Funct Integr Genomics (2006) 6: 300–309
DOI 10.1007/s10142-005-0015-y

ORIGINAL PAPER

Wanlong Li · Bikram S. Gill

Multiple genetic pathways for seed shattering in the grasses

Received: 6 September 2005 / Revised: 3 October 2005 / Accepted: 8 October 2005 / Published online: 11 January 2006

Abstract Shattering is an essential seed dispersal mechanism in wild species. It is believed that independent mutations at orthologous loci led to convergent domestication of cereal crops. To investigate genetic relationships of Triticeae shattering genes with those of other grasses, we mapped spike-, barrel- (B-type), and wedge-type (W-type) spikelet disarticulation genes in wheat and its wild relatives. The *Br1* gene for W-type disarticulation was mapped to a region delimited by *Xpsr598* and *Xpsr1196* on the short arm of chromosomes 3A in *Triticum timopheevii* and 3S in *Aegilops speltoides*. The spike- and W-type disarticulation genes are allelic at *Br1* in *Ae. speltoides*. The B-type disarticulation gene, designated as *Br2*, was mapped to an interval of 4.4 cM between *Xmwg2013* and *Xpsr170* on the long arm of chromosome 3D in *Aegilops tauschii*, the D-genome donor of common wheat. Therefore, B- and W-type disarticulations are governed by two different orthologous loci on group-3 chromosomes. Based on map position, orthologs of *Br1* and *Br2* were not detected in barley, maize, rice, and sorghum, indicating multiple genetic pathways for shattering in grasses. The implications of the mapping results are discussed with regard to the evolution of polyploid wheat and domestication of cereals.

Keywords Shattering · Domestication · Molecular mapping · Wheat · Grass

Systems for fruit and seed dispersal are critical to the survival of wild plants in nature, but the loss of natural dispersal mechanisms was essential to the domestication of wild plants for agriculture. Because of the importance of this trait to agriculture, many studies have tried to identify genes and elucidate genetic pathways associated with natural fruit and seed dispersal. In the model plant *Arabidopsis thaliana*, seed dispersal is controlled by the interaction of several MADS-box and homeodomain genes. *SHATTERPROOF1* (*SHP1*) and *SHATTERPROOF2* (*SHP2*) promote the development of a dehiscence zone at the valve–replum boundary and the lignification of cells adjacent to the dehiscence zone (Liljegren et al. 2000). The expression of *SHP1* and *SHP2* is regulated positively by the product of the MADS-box gene AGAMOUS (Savidge et al. 1995) and negatively by the product of the MADS-box gene FRUITFULL (Ferrandiz et al. 2000) and the REPLUMLESS homeodomain protein (Roeder et al. 2003). In tomato, seeds disseminate via fruit shedding, which depends upon the formation of abscission zones (AZs). Two genes, *jointless* and *jointless2*, are responsible for the AZ formation. *Jointless* is a MADS-box gene coding for a transcription factor (Mao et al. 2000). Recently, a gene coding for a homeodomain protein highly similar to *Arabidopsis* REPLUMLESS and responsible for shattering at the *qSH-1* locus was cloned from rice chromosome 1 (Konishi et al. 2005).

The polyploid wheats evolved in two lineages (see Fig. 1). The modern durum (*Triticum turgidum* subsp. *durum* L., AABB genomes, 2n=4x=28) and common (bread) wheat (*Triticum aestivum* subsp. *aestivum* L., AABBDD genomes, 2n=6x=42) comprise one lineage and have the genotype *brbrtgtgQQ*, where *Br* controls rachis brittleness, *Tg* controls glume toughness, and *Q* controls seed threshability. In wild ancestral wheats, shattering is caused by a brittle rachis, which is conferred by a dominant *Br* allele. A recessive mutant allele *br* at this locus produced a nonbrittle spike. The first cultivated wheats had nonbrittle spikes but had tough glumes (*Tg*) and were hulled (*q*). Mutations at the *Tg* and *q* loci produced modern free-threshing or naked wheats. The gene *Q* encodes an *APETALA2*-like transcription factor, which pleiotropically influences a number of domestication-related traits in addition to threshability (Faris et al. 2003).

The only cultivated tetraploid wheat in the second lineage is *Triticum timopheevii* (Zhuk.) Zhuk. subsp. *timopheevii* (AAGG genomes, 2n=4x=28), which is a hulled form with a tough rachis derived from its wild

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s10142-005-0015-y

W. Li · B. S. Gill (✉)
Wheat Genetics Resource Center,
Department of Plant Pathology, Kansas State University,
Manhattan, KS 66506-5502, USA
e-mail: bsgill@ksu.edu

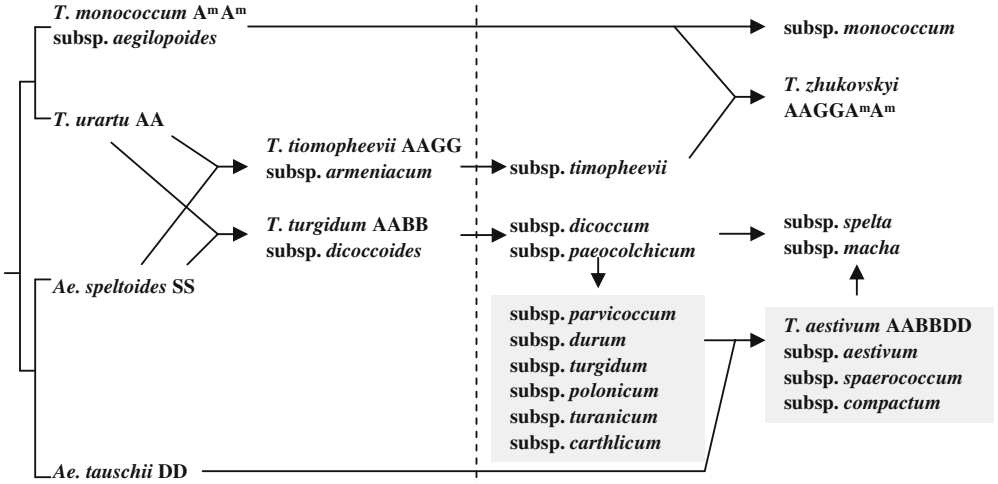

Fig. 1 Evolutionary relationships among different wheats and their domestication. The *vertical dashed line* separates the wild species (*left*) from the domesticated forms (*right*). The species and subspecies marked with *gray background* are free-threshing. The genome formula follows the species

shatter-prone ancestor *T. timopheevii* subsp. *armeniacum* (Jakubz.) MacKey (syn. *Triticum araraticum* Jakubz) by mutation at *Br* locus.

In the *Triticum–Aegilops* complex, shattering occurs by spike or spikelet disarticulation. In spike disarticulation, the rachis is brittle only near or at the base, and the whole spike detaches upon maturity as a dissemination unit. In spikelet disarticulation, the rachis is fragile at every joint, and the spike separates into individual spikelets at maturity. Spike disarticulation occurs only in the genus *Aegilops*, whereas spikelet disarticulation occurs in both *Aegilops* and *Triticum* (Kimber and Feldman 1987).

Spikelet disarticulation can be divided into two types, barrel- (B) and wedge-shaped (W), based on the products of disarticulation. B-type disarticulation results from breakage at the lower side of the junction of the rachis and spikelet base, and the adjacent rachis fragment remains attached behind each spikelet (Fig. 2). B-type disarticulation is observed in species containing the D genome (Kimber and Feldman 1987). In W-type disarticulation, the rachis fractures at the upper side of the junction of the rachis and spikelet base, and the associated rachis fragment remains attached below each spikelet (Fig. 2). W-type disarticulation occurs in species containing the A, B, G, S, or T genomes (Kimber and Feldman 1987) and in Tibetan semiwild wheat (SWW, Shao et al. 1983). In spelt wheat (*T. aestivum* subsp. *spelta* (L.) Thell.), the disarticulation types are associated with their origin. The European spelt has B-type, whereas the Iranian spelt has W-type disarticulation. It is speculated that European spelt originated from a cross between common wheat and emmer wheat (Tsunewaki 1968). One accession of SWW (Tsunewaki et al. 1990) and synthetic hexaploid wheat (McFadden and Sears 1946, Matsumoto et al. 1963) had both disarticulation types.

Alien chromosome substitution lines were used to localize the genes responsible for brittle rachis on chromosomes 3A and 3B of *T. turgidum* subsp. *dicoccoides* (Joppa and Cantrell 1990, Feldman 2001). Monosomic and telosomic analyses revealed that a single dominant gene on the short arm of chromosome 3D governs the brittle rachis of SWW (Chen et al. 1998). Telosomic analysis indicated the shattering genes were located at approximately 20 cM from the centromere on the short arms of chromosomes 3A and 3B of wild emmer and 3D of SWW, representing the orthologous locus *Br1* (Watanabe et al. 2002); however, intrachromosomal mapping using molecular markers has not been attempted. Little effort has been devoted to the genetic mapping of the genes for spike shattering and B-type spikelet shattering. Therefore, there is a lack of full understanding of the genetic relationships among the different types of shattering traits in the *Triticum–Aegilops* complex

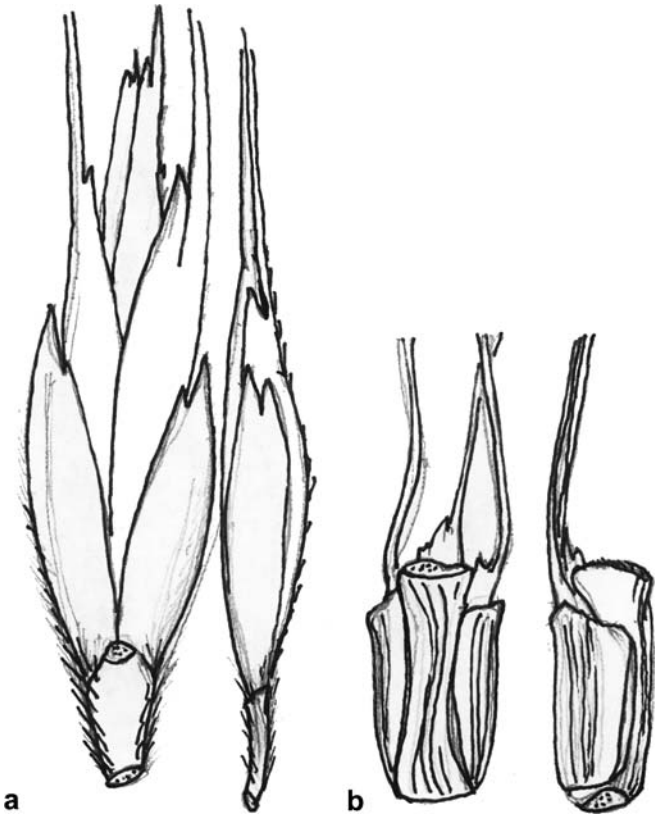

Fig. 2 Patterns of spikelet disarticulation: adaxial and lateral views of a W-type spikelet of *Triticum timopheevii* subsp. *armeniacum* (**a**) and a B-type spikelet of *Ae. tauschii* (**b**). Drawing by W.J. Raupp

and those of wheat with other grasses in terms of chromosome synteny. In this paper, we report the molecular mapping of the B-type gene in *Aegilops tauschii* and W-type shattering genes in *T. timopheevii* subsp. *armeniacum* and *Aegilops speltoides*, its implications for polyploid wheat evolution and cereal domestication, and genetic relationships among shattering genes of the Triticeae and the grasses.

Materials and methods

Plant materials

T. timopheevii subsp. *timopheevii* has a tough rachis, whereas its wild ancestor, *T. timopheevii* subsp. *armeniacum*, has a brittle rachis with W-type disarticulation. A cross was made between *T. timopheevii* subsp. *timopheevii* (accession Tim01, from Dr. M. Feldman, Weizmann Institute, Israel) and *T. timopheevii* subsp. *armeniacum* [accession TA39, Wheat Genetics Resource Center (WGRC), Kansas State University (KSU), Manhattan, Kansas]. The F_1 plants were bagged and allowed to self. The F_2 plants were scored for shattering. From these F_2 plants, a recombinant inbred line (RIL) population is under development using single-seed descent for fine mapping of the species-specific chromosome translocation breakpoints. Sixty nonshattering F_4 RILs were used for mapping the shattering gene in wild timopheevi wheat. Tim01 was used as a parent in developing a *T. timopheevii–Ae. tauschii* amphiploid (E.R. Sears, unpublished data) and, subsequently, *T. aestivum–T. timopheevii* chromosome disomic addition (DA) lines, where a pair of chromosomes or chromosome arms of *T. timopheevii* was added into the background of *T. aestivum* subsp. *aestivum* cv. Chinese Spring (CS) and disomic substitution (DS) lines, where one pair of CS chromosomes was replaced by the homoeologs of *T. timopheevii* (Brown-Guedira et al. 1996). Addition line CS-Tim01 DA3GS and substitution lines CS-Tim01 DS 2G(2B), DS3G(3B), DS4G(4B), DS5G(5B), DS6A (6A), and DS6G(6B) were also used in this research for assignment of shattering gene to a specific chromosome within a homoeologous group.

Ae. speltoides is a dimorphic species that contains two variants or morphological forms, var. *speltoides* and var. *ligustica*. *Ae. speltoides* var. *speltoides* has spike-type disarticulation, whereas *Ae. speltoides* var. *ligustica* has W-type disarticulation at maturity. A cross was made between TA1873 (*Ae. speltoides* var. *speltoides*) and TA1875 (*Ae. speltoides* var. *ligustica*). The F_1 plants showed W-type disarticulation similar to the *Ae. speltoides* var. *ligustica* parent. A population of 434 F_2 plants was grown in Root-trainers (Hummert International Horticultural Supplies, Earth City, MO, USA) in the greenhouse, and 58 plants were used to map the shattering gene.

For mapping B-type disarticulation, an F_2 population of *Ae. tauschii*, the D-genome donor of bread wheat, was developed using a nonshattering mutant (TA1604) and a shattering accession (AL8/78). TA1604 was collected from Afghanistan by Dr. R. Metzger and is maintained by the WGRC at KSU. AL8/78 was originally collected from Armenia (Dvorak et al 1998) and was supplied by Dr. J. Dvorak (University of California, Davis, CA, USA). We grew 138 F_2 plants in the greenhouse and used 118 for mapping. Genotypes of the 118 F_2 plants were determined by phenotypic evaluations of F_3 families consisting of 12 plants each, which allowed us to be confident at the 0.05 level of probability that all genotypic classes were observed.

Gene designations followed the rules of nomenclature as listed in Catalogue of Gene Symbols for Wheat (McIntosh et al. 1998). Thus, for brittle rachis, *Br1* is triplicated, and alleles of A-, B-, and D-genome origin are designated as *Br-A1*, *Br-B1*, and *Br-D1*, respectively, as suggested by Watanabe et al (2002). We detected a new locus for barrel-type shattering in *Ae. tauschii* and designated this gene as *Br2* and the *tauschii* allele as *Br-D2*.

RFLP clones

The clone ABG471 was provided by Dr. A. Kleinhofs (Washington State University, Pullman, WA, USA); BCD, CDO, and WG clones were provided by Dr. M.E. Sorrells (Cornell University, Ithaca, NY, USA); MWG and cMWG were provided by Dr. A. Graner (Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany); KSU clones were developed and maintained at WGRC; PSR clones were provided by Dr. M.D. Gale (John Innes Centre, Norwich, UK); UMC clones were obtained by H.W. Bass (Florida State University, Tallahassee, FL, USA); and barley expressed sequence tag (EST) clone HC06J13 (GenBank accession CB866622) was supplied by Dr. N. Stein (Institute of Plant Genetics and Crop Plant Research). The wheat EST clones used in this research were developed and mapped to deletion bins by the wheat EST project (Qi et al. 2004), and one copy of the bin-mapped EST clones is maintained at WGRC.

Data collection and molecular mapping

The phenotype (brittle or nonbrittle) scoring was performed by gentle hand tapping of mature spikes and subsequent squeezing of the nonbrittle spikes. In all cases, squeezing revealed that the nonbrittle spikes had tough rachis.

Protocols reported by Faris et al. (2000) were followed for DNA isolation, digestion, electrophoresis, blotting, and filter hybridization. Genetic maps were constructed with MAPMAKER (Lander et al. 1987) using a minimum logarithmic odds (LOD) of 3.0 for the marker ordering and the Kosambi mapping function (Kosambi 1944). To align the wheat linkage maps from this research with the finished rice genomic sequences, cDNA and EST sequences were retrieved from National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) and used as queries for BLASTn searches against rice bacterial artifi-

cial chromosome (BAC) and P1-derived artificial chromosome (PAC) sequences at The Institute for Genomic Research (TIGR, http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1). The rice PACs matched at an *E* value of 10^{-10} or less were considered carrying homologs of the wheat ESTs.

Results

Molecular mapping of *Br-A1*

The brittle rachis phenotype of *Br1* is dominant over the nonbrittle because the spikes of F_1 hybrids shattered into the W-type spikelets as observed in the wild timopheevi parent (Fig. 2). The F_2 population segregated 138 shattering and 42 nonshattering, which was a good fit to the monogenic segregation ratio of 3:1 (P=0.58). Thus, a single dominant gene governs shattering in *T. timopheevii*.

Considering that wild timopheevi exhibits the same disarticulation type as *T. turgidum* subsp. *dicoccoides* and SWW, we speculated that a gene orthologous to *Br1* confers shattering in wild timopheevi. *Br1* was mapped to a region approximately 20 cM from the centromere on the short arm of the group-3 chromosomes corresponding to deletion bin 3S-0.57-0.78 in hexaploid wheat. Three RFLP probes and 32 ESTs previously mapped to this bin were selected to survey for polymorphism between Tim01 and TA39. Because *T. timopheevii* is a tetraploid species, polymorphic loci may be located in either homoeologous chromosome 3A or 3G. To eliminate monomorphic and unlinked polymorphic markers, we adopted a modified segregant pooling strategy for the polymorphism survey. A bulk was constructed using 20 nonshattering (tough rachis) plants. DNA from the bulk and the parents was digested using five restriction enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Xba*I. Twenty-six of the 38 probes revealed polymorphism between the parents. For 13 of these, the polymorphic fragments of both parents were present in the bulk at equal intensities, indicating that these loci were not linked to the shattering trait. For the remaining 13 probes, polymorphic fragments from the nonshattering parent Tim01 were present in the bulk, but polymorphic fragments from TA39 were either absent from the bulk or present at a lesser intensity (Fig. 3a). Therefore, these 13 probes presumably detected loci linked to the shattering gene and were used for mapping in 58 nonshattering RILs. The shattering gene in *T. timopheevii* was located in an interval 6.4 cM distal to marker *XksuA6* and 28.6 cM proximal to marker *Xpsr1196* on the short arm of a homoeologous group-3 chromosome (Fig. 4), which falls in the same genomic region as the *Br1* ortholog. RFLP analysis of the CS-Tim01 DA3GS and CS-Tim01 DS3G(3B) did not assign the linked markers to chromosome 3G (Fig. 3b), suggesting that the shattering gene in wild timopheevi is located on chromosome 3A. Accordingly, the shattering gene from wild timopheevi wheat was designated as *Br-A1*. Alignment of the EST-derived markers with the rice genomic sequences indicated that ESTs BE446135 and BG604577 had homologs in a PAC (GenBank accession AP002743), and BE498786 and BE500000 had homologs in another PAC (GenBank accession AP003301) on rice chromosome 1 (data not shown).

Molecular mapping of *Br-S1*

The F_2 population of *Ae. speltoides* (from the cross var. *speltoides*/var. *ligustica*) segregated into 316 plants with *ligustica* (W-type) and 118 plants with *speltoides* (spike-type) types of disarticulation, which fit a 3:1 ratio (P=0.33) and confirmed that *ligustica* morphology is dominant over that of *speltoides* and governed by a single gene (Miczynski 1926, Schiemann 1928, Kihara and Lilienfeld 1932). All the ligustica segregants had W-type spikelet disarticulation, and all speltoides segregants had spike-type disarticulation.

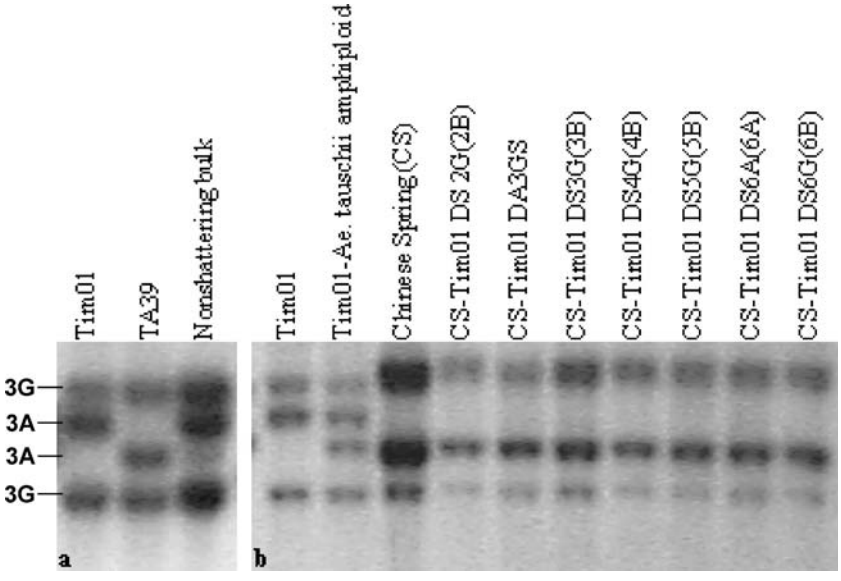

Fig. 3 Autograph of Southern blot hybridization using a group-3 specific wheat EST clone (GenBank accession BG604577) as a probe. **a** Polymorphic fragment from shattering parent TA39 is much weaker in the nonshattering bulk compared with that from nonshattering parent Tim01, suggesting that the polymorphism is linked with *Br1* locus. **b** The polymorphic fragment from Tim01 is not present in CS-Tim01 DA3GS and DS3G(3B), and therefore, the linkage group was assigned to chromosome 3A of *T. timopheevii*. The chromosome location of the *T. timopheevii* fragments was indicated on the *left*

Nonshattering recombinants were not observed. The data indicated that W-type disarticulation is allelic and dominant to the spike-type disarticulation. RFLP analysis of 58 F_2 individuals indicated that the shattering gene is located 9.9 cM distal to marker *Xabg471* and 32.3 cM proximal to marker *Xpsr1196*, falling within the genomic region of *Br1*. The shattering gene is orthologous to *Br1* and is designated as *Br1-S1* (Fig. 4).

Molecular mapping of *Br2*

The gene for B-type spikelet disarticulation in *Ae. tauschii* was mapped using a cross of a shattering parent (AL8/78, see Fig. 2) with a nonshattering natural variant (TA1604). Among the 118 F_2 plants, 76 had a brittle rachis and 42 had a nonshattering rachis. Thus, the segregation deviated from the expected monogenic ratio of 3:1 (P=0.011) with an

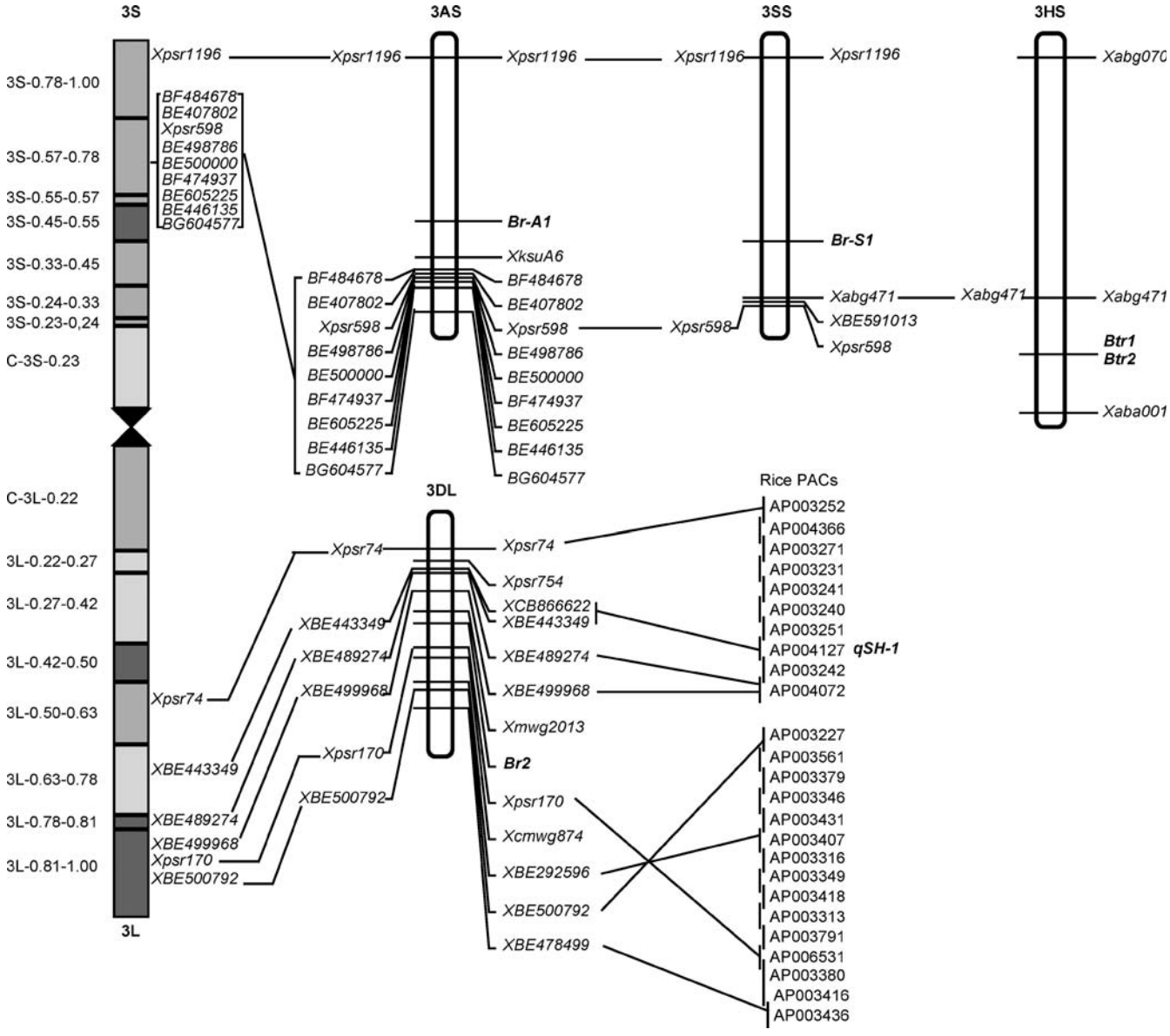

Fig. 4 The linkage mapping of shattering genes in reference to the consensus group-3 deletion map on the *left*. Each section of the consensus map represents a bin delimited by deletion breakpoints expressed as fraction of arm length from the centromere. The degree of shading is proportional to the frequency of recombination. To the *right* of the physical map are genetic maps of shattering genes in wheat and barley and the PAC physical map of rice. *Lines* connect the common or homologous markers in different maps. The *vertical bars* represent overlapped rice PACs with their GenBank accession numbers. Shattering genes are indicated in *bold type*. The map of 3AS of *T. timopheevii* shows the position of *Br-A1*. The map 3SS of *Ae. speltoides* shows the position of *Br-S1*. The 3DL map of *Ae. tauschii* shows the position of *Br2*. The 3HS map of barley showing the position of barley shattering gene *Btr1* and *Btr2* is reproduced from Komatsuda and Mano (2002) and Komatsuda et al. (2004). For 3AS, 3SS, and 3HS maps, the *top* of the maps is toward the telomere, and the *bottom* is toward the centromere. For 3DL map, the *top* of the maps is toward the centromere, and the *bottom* is toward the telomere

excess of nonshattering segregants. The F_3 progenies from the 118 F_2 plants were phenotyped to determine their genotypic constitution. All 42 nonshattering plants were homozygous. Among the 76 shattering F_2 plants, 20 were homozygous and the remaining 56 were heterozygous. These results imply that B-type spikelet disarticulation is governed by a single dominant gene in *Ae. tauschii*, and segregation distortion occurred in the vicinity of the brittle rachis gene, most probably resulting from the preferential transmission of gametes from the nonshattering parent.

We hypothesized that the shattering gene in *Ae. tauschii* was orthologous to *Br1* and selected RFLP probes previously mapped to the short arm of group-3 chromosomes and initiated linkage mapping. As mapping progressed, the shattering gene was found to be located on the long arm instead of the short arm of chromosome 3D, indicating that we detected a new shattering locus. Accordingly, we named the gene governing the B-type disarticulation in *Ae. tauschii* as *Br2*.

A total of 73 RFLP and EST-derived markers located on group-3 chromosomes were used to screen for polymorphism between the two parents. DNA was digested with six restriction enzymes, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I, and *Xba*I. Fifty-eight (79.5%) probes detected polymorphism with at least one enzyme, whereas the remaining 15 (20.5%) clones were monomorphic for all six enzymes. Of the 46 loci mapped, 33 were on chromosome 3D (Fig. 3). *Br2* was located in a 4.4-cM interval on the long arm of chromosome 3D, 1.5 cM distal to marker *Xmwg2013* and 2.9 cM proximal to marker *Xpsr170*. Markers flanking *Br2* showed segregation distortion as well, confirming the existence of a segregation-distorter locus in this region (Faris et al. 1998). Alignment with deletion maps of the wheat group-3 chromosomes indicated that *Br2* is located in the distal bin (0.81–1.00) of the 3D long arm (Fig. 4).

Alignment of the mapped cDNA- and EST-derived markers with rice genome sequences indicated conserved gene synteny between rice and wheat in the *Br2* region. Proximal to *Br2*, markers *Xpsr74*, *XCB866622*, *XBE443349*, *XBE489274*, and *XBE499968* showed perfect colinearity with sequences of ten overlapping PACs (1,168 kb) on rice chromosome 1 (Fig 4). Distal to *Br2*, markers *Xpsr170*, *XBE292596*, *XBE500792*, and *XBF478499* were also colinear with 15 overlapping PACs (1,596 kb) on rice chromosome 1, but the marker orientation was reversed (Fig. 4), indicating that an inversion occurred in this region between the wheat and rice genomes.

We mapped barley EST CB866622 because it showed high homology to the rice shattering gene *qSH-1*. We sequenced 1,116 bp of the EST clone, and it showed 99% identity to the last exon of *JuBel2*. In TIGR barley gene index database, CB866622 belongs to TC134032, and seven barley ESTs are anchored to the barley gene *JuBel2* (AF334759). *JuBel2* codes for homeodomain protein JUBEL2, is expressed in shoot apical meristem and immature inflorescence, and is mapped to barley chromosome 3H (Müller et al. 2001). *JuBel2* showed more than 80% nucleotide identity with *qSH-1* in all four exons and 50 bp of the first introns. JUBEL2 protein showed 55% of amino acid identity and 60% similarity to qSH-1 protein. The reduction of homology at protein sequence level was mainly due to 34 indels (Fig. 5 and Supplementary Fig 1). Of the 34 indels,

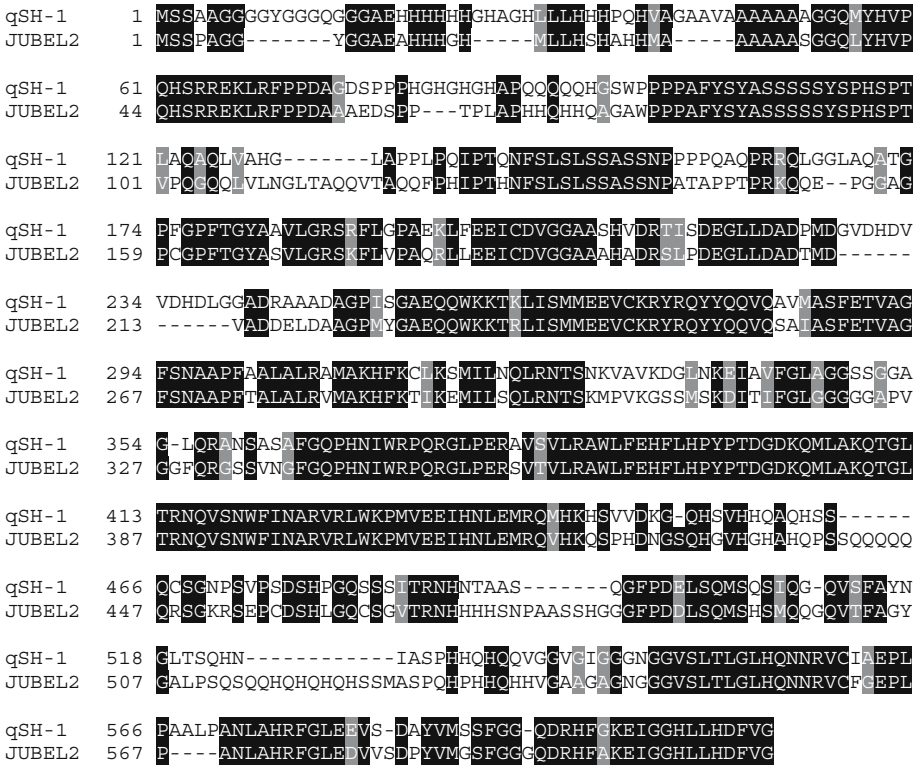

Fig 5 Alignment of amino acid sequences of barley JUBEL2 protein and rice shattering QTL qSH-1 protein. The positions of identical, similar, and deleted amino acids are indicated in *dark*, in *gray*, and by *dashes*, respectively. Although these genes are homologs, the barley shattering QTL maps elsewhere in the genome

19 deletions (totalling 114 bases) occurred in rice *qSH-1* and none caused shift of the open reading frame. Compared with rice *qSH-1*, barley *JuBel2* carried 15 deletions (totalling 113 bases), 11 of which caused a frameshift.

Discussion

Br1 orthology in the Triticeae

Many Triticeae species have been hybridized with wheat, and wheat–alien individual chromosome addition lines have been isolated. Because the *Br1* gene is dominant, its phenotype can be easily scored in these materials. Orthologous *Br1* genes in the investigated Triticeae species are located in group-3 chromosomes: 3S of *Ae. speltoides* (Friebe et al. 1999a,b), $3S^b$ of *Aegilops bicornis* (Riley et al. 1966, Urbano et al. 1988), $3S^l$ of *Aegilops longissima* Schweinf. & Muschl (Friebe et al. 1993, Urbano et al. 1988), $3S^{sh}$ of *Aegilops sharonensis* Eig (Urbano et al. 1988), $3S^s$ of *Aegilops searsii* (Friebe et al. 1995) and *Aegilops peregrina* (Hackel in J.Fraser) Maire & Weiller (Yang et al. 1996), $3M^g$ of *Aegilops geniculata* (Friebe et al. 1999a,b), 3N of *Aegilops uniaristata* Vis. (Miller et al. 1995), $3E^b$ of *Thinopyrum besarabicum* (Savul & Ravss) Löve (King et al. 1997), and 3V of *Dasypyrum villosum* Candargy (Urbano et al. 1988). Shattering in *Ae. uniaristata* is caused by spike-type disarticulation. During the transfer of an aluminum tolerance gene from chromosome 3N of *Ae. uniaristata* to chromosome 3A by homoeologous recombination, Iqbal et al. (2000) characterized six 3A–3N recombinant lines with RFLP markers and suggested that the brittle rachis gene for spike disarticulation was located very close to *Xpsr598* or between the loci *Xpsr598* and *Xpsr1196*. In this research, we localized *Br1* between markers *Xpsr1196* and *Xpsr598* on the short arm of chromosome 3A in *T. timopheevii* subsp. *armeniacum* and 3S of *Ae. speltoides*. These data strongly suggest that genes for spike-type and W-type are located in the same genomic region, supporting our results that they are allelic at *Br1* in *Ae. speltoides*.

Br1 alleles and the evolution of polyploid wheat

The tetraploid wheat sibling species *T. turgidum* (AABB) and *T. timopheevii* (AAGG) trace their origin to A-genome diploid *Triticum urartu* and S-genome diploid *Ae. speltoides* (Fig. 1). However, it has been a matter of considerable debate as to whether they had a monophyletic or a polyphyletic origin. The structure at the *Br1* locus in each lineage supports the diphyletic origin of tetraploid wheats (Mori et al. 2001; Jiang and Gill 1994). Our data show that the shattering gene in *T. timopheevii* is located only on 3AS and is orthologous to *Br1*. However, two genes, *Br-A1* on 3AS and *Br-B1* on 3BS, are responsible for the shattering trait in *T. turgidum* subsp. *dicoccoides* (Joppa and Cantrell 1990, Feldman 2001,Watanabe et al. 2002), suggesting that the B- and G-genome donors of polyploid wheat were different at the *Br1* locus. The B-genome donor of *T. turgidum* was probably related to *Ae. speltoides* var. *ligustica*, which has W-type disarticulation, and this gene is codominant to *Br-A1*. Thus, *T. turgidum* has codominant genes *Br-A1* on 3AS and *Br-B1* on 3BS that control shattering. However, the G genome of *T. timopheevii* most probably was derived from *Ae. speltoides* var. *speltoides*, which has spike-type disarticulation. Our data show that spike-type disarticulation is recessive to W-type, and hence, *T. timopheevii* expresses only *Br-A1* on 3AS. Possibly, *T. timopheevii* subsp. *armeniacum* still carries an allele for spike-type disarticulation at *Br-G1* locus, but it remained undetected because only W-type disarticulation was scored.

Different genetic pathways for B- and W-type disarticulations

From the mapping results, we conclude that the B- and W-type disarticulations are governed by different genes, *Br2* and *Br1*. These two types of spikelet disarticulation may involve different genetic pathways. This deduction receives additional support from previous investigations. W- and B-type disarticulations are codominant in an SWW accession (Tsunewaki et al. 1990) and in amphiploids between emmer and *Ae. tauschii* (McFadden and Sears 1946) or wild emmer and *Ae. tauschii* (Matsumoto et al. 1963). The observed codominance is suggestive of their unique genetic nature (or independence of expression).

In *T. aestivum* subsp. *spelta*, brittle rachis co-segregates with nonsquare spike morphology and tough glume and is inherited as part of the spelt syndrome (Leighty and Boshnakian 1921, Singh et al. 1957, MacKey 1966). These morphological characteristics are mainly controlled by a dominant allele at the *Q* locus on the long arm of chromosome 5A (Faris et al. 2003). The late E.R. Sears (unpublished data) substituted chromosome 5A from European spelt into *T. aestivum* subsp. *aestivum* cv. CS. The DS line of 5A from European spelt had speltoid spike morphology and a rachis as tough as that in CS (Muramatsu 1963), suggesting that the expression of B-type disarticulation in European spelt resulted from the interaction between the *q* allele and another gene, most probably *Br-D2*.

When chromosomes 3A and 3B of *T. turgidum* subsp. *dicoccoides* were individually introduced in *T. turgidum* subsp. *durum* cv. Langdon, which carries the *Q* allele, the spike has a brittle rachis controlled by *Br1* (Joppa and Cantrell 1990). SWW, which carries the *Br-D1* and *Q* alleles, has nonspeltoid spike morphology but shatters at maturity into W-type spikelets. Therefore, it is likely that the expression of W-type disarticulation is under the control of *Br1* and is independent of the *Q* locus.

Compared with W-type disarticulation, B-type disarticulation is confined to the D genome of *Ae. tauschii* and the D-genome-carrying polyploid species of *Triticum* and *Aegilops*. In *Aegilops*, all allopolyploid species having a

B-type disarticulation originated from hybridizations between *Ae. tauschii* and a parent of spike-type disarticulation including *Aegilops cylindrica* Host., *Aegilops crassa* Boiss., *Aegilops ventricosa* Tausch, *Aegilops juvenalis* (Thell.) Eig, and *Aegilops vavilovii* (Zhuk.) Chennav. In *Triticum*, the only form having B-type disarticulation is European spelt, which contains genomes AABBDD. This suggests that B-type disarticulation in the polyploid species is conferred by *Br-D2*.

Convergent domestication?

Comparative low-resolution genetic mapping of shattering quantitative trait loci (QTL) in the orthologous genomic regions in sorghum, maize, and rice led to the notion of convergent domestication of cereal crops by independent mutations at orthologous loci (Paterson et al. 1995). However, our studies reported here and the recent mapping data including comparative mapping of candidate genes for shattering loci do not support this hypothesis. *Br1* is located on the short arm of wheat group-3 chromosomes, which are syntenic to the short arm of rice chromosome 1 (Sorrells et al. 2003). Neither a corresponding shattering locus has been detected in the short arm of rice chromosome 1 nor in the homoeologous genomic regions of maize and sorghum (Paterson et al. 1995).

Within the Triticeae, barley has two complementary and closely linked shattering genes, *Btr1* and *Btr2*, both located on the short arm of chromosome 3H (Takahashi and Hayashi 1964; Franckowiak and Konishi 1997a,b). Molecular mapping located *Btr1* and *Btr2* in a region proximal to *Xabg471* on the short arm of chromosome 3H (Komatsuda and Mano 2002; Komatsuda et al. 2004; Fig. 3). *Br-S1* is distal to *Xabg471*, suggesting that *Br1* of wheat may not be orthologous to either *Btr1* or *Btr2* of barley, although more common markers between the two loci are needed for detailed comparative mapping.

Comparative mapping of shattering genes also did not support the hypothesis of convergent evolution. Recently, the shattering gene *qSH-1*, coding for a homeodomain protein, was cloned from the long arm of rice chromosome 1 (Konishi et al. 2005). Barley EST CB866622, part of barley gene *JuBel2*, which is homologous to *qSH-1*, was mapped 6.9 cM proximal to *Br-D2* in wheat. Furthermore, *XCB866622* and *Br2* are located in separate deletion bins on the physical map, suggesting their physical separation by at least 3% of the chromosome arm in wheat (Fig. 4). In barley, three head shattering QTL caused by weak rachis were located to short arm of chromosome 2H, centromeric region of 3H, and long arm of 5H (Kandemir et al. 2000). However, no shattering effect was detected in the distal region of 3H, where *JuBel2* resides. This suggests nonconvergent evolution of this homeodomain protein gene among these grass lineages.

Two shattering QTL were detected in homoeologous regions of maize chromosomes 3 and 8 and closely associated with markers UMC71 and UMC117 (Paterson et al. 1995). We sequenced these two maize clones, and they contain highly similar DNA sequences. A rice homolog was found 20.1 cM (4,608 kb) distal to *qSH-1* and 6.2 cM (1,618 kb) distal to *Xpsr170* on rice chromosome 1, suggesting the maize shattering QTL are not orthologous to *qSH-1* or *Br2*. These data suggest that *Br1* and *Br2* are not orthologous to the shattering loci detected in other grasses and are possibly unique to wheat.

Comparing our data with previously published data on sorghum, maize, rice, and barley as discussed above suggests that multiple genetic pathways are involved in controlling the disarticulation of mature inflorescences. Each pathway may consist of numerous components (potential shattering genes), and mutation of a single component may cause the nonshattering phenotype. Cereals were domesticated independently by different cultures, in different geographic regions, and at different times. Before their domestication, cereals coevolved for about 65 million years (Kellogg 2001), and the genomic environments changed dramatically during this time, increasing the opportunity for different shattering genes to evolve. These shattering genes were targeted in different domestication events challenging the concept of convergent domestication at the genetic level. As an example, wheat A-, B-, and D-genome diploids coevolved from a common ancestor approximately three million years ago (Huang et al. 2002), and this short time interval has already spawned a different disarticulation gene (*Br2*) in the D genome of *Ae. tauschii*.

Acknowledgements We thank Drs. Moshe Feldman and Jan Dvorak for supplying plant material; Drs. H.W. Bass, M.D. Gale, A. Graner, A. Kleinhofs, M.E. Sorrells, and N. Stein for providing RFLP probes; and John Raupp for excellent technical assistance. This paper is contribution number 05-302-J from the Kansas Agricultural Experiment Station.

References

Brown-Guedira GL, Badaevea ED, Gill BS, Cox TS (1996) Chromosome substitution of *Triticum timopheevii* in common wheat and some observation on the evolution of polyploid wheat species. Theor Appl Genet 93:1291–1298

Chen Q-F, Yen C, Yang JL (1998) Chromosome location of the gene for brittle rachis in the Tibetan weedrace of common wheat. Genet Resour Crop Evol 45:407–410

Dvorak J, Luo M-C, Yang Z-L, Zhang H-B (1998) The structure of the *Aegilops tauschii* gene pool and the evolution of hexaploid wheat. Theor Appl Genet 97:657–670

Faris JD, Laddomada B, Gill BS (1998) Molecular mapping of segregation distortion loci in *Aegilops tauschii*. Genetics 149:319–327

Faris JD, Haen KM, Gill BS (2000) Saturation mapping of a gene-rich recombination hot spot region in wheat. Genetics 154:823–835

Faris JD, Fellers JP, Brooks SA, Gill BS (2003) A bacterial artificial chromosome contig spanning the major domestication locus *Q* in wheat and identification of a candidate gene. Genetics 164:311–321

Feldman M (2001) Origin of cultivated wheat. In: Bojean AP, Angus WJ (eds) The world wheat book—a history of wheat breeding. Lavoisier, London, UK, pp 3–58

Ferrandiz C, Liljegren SJ, Yanofsky MF (2000) Negative regulation of the *SHATTERPROOF* genes by FRUITFULL during *Arabidopsis* fruit development. Science 289:436–438

Franckowiak JD, Konishi T (1997a) Stock number: BGS115. Barley Newsl 26:149

Franckowiak JD, Konishi T (1997b) Stock number: BGS116. Barley Newsl 26:150

Friebe B, Tuleen NA, Jiang J, Gill BS (1993) Standard karyotype of *Triticum longissimum* and its cytogenetic relationship with *T. aestivum*. Genome 36:731–742

Friebe B, Tuleen NA, Gill BS (1995) Standard karyotype of *Triticum searsii* and its relationship with other S-genome species and common wheat. Theor Appl Genet 91:248–254

Friebe B, Qi LL, Nasuda S, Zhang P, Tuleen NA, Gill BS (1999a) Development of a complete set of *Triticum aestivum–Aegilops speltoides* chromosome addition lines. Theor Appl Genet 101:51–58

Friebe B, Tuleen NA, Gill BS (1999b) Development and identification of a complete set of *Triticum aestivum–Aegilops geniculata* chromosome addition lines. Genome 42:374–380

Huang S, Sirikhachornkit A, Su X, Faris J, Gill BS et al (2002) Genes encoding plastid acetyl-CoA carboxylase and 3-phosphoglycerate kinase of the *Triticum*/*Aegilops* complex and the evolutionary history of polyploid wheat. Proc Natl Acad Sci U S A 99:8133–8138

Iqbal N, Reader SM, Caligari PD, Miller TE (2000) The production and characterization of recombination between chromosome 3N of *Aegilops uniaristata* and chromosome 3A of wheat. Heredity 84:487–492

Jiang J, Gill BS (1994) Different species-specific chromosome translocations in *Triticum timopheevii* and *T. turgidum* support the diphyletic origin of polyploid wheats. Chromosome Res 2:59–64

Joppa LR, Cantrell GR (1990) Chromosomal location of genes for grain protein content of wild tetraploid wheat. Crop Sci 30:1059–1064

Kandemir N, Kudrna DA, Ullrich SE, Kleinhofs A (2000) Molecular marker assisted genetic analysis of head shattering in six-rowed barley. Theor Appl Genet 101:203–210

Kellogg EA (2001) Evolutionary history of the grasses. Plant Physiol 125:1198–1205

Kihara H, Lilienfeld FA (1932) Genomanalyse bei *Triticum* and *Aegilops*. IV. Untersuchungen an *Aegilops* × *Triticum* und *Aegilops* × *Aegilops* Bastarden. Cytologia 3:384–456

Kimber G, Feldman M (1987) Wild wheat: an introduction. University of Missouri-Columbia, 142 pp

King IP, Law CN, Cant KA, Orford SE, Reader SM et al (1997) Tritipyrum, a potential new salt-tolerant cereal. Plant Breed 116:127–132

Komatsuda T, Mano Y (2002) Molecular mapping of the intermedium spike-c (*int-c*) and nonbrittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.). Theor Appl Genet 105:85–90

Komatsuda T, Maxim P, Senthil N, Mano Y (2004) High-density AFLP map of nonbrittle rachis 1 (*btr1*) and 2 (*btr2*) genes in barley (*Hordeum vulgare* L.). Theor Appl Genet 109:986–995

Konishi S, Lin SY, Fukuta Y, Izawa T, Sasaki T et al (2005) Molecular cloning of a major QTL, *qSH-1*, controlling seed shattering habit in rice. In: Plant and animal genome XIII abstract, W306

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

Lander E, Green P, Barlow A, Daley P, Stein L et al (1987) MAPMAKER: an interactive computer package for constructing primary linkage maps of experimental and natural populations. Genomics 1:174–181

Leighty CE, Boshnankian S (1921) Genetic behavior of the spelt form in crosses between *Triticum spelta* and *Triticum aestivum*. J Agric Res 7:335–364

Liljegren SJ, Ditta GS, Eshed Y, Saidge B, Bowman JL et al (2000) *SHATTERPROOF* MADS-box genes control seed dispersal in *Arabidopsis*. Nature 404:766–770

MacKey J (1966) Species relationship in *Triticum*. Proc 2nd Int Wheat Genet Symp (Hereditas Suppl) 2:237–276

Mao L, Begum D, Chuang H-W, Budiman MA, Szymkowiakish EJ et al (2000) *JOINTLESS* is a MADS-box gene controlling tomato flower abscission zone development. Nature 406:910–913

Matsumoto K, Teramura T, Tabushi J (1963) Development analysis of the rachis disarticulation in *Triticum*. Wheat Inf Serv 15–16:23–25

McFadden ES, Sears ER (1946) The origin of *Triticum spelta* and its free-threshing hexaploid relatives. J Hered 37:81–89, 107–116

McIntosh RA, Hart GE, Devos KM, Gale MD, Rogers WJ (1998) Catalogue of gene symbols for wheat. In: Slinkard AE (ed) Proc 9th Int Wheat Genet Symp, vol 5. University Extension Press, Saskatoon, Canada, pp 1–235

Miczynski K (1926) Etudes genetiques sur le genre *Aegilops*. I. Experiences avec l' *Aegilops speltoides* Jaub. Et Spach. Acta Soc Bot Pol 4:20–40

Miller TE, Reader SM, Mahmood A, Purdie MA, King IP (1995) Chromosome 3N of *Aegilops uniaristata*—a source of tolerance to high levels of aluminum for wheat. In: Li ZS, Xin ZY (eds), Proc 8th Int Wheat Genet Symp 1993. China Agricultural Scientech Press, Beijing, China, pp 1037–1042

Mori N, Liu Y-G, Tsunewaki K (2001) Wheat phylogeny determined by RFLP analysis of nuclear DNA. 2. Wild tetraploid wheats. Theor Appl Genet 90:129–134

Müller J, Wang Y, Franzen R, Santi L, Salamini F, Rohde W (2001) In vitro Interactions between barley TALE homeodomain proteins suggest a role for protein–protein associations in regulation of *Knox* gene function. Plant J 27:13–23

Muramatsu M (1963) Dosage effect of the spelta gene *q* of the hexaploid wheat. Genetics 48:469–482

Paterson AH, Lin Y-R, Li Z, Schertz KF, Doebley JF et al (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 269:1714–1718

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

Riley RG, Kimber G, Law CN (1966) Correspondence between wheat and alien chromosomes. Annu Rep Plant Breed Inst 1964–1965:108–109

Roeder AH, Ferrandiz C, Yanofsky MF (2003) The role of the REPLUMLESS homeodomain protein in patterning the *Arabidopsis* fruit. Curr Biol 13:1630–1635

Savidge B, Rounsley SD, Yanofsky MF (1995) Temporal relationship between the transcription of two *Arabidopsis* MADS box genes and the floral organ identity genes. Plant Cell 7:721–733

Schiemann E (1928) Zytologische und pflanzen-geographische Beitrage zur gattung *Aegilops* (II. Mitteilung). Ber Dtsch Bot Ges 46:107–123

Shao Q, Li C, Basang C (1983) Semiwild wheat from Xizang (Tibet). In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp. Plant Germ Plasm Institute, Kyoto, Japan, pp 111–114

Singh HB, Anderson E, Pal PB (1957) Studies in the genetics of *Triticum vavilovii* Jackub. Agron J 49:4–11

Sorrells ME, La Rota M, Bermudez-Kandianis CE, Greene RA, Kantety R et al (2003) Comparative DNA sequence analysis of wheat and rice genomes. Genome Res 13:1818–1827

Takahashi R, Hayashi J (1964) Linkage study of two complementary genes for brittle rachis in barley. Ber Ohara Inst Landwirtsch Biol Okayama Univ 12:99–105

Tsunewaki K (1968) Origin and phylogenetic differentiation of bread wheat revealed by comparative gene analysis. In: Finlay KW, Shepherd KW (eds) Proc 3rd Int Wheat Genet Symp. Australian Academy of Science, Canberra, Australia, pp 71–85

Tsunewaki K, Yamada S, Mori N (1990) Genetical studies on a Tibetan semi-wild wheat, *Triticum aestivum* ssp. *tibetanum*. Jpn J Genet 65:353–365

Urbano M, Resta P, Benedettelli SB, Blanco A (1988) A *Dasypyrum villosum* (L.) Candargy chromosome related to homoeologous group 3 of wheat. In: Miller TE, Koebner RMD (eds) Proc 7th Int Wheat Genet Symp, vol 1. Institute of Plant Science Research, Cambridge, UK, pp 169–173

Watanabe N, Sugiyama K, Yamagishi Y, Sakata Y (2002) Comparative telosomic mapping of homoeologous genes for brittle rachis in tetraploid and hexaploid wheats. Hereditas 137:180–185

Yang YC, Tuleen NA, Hart GE (1996) Isolation and identification of *Triticum aestivum* L. em. Thell. cv. Chinese Spring–*T. peregrinum* Hackel disomic addition lines. Theor Appl Genet 92:591–598