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; Frieba 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. kuekjianum 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\textsubscript{1} on the short arm of chromosome 3D (Chen et al. 1999a, b). 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 & Ikehata* 2000). *Br*$_2$, *Br*$_3$ and *Br*$_3$ genes determine the wedge type disarticulation of *Triticum* species. *Watanabe et al.* (2003) located *Br*$_2$, *Br*$_3$ 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/traitss 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*$_2$, *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 (RICLs) 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 recombinated chromosome consisting of portions of the DIC 3A chromosome and portions of the LDN 3A chromosome. Each recombinated 3A chromosome pair should differ from all other recombinated 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 recombinated 3A chromosomes (Joppa, personal communication). To map *Br*$_2$, Langdon (LDN) was crossed with LDN(DIC 3B). F$_1$ plants were bagged just before flowering to obtain F$_2$ seeds. To map *Br*$_2$ (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*$_{r1}$. *T. aestivum* cv. Bet Hashita was crossed with R-61 to map *Br*$_{r2}$. F$_1$ plants were crossed with Bet Hashita to obtain B$_1$F$_2$ 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*$_2$, *Br*$_2$, *Br*$_3$ and *Br*$_3$. 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 F2 populations independently of each other.

**RESULTS AND DISCUSSION**

Out of 85 F2 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), $\chi^2$ 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), $\chi^2$ 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 F2 plants from Langdon/LDN(DIC 3B), 40 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 $Br_6^1$. 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). $\chi^2$ values ranged from 0.531 to 2.701. The $Br_6^1$ locus was distally located on chromosome 3DS, and was linked with the centromeric marker Xgdm72 (27.5 cM).

For Aegilops tauschii, out of 95 F2 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 $Br_6$ on chromosome 3DS. The segregation of these microsatellite markers confirmed the expected 1:2:1 ratios (df = 2), $\chi^2$ values ranging from 3.379 to 5.147. The genetic map location of the $Br_6$ locus is shown in Figure 1: $Br_6$ was distally 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 $Br_6^1$ for brittle rachis is located on chromosome 3D. Out of 56 F2 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 $Br_6^1$. 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). $\chi^2$ values ranged from 0.531 to 2.701. The $Br_6^1$ locus was distally located on chromosome 3DS, and was linked with the centromeric marker Xgdm72 (27.5 cM).
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 (*M. F. & S. 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 2. Linkage maps for the genes for brittle rachis on the short arm of chromosome 3D of *Aegilops tauschii* (left) and R-61 (right).](image2)

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

![Figure 3. Types of disarticulation of rachides in *Aegilops tauschii* (barrel type) and R-61 (wedge type).](image3)

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 Takata 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. tauschii* 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.

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References


Souhrn


Rozpadavost vřetena klasu je adaptací vlastnosti planých druhů Poaceae, umožňující přirozený rozpad klasu na klásky a jejich šíření. Geny pro rozpadavost klasu se v Triticace Nacházejí v 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 Br1 na chromozomu 3AS byl ve vazbě s centromerickým markerem Xgwm32 ve vzdálenosti 13,3 cM. Gen Br2 byl lokalizován na chromozomu 3BS, nacházel se ve vazbě s centromerickým markerem Xgwm72 ve vzdálenosti 14,2 cM. Gen Br3 byl lokalizován na chromozomu 3DS. Jeho vzdálenost od centromerického markeru Xgdm72 byla 23,6 cM. Lokusy Br1, Br2 a Br3 podmiňují rozdíly ve způsobu spojení vřetének klásků s vřetennem klasu, což se po rozpadu klasu projevuje rozdíly ve velikosti fragmentů kláskového 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 Br1 na chromozomu 3DS. Br1 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 vyhnovala klinovitým typem rozpadu. Gen (prozatím označený jako Br61) pro klinovitý 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

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