Comparative Mapping of Genes for Brittle Rachis in *Triticum* and *Aegilops*

NOBUYOSHI WATANABE[1], NAOTO TAKESADA[1], YUKO FUJII[1] and PETR MARTINEK[2]

[1]Faculty of Applied Biological Sciences, Gifu University, Gifu, Japan; [2]Agrotest, Agricultural Testing, Advisory Services and Research, Ltd., Kroměříž, Czech Republic

Abstract: The brittle rachis phenotype is of adaptive value in wild grass species because it causes spontaneous spike shattering. The genes on the homoeologous group 3 chromosomes determine the brittle rachis in *Triticeae*. A few genotypes with brittle rachis have also been found in the cultivated *Triticum*. Using microsatellite markers, the homoeologous genes for brittle rachis were mapped in hexaploid wheat (*Triticum aestivum* L.), durum wheat (*Triticum turgidum* L. conv. *durum* /Desf./) and *Aegilops tauschii* Coss. On chromosome 3AS, the gene for brittle rachis, Br_2, was linked with the centromeric marker, *Xgwm32*, at 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 so 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. A D-genome introgression line, R-61, was derived from the cross Bet Hashita/*Ae. tauschii*, whose rachis disarticulated as a wedge type. The gene for brittle rachis of R-61, tentatively designated as Br^{61}, was distally located on chromosome 3DS, and was linked with the centromeric marker, *Xgdm72* (27.5 cM). We discussed how the brittle rachis of R-61 originated genetically.

Keywords: 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*, several reports were published in which the brittle rachis was 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; YANG *et al*. 1996; KING *et al*. 1997; FRIEBE *et al*. 1999a, b). A few accessions with brittle rachis have been found in cultivated *Triticum*. 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). There exist two types of disarticulation of rachis in hexaploid species of *Triticum*. The rachis of European spelt (*T. spelta* L.) disarticulates as a barrel type, but Iranian spelt (*T. spelta* ssp. *kuckuckianum* Gokg.), *T. macha* Dekapr. & Menabde and *T. vavilovii* (Thum) Jakubz disarticulate as wedge types. 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*.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 2532885901 and the grant for the Initiative Research 2002 from Gifu University to N. Watanabe.

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 the wedge type disarticulation of *Triticum* species. Watanabe *et al*. (2003) 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. *Ae. tauschii* is an excellent source of useful genes/traits for developing new wheat cultivars. Cox *et al*. (1990) made crosses between wheat and *Aegilops tauschii* to introduce its genes into the wheat genome. Fritz *et al.* (1995a, b) assessed the effects of an introgressed segment of *Ae. tauschii* in winter wheat × *Ae. tauschii* populations. There may be a possibility that the gene for the brittle rachis of *Ae. tauschii* was introduced into contemporary wheat cultivars. We found that the rachis of an introgression line, R-61 (Bet Hashita/*Ae. tauschii*), was brittle whereas the rachides of synthetic hexaploid wheat accessions, *T. durum*/*Ae. tauschii*, are usually tough. In the present study, we used microsatellite markers to map the genes for brittle rachides in *Triticum* and *Aegilops*.

MATERIAL 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). Thus in the line LDN(DIC 3A), chromosome 3A from Langdon was replaced by its equivalent in emmer wheat (Joppa & Williams 1988). To develop RICL's for DIC 3A, LDN(DIC 3A) was crossed with LDN and several F_1 plants were grown. Pollen from F_1 plants was used to pollinate emasculated heads of a LDN-D genome chromosome substitution line, LDN 3D(3A). The crossed seeds were grown in individual pots in a greenhouse and were selfed and sampled to determine chromosome pairing (13″ + 2′) at metaphase one (MI) of meiosis. One of the univalents was chromosome 3D and the other was a recombined chromosome consisting of portions of the DIC 3A chromosome and portions of the LDN 3A chromosome. Each recombined 3A chromosome pair should differ from all other recombined 3A chromosomes unless crossovers were identical. Several selfed seeds from each F_1 plant were grown in individual pots in a greenhouse. Each F_2 plant that had 14″ at MI of meiosis was crossed with double ditelosomic 3A line of LDN ($2n = 26 + 2tL + 2tS$) and the testcrosses were grown in the greenhouse to differentiate F_2 plants with a pair of 3D chromosomes from those with a homozygous pair of recombined 3A chromosomes (Joppa, personal communication). 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 gene for the brittle rachis of a *T. aestivum-Ae. tauschii* introgression line (R-61) was tentatively designated as Br^{61}. *T. aestivum* cv. Bet Hashita was crossed with R-61 to map Br^{61}. F_1 plants were crossed with Bet Hashita to obtain B_1F_1 seeds.

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) 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. *Xbarc* microsatellite markers in wheat were available from Song *et al.* (2005). Polymerase chain reactions (PCR) were performed with minor modification as described by Plaschke *et al*. (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/).

Assessment of brittle rachis. The trait of brittle rachis of tetraploid and hexaploid wheats was defined as a spike having a rachis that disarticulated when the tip of the spike was bent by up to 45° relative to the peduncle. The trait of the brittle rachis of *Ae. tauschii* was defined as a spike having

a rachis that naturally disarticulated after ripening. Two observers classified rachis fragility in the F_2 populations independently of each other.

RESULTS AND DISCUSSION

Out of 85 F_2 plants from N67/KU510, 20 had tough rachis. The segregation ratio of brittle rachis confirmed the expected 3:1 ratio ($\chi^2 = 1.979$). 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). Out 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 the centromeric marker *Xgwm5 – Xgwm32 –* Br_2 *– Xgwm779* on chromosome 3AS (Figure 1). Out 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 ratio ($\chi^2 = 0.222$). 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 Fig. 1, Br_3 was distally located on chromosome 3BS, and was linked with the centromeric marker *Xgwm72* (14.2 cM).

For *Aegilops tauschii*, out 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 ratio ($\chi^2 = 0.172$). 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).

It is of interest that R-61 arose from the hybridisation between hexaploid wheat cultivar Bet Hashita and *Ae. tauschii* in order to introduce an eyespot resistance gene from *Ae. tauschii* (Kushnir, personal communication). It was most likely that Br^{61} for brittle rachis is located on chromosome 3D. Out of 56 F_2 plants from Bet Hashita*2/R-61, 27 had tough rachis. The segregation ratio of brittle rachis confirmed the expected 1:1 ratio ($\chi^2 = 0.071$, df = 1). Four polymorphic markers which detect a single locus on chromosome 3DS were used for mapping Br^{61}. The results indicate that R-61 had a segment of chromosome 3D of *Ae. tauschii*. The segregation of these microsatellite markers confirmed the expected 1:1 ratios (df = 1). χ^2 values ranged from 0.531 to 2.701. The Br^{61} locus was distally located on chromosome 3DS, and was linked with the centromeric marker *Xgdm72* (27.5 cM).

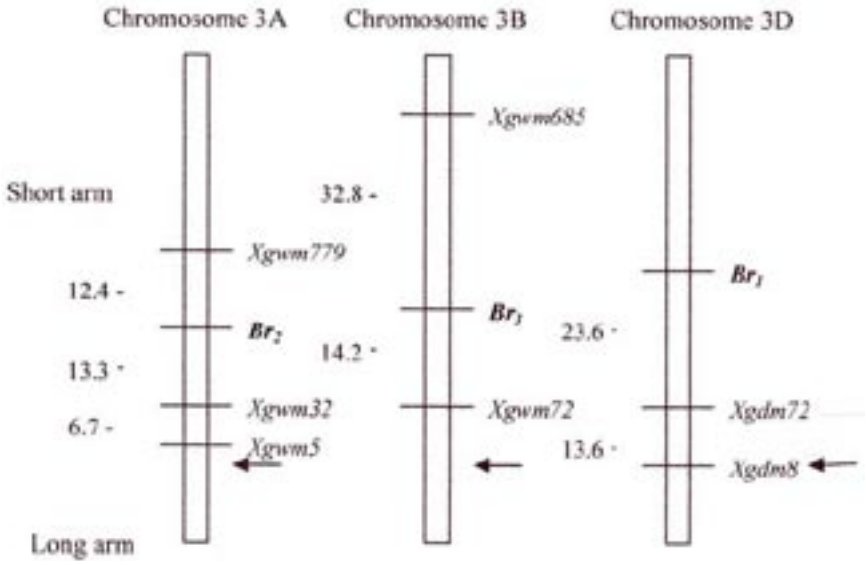

Figure 1. Linkage maps for the genes for brittle rachis on the short arm of homoeologous group 3 chromosomes

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

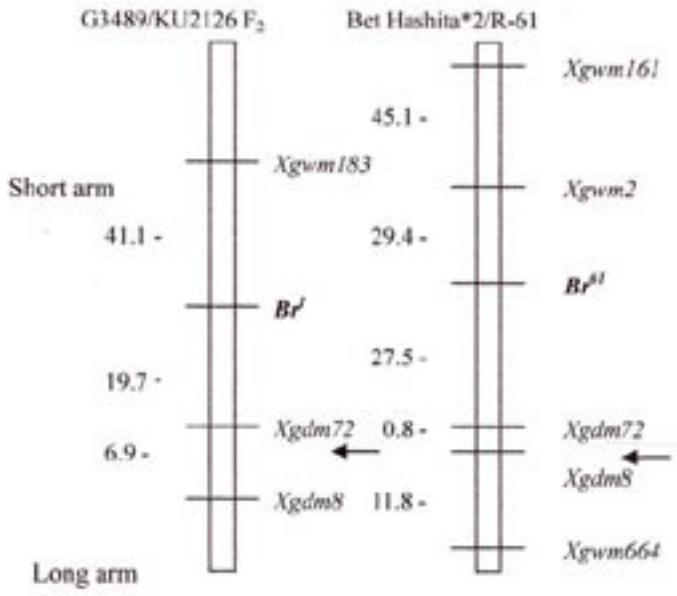

Figure 2. Linkage maps for the genes for brittle rachis on the short arm of chromosome 3D of *Aegilops tauschii* (left) and R-61 (right)

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

Summarising the results of the telosomic mapping of Br_1, Br_2 and Br_3 by Watanabe *et al*. (2003) and the results of the present study, Br_1, Br^t and Br^{61} were found to be located on chromosome 3DS with similar distances to centromeres. Scant attention has been paid to the function of the gene Br^t of *Ae. tauschii* since the development of synthetic hexaploid wheat (McFadden & Sears 1946). The rachis of Tetra Canthatch, which is a cytologically extracted form of hexaploid cultivar Canthatch (AABB, $2n = 4x = 28$), was tough, whereas those of *Ae. tauschii* accessions were brittle. Five synthetic hexaploid wheat accessions (Tetra Canthatch/*Ae. tauschii*), which were developed by Dr. E. R. Kerber, have tough rachides (Watanabe 1983). All synthetic hexaploid wheat accessions of 'Langdon' durum/*Ae. tauschii* had tough rachides (Xu, personal communication). The *Br/br* gene complex

Figure 3. Types of disarticulation of rachides in *Aegilops tauschii* (barrel type) and R-61 (wedge type)

The rachis of *Ae. tauschii* KU2126 (*left*) breaks at the node and creates a barrel-shaped spikelet, whereas the rachis of R-61 (*centre*) breaks at the node above the insertion point of the spikelet and creates a wedge-shaped spikelet unit attached to the rachis internode beneath. The rachis of Bet Hashita (*right*) was tough

on chromosomes 3A and 3B is epistatic to Br^t of *Ae. tauschii*. This finding is in accordance with the finding of Taketa and Takeda (1997), who showed that the dominant brittle rachis gene located on 3H chromosome of wild barley (*Hordeum spontaneum*) was not expressed in wheat-barley hybrids.

As shown in Figure 3, the rachis of R-61 breaks at the node above the insertion point of the spikelet and creates a wedge-shaped spikelet unit attached to the rachis internode beneath, whereas the rachis of *Ae. tauschi* breaks at the node and creates a barrel-shaped spikelet. It was evident that the recombination around the regions of Br_1 locus and Br^t locus created the wedge type disarticulation of R-61. This suggests that either intralocus recombination at the complex locus determining brittle rachis or recombination of closely linked locus/loci for brittle rachides was responsible for the brittle rachis of R-61.

Acknowledgement. We acknowledge Dr. M.S. Röder, IPK-Gatersleben, for providing us unpublished primer sequences of microsatellite markers, Dr. L.R. Joppa, Agricultural Research Service, U.S. Department of Agriculture, Fargo, North Dakota, USA, Dr. J.G. Waines, University of California, Riverside, California, USA, Dr. Uri Kushnir, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, and Dr. T. Kawahara, the Plant Germplasm Institute, Kyoto University, Mozume, Japan, for providing the seed samples for the experiments.

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Received for publication April 29, 2005
Accepted May 20, 2005

Souhrn

Watanabe N., Takesada N., Fujii Y., Martinek P. (2005): **Srovnávací mapování genů pro rozpadavost klasového vřetena u** *Triticum* **a** *Aegilops*. Czech J. Genet. Plant Breed., **41**: 39–44.

Rozpadavost vřetena klasu je adaptační vlastností planých druhů *Poaceae,* umožňující přirozený rozpad klasu na klásky a jejich šíření. Geny pro rozpadavost klasu se u *Triticeae* nacházejí ve třetí homeologické skupině chromozomů. Několik forem s rozpadavým vřetenem klasu se vyskytuje i u kulturní pšenice. Geny pro rozpadavost klasu byly u hexaploidní pšenice (*Triticum aestivum* L.), pšenice tvrdé (*Triticum turgidum* L. conv. *durum* /Desf./) a *Aegilops tauschii* Coss. mapovány pomocí mikrosatelitních markerů. Gen pro rozpadavý klas Br_2 na chromozomu 3AS byl ve vazbě s centromerickým markerem *Xgwm32* ve vzdálenosti 13,3 cM. Gen Br_3 byl lokalizován na chromozomu 3BS, nacházel se ve vazbě s centromerickým markerem *Xgwm72* ve vzdálenosti 14,2 cM. Gen Br_1 byl lokalizován na chromozomu 3DS. Jeho vzdálenost od centromerického markeru *Xgdm72* byla 23,6 cM. Lokusy Br_1, Br_2 a Br_3 podmiňují rozdíly ve způsobu spojení vřetének klásků s vřetenem klasu, což se po rozpadu klasu projevuje rozdíly ve velikosti fragmentů článků klasového vřetena pod každým kláskem. U *Aegilops tauschii* jsou všechna napojení klásků na klasové vřeteno rozpadavá, takže zralý klas se rozpadá do válcových útvarů. Tento typ rozpadu je podmíněn dominantním genem Br^t na chromozomu 3DS. Br^t je ve vazbě s centromerickým markerem *Xgdm72* ve vzdálenosti 19,7 cM. Introgrese D genomu u linie R-61, vytvořené z křížení Bet Hashita/*Ae. tauschii*, se však vyznačovala klínovitým typem rozpadu. Gen (prozatímně označený jako Br^{61}) pro klínovitý typ rozpadu klasového vřetena u R-61 byl nalezen na distální části chromozomu 3DS ve vazbě s centromerickým markerem *Xgdm72* ve vzdálenosti 2,5 cM. Je diskutován původ genetického založení rozpadavosti klasu u linie R-61.

Klíčová slova: rozpadavost vřetena; homeologické geny; mapování; *Triticum*; *Aegilops*

Corresponding author:

Dr. Nobuyoshi Watanabe, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan
tel.: + 81 58 293 28 52, fax: + 81 58 293 28 52, e-mail: watnb@cc.gifu-u.ac.jp