The Genes on Homoeologous Group 3 Chromosomes Determine Brittle Rachides in *Triticum* and *Aegilops*

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Abstract: Using microsatellite markers, the homoeologous genes for brittle rachis were mapped in hexaploid wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L.) and *Aegilops tauschii* Coss. On chromosome 3AS, the gene for brittle rachis, Br_2 was linked with the centromeric marker, *Xgwm32* in the distance of 13.3 cM. Br_3 was located on chromosome 3BS and linked with the centromeric marker, *Xgwm72* (14.2 cM). Br_1 was located on chromosome 3DS. The distance from the centromeric marker *Xgdm72* was 23.6 cM. The loci Br_1, Br_2 and Br_3 determine disarticulation of rachides above the junction of the rachilla with the rachis such that a fragment of rachis is attached below each spikelet. The rachides of *Ae. tauschii* are brittle at every joint, so that the mature spike disarticulates into barrel type. The brittle rachis was determined by a dominant gene, Br^t, which was linked to the centromeric marker, *Xgdm72* (19.7 cM) on chromosome 3DS.

Key words: brittle rachis; homoeologous genes; mapping; *Triticum*; *Aegilops*

The brittle rachis character, which causes spontaneous spike shattering, is of adaptive value in wild grass species. In *Triticeae*, there have been several reports in which brittle rachis is claimed to be controlled by the genes on homoeologous group 3 chromosomes using chromosome additions and chromosome substitutions to *Triticum aestivum* (Riley *et al*. 1966; Urbano *et al*. 1988; Miller *et al*. 1995; King *et al*. 1997; Friebe *et al*. 1999a, b; Yang *et al*. 1996). Since the development of synthetic hexaploid wheat by McFadden and Sears (1946), brittleness of rachis has been regarded as a pleiotropic effect of the spelt gene (*q*) located on chromosome 5A (Cao *et al*. 1997). Tibetan landraces of common wheat (Shao 1980, 1983) have brittle rachides controlled by Br_1 on the short arm of chromosome 3D (Chen *et al*. 1998). The brittle rachis of wild emmer *Triticum dicoccoides* Koern. is controlled by two dominant genes, Br_2 and Br_3, which are located on chromosomes 3A and 3B (Watanabe & Ikebata 2000). Br_1, Br_2 and Br_3 genes determine wedge type disarticulation of *Triticum* species. Watanabe *et al*. (2002) located Br_1, Br_2 and Br_3 genes on the short arms of homoeologous group 3 chromosomes using telosomic lines. Metger and Silbaugh (1968/1969) found that the rachis of *Aegilops tauschii* KU2086, which was collected near Firuzkuh, Afghanistan, in 1956, was non-brittle. In the present study, we used microsatellite markers to map the genes for brittle rachides in *Triticum* and *Aegilops*.

MATERIALS AND METHODS

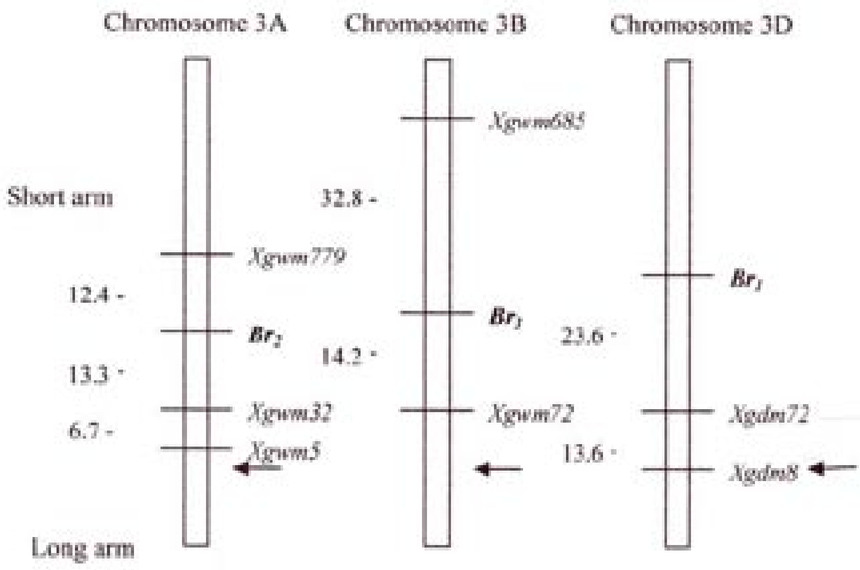
Plant materials. To map Br_1, *T. aestivum* cv. Novosibirskaya 67 (N67) was crossed with *T. aestivum* cv. KU510, whose rachis is brittle. F_1 plants were bagged just before flowering to obtain F_2 seeds. To map Br_2, we used Langdon (LDN), a LDN chromosome substitution line, LDN(DIC 3A) and 82 recombinant inbred chromosomal lines (RICL's) for

DIC 3A developed by Dr. L.R. Joppa. In the LDN durum chromosome substitution lines, a pair of LDN chromosomes was replaced with a pair of chromosomes from wild emmer wheat, *T. dicoccoides* (DIC). To map *Br_3*, Langdon (LDN) was crossed with LDN(DIC 3B). F_1 plants were bagged just before flowering to obtain F_2 seeds. To map *Br^t* (Brittle rachis of *Aegilops tauschii*), G3489, the tough rachis variant of *Ae. tauschii*, was crossed with *Ae. tauschii* KU2126. F_1 plants were grown in the greenhouse and were bagged just before flowering to obtain F_2 seeds. The trait of brittle rachis of tetraploid and hexaploid wheats was defined as a spike having a rachis that disarticulated when the tip of a spike was bent by up to 45° relative to the peduncle. The trait of brittle rachis of *Ae. tauschii* was defined as a spike having a rachis that naturally disarticulated after ripening.

Microsatellite mapping of genes for brittle rachides. Nuclear DNA was isolated from leaves of single plants using the Qiagen Dneasy mini kit procedure. Wheat microsatellite markers located on the short arms of homoeologous group 3 chromosomes (Röder *et al*. 1998; Song *et al*. 2005) were chosen to map Br_1, Br_2, Br_3 and Br^t. Further microsatellite markers were provided by Dr. M. S. Röder under the aegis of a material transfer agreement between Gifu University and IPK-Gatersleben, Germany. Polymerase chain reactions (PCR) were performed with minor modification as described by Plaschke *et a*l. (1995). After electrophoresis of PCR products in 10% acrylamide gel, amplified fragments were detected by silver staining. Multipoint linkage values in centiMorgans (cM) were calculated using Map Manager QTX (http://mapmgr.roswellpark.org/).

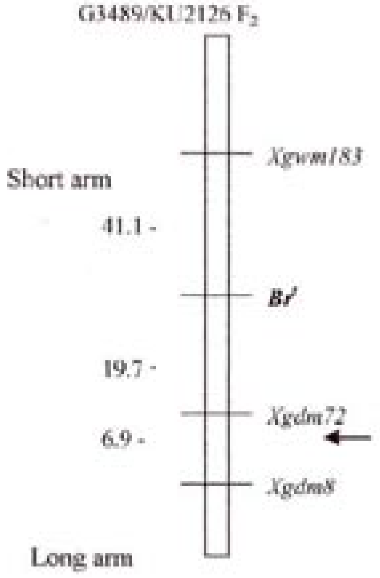
RESULTS AND DISCUSSION

Of 85 F_2 plants from N67/KU510, 20 had tough rachis. The segregation ratio of brittle rachis confirmed the expected 3:1 (χ^2 = 1.979). Two polymorphic markers which detect a single locus were used to map *Br_1* on chromosome 3DS. The segregation of these microsatellite markers confirmed the expected 1:2:1 ratios (df = 2), χ^2 values being 2.365 for *Xgdm72* and 2.859 for *Xgdm8*. The genetic map location of the *Br_1* locus is shown in Figure 1: *Br_1* was distally located on chromosome 3DS, and was linked with the centromeric marker, *Xgdm72* (23.6 cM). Of 82 RICL's for DIC 3A, 44 lines had tough rachis. To map *Br_2* on chromosome 3AS, three polymorphic markers, which detect a single locus, were used. The segregations of rachis brittleness and three microsatellite markers confirmed the expected 1:1 ratios (df = 1), χ^2 values ranging from 0.439 to 3.2. The established gene order was centromeric marker, *Xgwm5* – *Xgwm32* – *Br_2* – *Xgwm779* on chromosome 3AS (Figure 1). Of 150 F_2 plants from Langdon/LDN(DIC 3B), 40 had tough rachis. The segregation ratio of brittle rachis confirmed the expected 3:1 (χ^2 = 0.222). Two polymorphic markers which detect a single locus were used to map *Br_3* on chromosome 3BS. The segregation of these microsatellite markers confirmed the expected 1:2:1 ratios (df = 2), χ^2 values being 0.231 for *Xgwm72* and 1.627 for *Xgwm685*. As shown in Figure 1, *Br_3* was distally located on chromosome



Distances are shown in cM. Arrow indicates the putative position of the centromere of each chromosome

Figure 1. Linkage maps for the genes for brittle rachis on the short arm of homoeologous group 3 chromosomes



Distances are shown in cM. Arrow indicates the putative position of the centromere of each chromosome

Figure 2. Linkage maps for the genes for brittle rachis on the short arm of chromosome 3D of *Aegilops tauschii*

3BS, and was linked with the centromeric marker, *Xgwm72* (14.2 cM).

For *Aegilops tauschii*, of 95 F_2 plants from G3489/KU2126, 22 had tough rachis. The segregation ratio of brittle rachis was consistent with the expected 3:1 (χ^2 = 0.172). Three polymorphic markers which detect a single locus were used to map Br^t on chromosome 3DS. The segregation of these microsatellite markers confirmed the expected 1:2:1 ratios (df = 2), χ^2 values ranging from 3.379 to 5.147. The genetic map location of the Br^t locus is shown in Figure 2: Br^t was located on chromosome 3DS, and was linked with the centromeric marker, *Xgdm72* (19.7 cM). Br_1 and Br^t on chromosome 3DS with similar distances to centromeres determined the different ypes of brittle rachides. Scant attention has been given to the function of the gene Br^t of *Ae. tauschii* since the development of synthetic hexaploid wheat (McFadden & Sears 1946). It is required further experiment to assess the discrepancy of disarticulation.

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