	558	9.40E-26	OS12g02094	OJ1769_D07
		11S	0.5	545	1.70E-18	OS11g02150	OSJNBa0025K19
BF202268	C-5L-0.60	12S	0.4	415	1.20E-12	OS12g01680	OSJNBa0052H10
		11S	0.4	348	1.60E-09	OS11g01600	OSJNBa0010K05
BE398438	C-5L-0.60	12S	0.3	312	7.80E-08	OS12g01390	OSJNBb0068K19
		11S	0.2	299	3.00E-07	OS11g01380	OSJNBa0032J07
BE442814	5L-0.29-0.35	12S	0.3	670	2.50E-29	OS12g01390	OSJNBb0068K19
		11S	0.2	695	2.00E-30	OS11g01380	OSJNBa0032J07
BM138668	5L-0.35-0.55	12S	0.2	1889	1.90E-79	OS12g01360	OSJNBb0068K19
		11S	0.2	1869	1.50E-78	OS11g01330	OSJNBa0032J07
BF201857	C-5L-0.60	12S	0.1	555	3.80E-55	OS12g01170	OSJNBb0077A02
		11S	0.1	564	1.50E-55	OS11g01170	OSJNBa0029D01

* Diagnostic marker which detects the inversion in the chromosome 5A. **centromeric BAC of rice chromosome 11.