High-resolution linkage mapping for the non-brittle rachis locus
btr1 in cultivated × wild barley (*Hordeum vulgare*)

Perumal Azhaguvel^a, Dhanasekaran Vidya-Saraswathi^{a,b}, Takao
Komatsuda^{a,*}

^aNational Institute of Agrobiological Sciences,
2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, JAPAN

^bFaculty of Horticulture, Chiba University, 648 Matsudo, JAPAN

*Corresponding author. Tel.: +81-29-838-7482; Fax: +81-29-838-
7054.

E-mail address: takao@affrc.go.jp (T. Komatsuda).

(P. Azhaguvel and D. Vidya Saraswathi contributed equally to this
work)

Abstract

Brittle rachis is an important trait to elucidate the
domestication process in barley. Brittle rachis in wild barley
(*Hordeum vulgare* ssp. *spontaneum* (C. Koch.) Thell) is controlled

by two dominant complementary genes, *Btr1* and *Btr2*. Cultivated barley (*H. vulgare* L. ssp. *vulgare*) lost the brittle rachis character during domestication as a result of mutation at the *Btr1* or *Btr2* locus. In this study, a high-resolution map of the *btr1* locus was constructed using an F₂ population of cultivar (cv. 'Kanto Nakate Gold') × wild barley (line OUH602). We cloned and sequenced 26 AFLP markers linked with the *btr1* and *btr2* loci. Ten converted STS markers were located on the short arm of chromosome 3H only, and at least 9 of the 10 STS markers were allelic with their original AFLPs. Efficient conversion of co-dominant STS markers using BAC clones was successful. No suppression of recombination was observed in the *btr1* region even though wild barley was used as one of the parents. Initial results of BAC screening confirmed the resolution power of the developed high resolution map.

Keywords: Barley; Non brittleness; STS markers; High-resolution map

1. Introduction

Brittle rachis is one of the most important traits in studies of the evolutionary history of barley [1,2]. Two dominant complementary genes, *Btr1* and *Btr2*, are necessary for the formation of a brittle rachis in wild-type barley (*Hordeum vulgare* ssp. *spontaneum* (C. Koch.) Thell), and the two genes are tightly linked on chromosome 3H [3]. Cultivated barley (*H. vulgare* ssp. *vulgare* L.) lost the brittle rachis characteristic during the process of domestication, by acquiring the recessive *btr1* and *btr2* alleles. Takahashi [4] reported that the *btr1* allele is present in most occidental cultivars whereas the *btr2* allele is present in most oriental cultivars. The non-brittle rachis 1 locus (*btr1*) was recently located in a molecular linkage map of the short arm of chromosome 3H [5]. A high-density amplified fragment-length polymorphism (AFLP) map of the *btr1* and *btr2* loci, which consisted of 84 AFLP markers in a 6.7-cM region, was constructed using recombinant inbred lines (RILs) of Azumamugi (oriental) × Kanto Nakate Gold (occidental) [6]. The parent Azumamugi is likely to have inhibitor genes in chromosomes 5H and 7H, which are affecting brittleness other than *btr2* [6]. This complicated the scoring of brittle verses non-brittle in the crosses involving Azumamugi as one of the parents. Senthil and Komatsuda [7] reported mapping of non-brittle rachis genes using wild and cultivar crosses and suggested the wild barley as one of the parents in the crosses helped to get less ambiguous phenotypic scores.

AFLP markers are abundant in nature and are used for the construction of genetic linkage maps and in genetic diversity studies. This approach requires no specific prior knowledge about the genome, but despite this advantage, AFLP is not a convenient marker system to be used for large segregating populations because of its complex protocol. Another major limitation of AFLP is that its reliance on dominant-recessive type markers decreases the amount of information in linkage analysis. Methods for converting AFLP markers into sequence-tagged sites (STSs) were thus developed and utilized in many mapping programs [8-11]. The objective of the present study was to convert AFLP markers linked to the *btr1* and *btr2* loci into STSs and map them in a new mapping population of cultivar X wild and thereby construct a high-resolution linkage map of the *btr1* locus, which is a crucial step towards cloning of the *btr1* gene.

2. Materials and Methods

2.1. Plant material

Seeds of the 'Azumamugi' (AZ) and 'Kanto Nakate Gold' (KNG) cultivars were obtained from the Barley Breeding Laboratory of the National Institute of Crop Science, Tsukuba. AZ is a six-rowed cultivar with a *Btr1Btr1btr2btr2* genotype and KNG is a two-rowed cultivar with a *btr1btr1Btr2Btr2* genotype. Seeds of a wild barley line 'OUH602' (OUH) were obtained from the Research Institute for Bioresources of Okayama University, Kurashiki. OUH

has a *Btr1Btr1Btr2Btr2* genotype. We performed a cross between KNG and OUH, and self-pollinated the F_1 plants to raise 672 F_2 plants. Plants were grown over the winter in the field at NIAS, Tsukuba, because some materials required vernalization before flowering. Wheat (*Triticum aestivum* L. cv. 'Chinese Spring' (CS))-barley (cv. 'Betzes' (BZ)) chromosome addition lines (CALs) and their parental lines were kindly provided by Dr. A.K.M.R. Islam (University of Adelaide, SA, Australia).

2.2. Evaluation of rachis brittleness

Evaluation of rachis brittleness in the field was done after maturation of the plants by gently pulling apart the awns of the two main rows. Brittleness was scored quantitatively (%) as $100 \times (\text{number of disarticulated spike rachis nodes}) / (\text{number of rachis nodes in a spike})$, and classified plants with a value from 0% to 20% as non-brittle, and plants with a value of 80% and above as brittle. There were no plants with intermediate values. At least three spikes were evaluated for each 600 F_2 plants of KNG $\times$ OUH. We evaluated 30 F_3 plants derived from each 23 F_2 recombinant in the interval between e5m15-09STS and e39m23-11STS in the following year.

2.3. Cloning and sequencing of the AFLP bands

Twenty-six AFLP bands linked to the *btr1* and *btr2* loci [6,7] were taken for cloning (Table 1). Genomic DNA from fresh leaf

tissues was extracted by means of the SDS (sodium dodecyl sulfate)-method, as described previously [12]. We performed AFLP reactions following standard methods [13], using *EcoRI* and *MseI* for the digestion of genomic DNAs. A small 7% polyacrylamide slab gel electrophoresis system (14) with TBE (Tris base plus boric acid plus EDTA) buffers was used as described previously [6]. We detected AFLP bands by means of SYBR Gold staining (Molecular Probes, Eugene, Oregon, USA). AFLP fragments were purified and cloned in pCRII-TOPO (Invitrogene, Carlsbad, California, USA) following previously described methods [11]. We sequenced two to four clones per AFLP fragment using -21 M13 Forward (5'-TGTAACGACGCGCCAGT-3') and M13 Reverse primers (5'-CAGGAAACAGCTATGACC-3'). The nomenclature of the AFLP fragments followed the method of Mano et al., [14]. Blast search was performed against GenBank (www.ncbi.nlm.nih.gov) and TIGR (www.tigr.org) database.

2.4. PCR of STS

The PCR (polymerase chain reaction) mixture (10 μ L) contained 20 ng of genomic DNA, 300 nM each primer, 200 μ M each dNTP (dATP, dCTP, dGTP, and dTTP), 25 mM TAPS (N-tris(hydroxymethyl)methyl-3-amino-propanesulphonic acid, pH 9.3), 50 mM KCl, 1 mM 2-mercaptoethanol, 1 to 4 mM $MgCl_2$ (Table 2), and 0.25 U *ExTaq* DNA polymerase (Takara, Tokyo). Amplification was done in a GeneAmp PCR System 9700 (ABI, Tokyo). The standard PCR conditions were as follows: denaturing at 94 °C for 5 min; followed by 30 cycles of

denaturing at 94 °C for 30 s, annealing at 56 °C to 62 °C depending on sequence for 30 s (Table 2), and extension at 72 °C for 30 s; followed by final incubation at 72 °C for 7 min. Amplified DNAs, after treatment with restriction enzymes if necessary, were separated in 1.8% (w/v) agarose (Iwai Kagaku, Tokyo) or, for the separation of fragments shorter than 60 bp, in 3% or 4% (w/v) Metaphor agarose (Cambrex, Rockland, Maine, USA). Gels were stained with ethidium bromide.

2.5. Analysis of allelic diversity

Parental PCR products were recovered from the agarose gels using the Qiaquick DNA extraction kit (Qiagen, Tokyo) and sequenced these products using BigDye terminator version 1.0 (ABI, Tokyo). DNA sequences were aligned using CLUSTAL W [15] to search for polymorphisms. We used the Webcutter software (www.firstmarket.com/cutter/cut2.html) to search restriction sites in order to detect polymorphisms between parents.

2.6. BAC library screening and sequencing

Morex barley BAC library [16] was screened by PCR using converted STS markers e09m25-08STS and e23m23-07STS. BAC cultures were grown in LB medium containing 12.5 mg/L of chloramphenicol, and BAC DNAs were prepared using the alkaline-lysis method [17]. The e09m25-08STS primers (Table 2) were used for sequencing internal regions flanking the AFLP fragment in positive BAC. Both

ends of a BAC selected by e23m23-07STS marker were sequenced. DNAs were sequenced according to the manufacturer's instructions provided with BigDye terminator version 3.1 (ABI, Tokyo).

2.7. Linkage analysis

We used 99 RILs of AZ × KNG [6] for our tests of allelism between AFLPs and STSs. We analyzed 672 segregating F₂ plants from KNG × OUH using STS markers, and constructed a linkage map using MAPMAKER/EXP version 3.0 [18,19]. Kosambi's mapping function was used to estimate map distance [20].

3. Results

3.1. DNA Sequences of AFLP fragments

DNA sequences of 26 AFLP fragments previously linked to btr1/btr2 loci [6,7] were determined (Table 1). The sizes ranged between 81 and 1247 bp. All the fragments contained an *EcoRI*-adapter sequence on one end and an *MseI*-adapter sequence on the other end. Blastn search for the DNA sequences against the GenBank revealed 10 AFLP fragments to be homologous with Triticeae retrotransposons (Table 1). A blastn search against TIGR ("Gramineae Repeat") showed almost the same result (data not shown). Translated sequences revealed no significant homologies for all AFLP sequences in protein database by blastx with the exception of e50m21-01 ($4e^{-10}$) and e57m10-07 ($4e^{-24}$) which showed

homologies with polyproteins of *Oryza sativa* and *Oryza australiensis* respectively (data not shown). Excluding small fragments and highly repeated sequences, a total of 18 AFLP fragments were considered for conversion to STS markers.

3.2. Redundancy of the STSs

Oligonucleotides (21-mers) were synthesized according to the DNA sequences of the 18 AFLPs and used for analysis of wheat-barley CALs (Table 2). Amplicons of ten STS markers were assigned to chromosome 3H by CALs (e04m55-09STS, e05m15-09STS, e06m49-08STS, e09m25-08STS, e23m23-07STS, e39m23-11STS, e40m19-08STS, e45m11-11STS, and e50m21-01STS) or linkage analysis using RILs (e30m07-09STS) (Table 2). Amplicons of four STS markers (e46m23-09STS, e37m30-10STS, e50m12-04STS, and e53m62-09STS) were assigned to two or more chromosomes, including 3H. Since three STS markers (e48m02-08STS, e58m61-09-1STS, and e60m22-07STS) amplified DNAs of the same size in both CS and BZ, we were not able to determine their chromosomal location by this system. The e24m42-08STS produced an amplicon on chromosome 2H only (Table 2), indicating that an incorrect fragment had been cloned.

3.3. Co-segregation of STS markers with the original AFLPs

Of the 10 STS markers assigned to chromosome 3H (including e30m07-09STS), 7 showed a presence-absence polymorphism between AZ and KNG (Table 2). We sequenced the amplicons of the remaining

3 STSs, and appropriate restriction enzymes generated three co-dominant markers between AZ and KNG (Table 2). Co-segregation of 9 STSs (but not e06m49-08STS) with their original AFLPs was determined using the AZ × KNG RILs (Table 2). We had considerable difficulty scoring e06m49-08STS in the RILs, and could not confirm their co-segregation. The major amplicon of this STS was assigned to chromosome 3H, while very weak amplicons were also assigned to chromosomes 5H and 6H of BZ (data not shown), probably because of its homology with MITE (miniature inverted repeat transposable element) (9e-55). Nevertheless 26 RILs showed consistency between e06m49-08 and e06m49-08STS scores.

3.4. High-resolution mapping using the KNG × OUH F₂ population

Developed dominant and co-dominant STS markers were used to analyze the KNG × OUH 672 segregating F₂ plants (Table 2). Initially, we used four STS markers: The e05m15-09STS and e39m23-11STS markers apparently flanked the *btr1* locus in the AZ × KNG population (Fig. 1a), but they were dominant markers (Table 2). The orientation of e23m23-07STS and e50m21-01STS with respect to *btr1* was not clear in the AZ × KNG population (Fig. 1a), but they were co-dominant markers (Table 2) and expected to recombine with the *btr1* locus in the large-scale KNG × OUH F₂ population. The segregation ratio of all four markers did not significantly deviate from the expected 3:1 or 1:2:1 ratio, but each deviated

slightly in favor of OUH: e05m15-09STS (chi-squared = 1.36, $0.2 < P < 0.5$), e23m23-07STS (chi-squared = 2.82, $0.2 < P < 0.5$), e50m21-01STS (chi-squared = 3.86, $0.1 < P < 0.2$), and e39m23-11STS (chi-squared = 0.4, $0.5 < P < 0.8$). Since we lost 72 F_2 plants to a heavy frost in the field after the DNA analysis, we scored brittleness for the surviving 600 F_2 plants into a segregated population of 475 brittle-rachis plants and 125 non-brittle-rachis plants after maturation. The segregation ratio (3.8:1) differed significantly from the expected 3:1 ratio (chi-squared = 5.55, $0.02 < P < 0.05$) in favor of the OUH allele. The constructed map is shown in Fig. 1B.

A total of 23 F_2 plants were detected as recombinants between any two markers within the analyzed interval (2.8 cM). Using these 23 recombinants, we integrated e40m19-08STS and e09m25-08STS into the map. In the following year, we evaluated brittleness for 30 F_3 plants derived from each F_2 recombinant, which allowed complete classification of three genotypes for the *btr1* locus. All the molecular markers recombined with the *btr1* locus, allowing us to order them within this high-resolution map (Fig. 1B). There were no contradictions between the marker order and the previously reported results (Fig. 1A), but resolution was much increased in the new map (Fig. 1B).

3.5. Conversion of dominant STS markers into co-dominant STSs

The e09m25-08STS and e23m23-07STS cosegregated in the KNG x OUH map (Fig. 1B), and e09m25-08STS was obviously located closer

to the *btr1* and *btr2* loci than e23m23-07STS as shown in the AZ x KNG map (Fig. 1A). The e09m25-08STS was a dominant marker (Table 2). We used the Morex barley BAC library to obtain an extended length to generate a co-dominant marker for the e09m25-08STS. PCR screening of the BAC library with e09m25-08STS identified two clones, 679M06 and 708H16. Then the two primers of e09m25-08STS were independently used to sequence the BACs and obtain an extended length on both sides of AFLP fragments. A total of 990 bp was obtained in 679M06. We designed several primer pairs on the basis of the extended length to amplify all the alleles of AZ, KNG, and OUH. We found a primer pair M679M06a620U037 (5'-AGAAGCTCACAGGGTTAGAAT-3') and M679M06a990L643 (5'-TACGAGGAGCTGGTCAAGGAA-3') to be able to amplify all the parental lines. Sequence variations in both the *MseI* and *EcoRI* sites were confirmed, which generated an AFLP fragment e09m25-08. Later we found a primer M679M06a990U073 (5'-TTGTGAAGGCTCTCCAGAGTC-3') to generate more strong and stable PCR products (named e09m25-08STS-Ext) than the M679M06a620U037. We used the e09m25-08STS-Ext for analyzing segregating F2 population. Restriction enzyme digestion using either *HinfI* or *TaqI* generated a co-dominant marker polymorphic between AZ and KNG and between OUH and KNG, which co-segregated with the e09m25-08STS (Fig. 1b).

3.6. Development of physical contig for the co-segregating markers

The STS markers e09m25-08STS and e23m23-07STS are co-segregating in the high-resolution map (Fig. 1). A physical contig was constructed to evaluate the resolution power of the genetic map. The STS marker e09m25-08 identified two BAC clones 679J21 and 708H16, whereas e23m23-07STS identified 174J19 and 592A03. End-sequence of the 679J21 was converted to a PCR marker 679J21-R-STS using primers 5'- TAGCTATCAATAAGTCCCATC -3' and 5'- GTACAGTGACTAAAGTGCATG -3'. The 679J21-R-STS co-segregated with e09m25-08STS and e23m23-07STS, and hit the 174J19 and 708H16, which allowed connection of the two BAC contigs (Fig. 1).

4. Discussion

AFLP markers were converted into STSs to exploit the potentiality of simple and co-dominant nature of STS markers [8-11]. The *Pst*I-*Mse*I combination was more efficient than the *Eco*RI-*Mse*I combination for detecting AFLPs in barley and maize [21,22], whereas the difference was not significant in wheat [23]. Since *Pst*I is sensitive to methylation, it has been assumed that using *Pst*I would decrease the ratio of repetitive DNA sequences in cereal AFLPs [10,24,25]. However, few AFLPs were converted to STSs in these studies. Shan et al. [26] cloned 26 AFLP markers generated by means of the *Eco*RI-*Mse*I combination in wheat or barley, and were able to develop 6 STSs markers (23%) that could be assigned to specific chromosomes. In the present study, the efficiency of the conversion was 38% (10/26). He et al. [11] successfully converted all five AFLP fragments in their study,

which had been generated by means of the *EcoRI-MseI* combination, into five STS markers that were monogenically inherited. These results suggest a range of conversion efficiencies when the *EcoRI-MseI* combination is used in barley and wheat.

Among the 18 primer pairs that we generated, 13 did not include known repeat-DNA sequences: 7 of these 13 (54%) generated amplicons that were assigned to a single chromosome (3H), whereas 6 generated amplicons that were assigned to two or more chromosomes (Table 2). In the remaining 5 AFLPs, one side of the primer pair included a repeated DNA sequence: 3 of the 5 (60%) generated amplicons assigned to a single chromosome (3H). Therefore, the difference between the two primer-design methods was not significant. A homology search of DNA sequences and chromosome assignment of the STS markers were thus complementary and useful methods for detecting repeat sequences in barley. Our study suggested that e37m30-10STS, e46m23-09STS, e50m12-04STS, and e53m62-09STS included DNA sequences that were repeated in the barley genome. However, these finding requires further study before we can draw firm conclusions about their meaning.

We detected polymorphisms between AZ and KNG for all 10 STS markers that we assigned to chromosome 3H. This result suggests that AZ and KNG are useful for developing and mapping STS markers for the *btr1* and *btr2* loci. AZ has two inhibitor QTLs (quantitative trait loci) in addition to the *btr2* locus for non-brittle rachis, indicating that the AZ × KNG combination is not suitable for developing a high-resolution map of the *btr1* and *btr2* loci [6]. However, the parents were highly polymorphic

regarding the *btr1* and *btr2* loci, and the STS markers developed here can be used in other populations from different crosses, such as KNG × OUH. For instance, e23m23-07, e40m19-08, and e50m21-01, being polymorphic between AZ and KNG, were monomorphic between KNG and OUH [7], but all three were converted into STS markers polymorphic between KNG and OUH (Table 2). Brugmans et al. [27] explained the methods used to obtain SNPs (single-nucleotide polymorphisms) that originally caused the AFLPs, enabling them to obtain co-dominant markers from monomorphic STS markers. In the present study, the BAC internal sequence was demonstrated to be useful for the conversion of the dominant e09m25-08STS into a co-dominant marker. It is interesting to see the sequence polymorphism between parents at the e09m25-08STS locus (less than 600 bp). Most of these identified sequence polymorphisms are specific to differentiate occidental and oriental types allowing them to be differentiated [28]. Sequencing of wild-type barley collections together with occidental and oriental cultivars is currently in progress, and the results will be published elsewhere.

Strongly suppressed recombination was previously reported in wild-type × cultivar crosses of barley [29]; however, recombination was not suppressed at the *btr1* locus in the KNG × OUH F₂ population. STS marker e09m25-08STS was mapped 0.21 cM distal from the *btr1* locus and e50m21-01STS was mapped 0.63 cM proximal in the high-resolution map (Fig. 1b). Since both flanking markers recombined with the *btr1* locus, there was no

evidence to suppose the existence of chromosomal rearrangement at the *btr1* locus, at least not between KNG and OUH.

Initial BAC screening results suggested reasonable resolution power of the present genetic map, as we aligned physical contig for the co-segregating markers. Künzel et al. [30] reported increased recombination in the marker interval between MWG577 and ABG462 region of 3HS. The *btr1/btr2* region falls under this marker interval and the estimated physical/genetic distance ratio was 1.0-4.4 Mb/cM [30]. The average size of each BAC is 100 kb [16], so the physical distance between e09m25-08STS and e23m23-07STS should be a maximum of 200 kb. Although there were no recombinations between the two markers, one recombination will make the map distance of 0.07 cM, therefore physical/genetical ratio for this region can be estimated minimum 200 kb/0.07 cM = 3Mb/cM. However, it is difficult to prove the hypothesis rate of recombination unless the completion of contig of *btr1* flanking markers. The new co-dominant STS markers e09m25-08STS-Ext and e50m21-01STS still flank a 0.84 cM and the markers may not be optimal to start chromosome walking. Since barley chromosome 3H and rice chromosome 1 are colinear over their entire length [31,32], marker enrichment could be achieved by using barley EST resources and barley-rice colinearity. Ten AFLP markers co-segregated with the *btr1* and *btr2* loci [6] and we were able to convert only four of them in this study, but remaining six were not because of their small fragments or repeated elements. These six AFLPs can be used for fine mapping, but five of the six AFLPs were monomorphic between KNG and OUH. The population of 672 F₂

plants is moderate and more is needed. The AFLP-derived STS markers will be used for further high-resolution mapping, which is essential for map-based cloning of the *btr1* genes.

Acknowledgments

The authors thank Dr. N. Senthil, Dr. Congfen He, and Dr. Y. Turuspekov of our laboratory for their technical assistance and suggestions in our laboratory protocols, and Mr. M. Sameri of our laboratory for his assistance in our field experiments. This study was supported in part by the CREST program of the Japan Science and Technology Corporation (JST). P. Azhaguvel was a research fellow supported by the Japan Society for the Promotion of Science (JSPS).

References

- [1] R. Takahashi, The origin and evolution of cultivated barley, in: M. Demerec, (ed.), *Advances in Genetics*, vol. 7, Academic, New York, 1955, pp. 227-266.
- [2] Rv. Bothmer, N. Jacobsen, Origin, taxonomy, and related species. in: D. Rasmusson (ed.), *Barley*. ASA Agronomy Monograph, vol. 26, American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, 1985, pp. 19-56.

- [3] R. Takahashi, J. Hayashi, Linkage study of two complementary genes for brittle rachis in barley, Ber Ohara Inst Landwirtsch Biol. Okayama Univ. 12 (1964) 99-105.
- [4] R. Takahashi, Further studies on the phylogenetics differentiation of cultivated barley, Barley Genetics 1 (1963) 19-26.
- [5] T. Komatsuda, Y. Mano, Molecular mapping of the intermedium spike-c (*int-c*) and non-brittle rachis 1 (*btr1*) loci in barley (*Hordeum vulgare* L.), Theor. Appl. Genet. 105 (2002) 85-90.
- [6] T. Komatsuda, P. Maxim, N. Senthil, Y. Mano, High-density AFLP map of nonbrittle rachis 1 (*btr1*) and 2 (*btr2*) genes in barley (*Hordeum vulgare* L.), Theor. Appl. Genet. 109 (2004) 986-995.
- [7] N. Senthil and T. Komatsuda, Comparative mapping of non-brittle rachis genes *btr1* and *btr2* using wild x Occidental and wild x oriental cultivar populations in barley *Hordeum vulgare* L., Euphytica 145 (2005) 215-220.
- [8] N.G. Paltridge, N.C. Collins, A. Bendahmane, R.H. Symons, Development of YLM, a co-dominant PCR marker closely linked to the *Yd2* gene for resistance to barley yellow dwarf disease, Theor. Appl. Genet. 96 (1998) 1170-1177.
- [9] L. Decousset, S. Griffiths, R.P. Dunford, N. Pratchett, D.A. Laurie, Development of STS markers closely linked to the *Ppd-H1* photoperiod response gene of barley (*Hordeum vulgare* L.), Theor. Appl. Genet. 101 (2000) 1202-1206.

- [10] J.S. Weerasena, B.J. Steffenson, A.B. Falk, Conversion of an amplified fragment length polymorphism marker into a co-dominant marker in the mapping of the *Rph15* gene conferring resistance to barley leaf rust, *Puccinia hordei* Otth., Theor. Appl. Genet. 108 (2003) 712-719.
- [11] C. He, B.E. Sayed-Tabatabaei, T. Komatsuda, AFLP targeting of the 1-cM region conferring the *vrs1* gene for six-rowed spike in barley, *Hordeum vulgare* L., Genome 47(2004)1122-1129.
- [12] T. Komatsuda, I. Nakamura, F. Takaiwa, S. Oka, Development of STS markers closely linked to the *vrs1* locus in barley, *Hordeum vulgare*, Genome 41 (1998) 680-685.
- [13] P. Vos, R. Hogers, M. Bleeker, M. Reijans, T. van de lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper, M. Zabeau, AFLP: a new technique for DNA fingerprinting, Nucleic Acids Res. 23 (1995) 4407-4414.
- [14] Y. Mano, S. Kawasaki, F. Takaiwa, T. Komatsuda, Construction of a genetic map of barley (*Hordeum vulgare* L.) cross 'Azumamugi' x 'Kanto Nakate Gold' using a simple and efficient amplified fragment-length polymorphism system, Genome 44 (2001) 284-292.
- [15] J.D. Thompson, D.G. Higgins, T.J. Gibson, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice, Nucleic Acid Res. 22 (1994) 4673-4680.

- [16] Y. Yu, J.P. Tomkins, R. Waugh, D.A. Frisch, D. Kudrna, A. Kleinhofs, R.S. Brueggeman, G.J. Muehlbauer, R.P. Wise, R.A. Wing, A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes, *Theor. Appl. Genet.* 101 (2000) 1093-1099.
- [17] J. Sambrook, D.W. Russel, *Molecular cloning: a laboratory manual*. 3rd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2001.
- [18] E.S. Lander, P. Green, J. Abrahamson, A. Barlow, M.J. Daly, S.E. Lincoln, L. Newburg, MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations, *Genomics* 1 (1987) 174-181.
- [19] S.E. Lincoln, M.J. Daly, E.S. Lander, *Constructing genetic linkage maps with MAPMAKER/EXP 3.0*. Whitehead Institute for Biomedical Research Technical Report, Cambridge, 1993.
- [20] D. Kosambi, The estimation of map distances from recombination values, *Ann. Eugen.* 12 (1944) 172-175.
- [21] W. Powell, W.T.B. Thomas, E. Baird, P. Lawrence, A. Booth, B. Harrower, J.W. McNicol, R. Waugh, Analysis of quantitative traits in barley by the use of amplified fragment length polymorphisms, *Heredity* 79 (1997) 48-59.
- [22] M. Vuylsteke, R. Mank, B. Brugmans, P. Stam, M. Kuiper, Further characterization of AFLP[®] data as a tool in genetic diversity assessments among maize (*Zea mays* L.) inbred lines, *Mol Breed* 6 (2000) 265-276.

- [23] B.A. Barrett, K.K. Kidwell, AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest, *Crop Sci.* 38 (1998) 1261-1271.
- [24] Y.G. Cho, M.W. Blair, O. Panaud, S.R. McCouch, Cloning and mapping of variety-specific rice genomic DNA sequences: amplified fragment length polymorphism (AFLP) from silver-stained polyacrylamide gels, *Genome* 39 (1996) 373-378.
- [25] R. Prins, J.Z. Groenewald, G.F. Marais, J.W. Snape, R.M.W. Koebner, AFLP and STS tagging of *Lr19*, a gene conferring resistance to leaf rust in wheat, *Theor. Appl. Genet.* 103 (2001) 618-624.
- [26] X. Shan, T.K. Blake, L.E. Talbert, Conversion of AFLP markers to sequence-specific PCR markers in barley and wheat, *Theor. Appl. Genet.* 98 (1999) 1072-1078.
- [27] B. Brugmans, R.G.M. van der Hulst, R.G.F. Visser, P. Lindhout, H.J. van Eck, A new and versatile method for the successful conversion of AFLP markers into simple single locus markers, *Nucleic Acids Res.* 31 (2003) e55(9 pp).
- [28] P. Azhaguvel, D. Vidya-Saraswathi, T. Komatsuda, Conversion of AFLP markers linked to the non-brittle rachis locus *btr1/btr2* into STS markers for high-resolution linkage mapping and phylogenetic study using *btr1*-linked markers in barley, in: *Proceeding of the 8th Gatersleben Research Conference - Genetic Diversity & Genome Dynamics in Plants*, Chateau Meisdorf/IPK Gatersleben, Germany, 2005, pp. 93.

- [29] R. Görg, K. Hollricher, P. Schulze-Lefert, Functional analysis and RFLP-mediated mapping of the *Mlg* resistance locus in barley, *Plant J.* 3 (1993) 857-866.
- [30] G. Künzel, L. Korzun, A. Meister, Cytologically intergrated physical restriction fragment length polymorphism maps for the barley genome based on translocation breakpoints, *Genetics* 154 (2000) 397-412.
- [31] G. Moore, K.M. Devos, Z. Wang, M.D. Gale, Grasses, line up and form a circle, *Curr. Biol.* 5 (1995) 737-739.
- [32] D.W. Smilde, J. Haluskova, T. Sasaki, A. Graner, New evidence for the syntenry of rice chromosome 1 and barley chromosome 3H from rice expressed sequence tags, *Genome* 44 (2001) 361-367.

Figure legend

Fig 1. (A) High-density map of the *btr1* and *btr2* loci in 99 RILs of 'Azumamugi' × 'Kanto Nakate Gold', modified after Komatsuda and Mano (2002) and Komatsuda et al. (2004). All the 23 AFLPs that we sequenced are shown in Table 1. Allelism was confirmed for nine AFLP-STS pairs. ABG047 and ABA001 are STS markers flanking the *btr1* and *btr2* loci. (B) High-resolution map of the *btr1* locus in 672 F₂ plants of 'Kanto Nakate Gold' × OUH602. Six STS markers converted from AFLPs were used for the mapping. A physical contig was made for the co-segregating markers.

Table 1. AFLP markers for the *btr1* and *btr2* loci, their DNA sequences (plus GenBank/EMBL/DDBJ accession numbers), and results of a blastn search

AFLP marker	Dominant parent	Size (bp) ^a	Acc. No	Blastn with the Genbank	E value ^b
e04m55-09	KNG	202	#####	-	-
e05m15-09	AZ	204	#####	-	-
e06m49-08	KNG	278	#####	<i>Hordeum vulgare</i> transposon MITE	9.0e-55
e09m25-08	KNG	245	#####	-	-
e19m27-11	KNG	131	#####	-	-
e23m12-13-1	AZ	101	#####	-	-
e23m12-13-2	KNG	100	#####	-	-
e23m23-07	KNG	318	#####	<i>Hordeum chilense</i> RAPD marker IAS-pHc4-3	2.0e-22
e23m44-13	KNG	96	#####	-	-
e24m42-08	AZ	285	#####	<i>Hordeum vulgare</i> ssp. <i>vulgare</i> Morex BAC clone 773K14	8.0e-06
e26m58-13	KNG	81	#####	-	-
e30m07-09	AZ	204	#####	-	-
e34m11-10	KNG	160	#####	-	-
e37m30-10	KNG	150	#####	-	-
e39m23-11	KNG	133	#####	<i>Hordeum vulgare</i> Gypsy type retrotransposon BAGY-1	5.0e-17
e40m19-08	KNG	284	#####	-	-
e45m11-11 ^c	KNG	143	#####	-	-
e46m23-09	KNG	203	#####	<i>Hordeum vulgare</i> Gypsy type retrotransposon BAGY-1	2.0e-24
e48m02-08	KNG	289	#####	-	-
e50m12-04	KNG	610	#####	<i>Triticum turgidum</i> LTR retrotransposon Sabrina	5.0e-09
e50m21-01	KNG	1247	#####	-	-
e53m62-09	KNG	237	#####	<i>Triticum turgidum</i> Gypsy LTR retrotransposon Laura	2.0e-15
e57m10-07	AZ	318	#####	<i>Hordeum vulgare</i> chromosome 5 BAC 635P2	0.0e-113
e58m61-09-1	KNG	223	#####	-	-
e60m22-07	KNG	365	#####	<i>Aegilops tauschii</i> LZ-NBS-LRR class RGA, HCBT	2.0e-35
e62m32-08	AZ	243	#####	<i>Hordeum roshevitzii</i> BARE-1 long terminal repeat DNA	1.0e-47

^a Amplicon size including AFLP primers^b Stringency threshold = 1e-04 in this table^c A blastn search against TIGR database (Gramineae Repeat) showed a homology with *Hordeum vulgare* Sukkula retrotransposon with a p value of 1.1e-08

Table 2. STS markers for the btr1 and btr2 loci, and their chromosomal locations and polymorphism

STS maker	<i>Eco</i> RI-side primer	<i>Mse</i> I-side primer	Anneal (°C)	MgCl ₂ (mM)	Size (bp)	DNA amplification by PCR													Enzyme	Restriction analysis			A	
						wheat	CALs							barley						(bp)				
							CS	2H	3H	4H	5H	6H	7H	BZ	AZ	KNG	OUH	AZ			KNG	OUH		
e04m55-09STS	AATGTTTAGAGTTTGTTTGGT	TTCGTGAAGTAGATTTTGGGG	60	2.5	111	-	-	+	-	-	-	-	-	+	+	+	+	<i>Msl</i> I	56+33+2;	56+55	56+55			
e05m15-09STS	GATCACGCTAGGAATTGTCTT	AGGTGATACTGTTGTCCGTCT	60	2.5	156	-	-	+	-	-	-	-	-	+	+	-	+	<i>Ava</i> II	107+49	-	155			
																		<i>Apa</i> I	156	-	105+50			
e06m49-08STS	<u>ATATATCATTAGAAACCTTAG</u>	CATTGAGGAGAAAAAAGTGTA	58	4.0	174	-	-	+	-	-	-	-	-	+	-	+	+	Mono	-	174	174			
e09m25-08STS	CGCTCACCCGCTGTCCCGGCC	AATGAAACTAACGCTAATACT	62	4.0	194	-	-	+	-	-	-	-	-	+	-	+	-							
e23m23-07STS	<u>TGTGCTGCAATTACCGGTTCC</u>	GCACCCCTTCACGCATCTCAA	60	2.5	252	-	-	+	-	-	-	-	-	+	-	+	+	<i>Acc</i> II	-	252	169+83			
e24m42-08STS	CCAAAGATGCCCGGCCAGAT	GCAGTAGAAAGATCAAGCCAA	60	2.5	196	-	+	-	-	-	-	-	-	+	+	+	+							
e30m07-09STS	CAGATGCCATTTGAACAGAGT	TCCGATGATGCTATATTCTTG	60	1.5	160	-	-	-	-	-	-	-	-	-	+	-	-	-						
e37m30-10STS	GGTTAGAAAGCAAAATAGTCG	ATATGGTCTTGGTAGCATGTC	58	1.5	106	-	+	+	-	+	+	-	-	+	+	+	+							
e39m23-11STS	AAGGATAAAGCACTAGAGCAA	<u>TGAGACCACAAAGGGATGCGA</u>	63	2.5	91	-	-	+	-	-	-	-	-	+	-	+	-							
e40m19-08STS	TCCCATTACAGAAAAACACAG	TCCCCAACAAAGATAGGTATTT	58	1.5	236	-	-	+	-	-	-	-	-	+	+	+	+	<i>Dra</i> I	207+29	236	207+29			
e45m11-11STS	CCGGTGCTTCCTTCAATTTTT	TGCAATGATGTAGATGAAGCT	62	1.5	103	-	-	+	-	-	-	-	-	+	-	+	+	Mono	-	103	103			
e46m23-09STS	CACCTTTGTTACTATTGTTAC	<u>CAAAGGGATGCGATGTGTTAC</u>	58	1.5	110	-	-	+	-	-	-	-	+	+	+	+	-							
e48m02-08STS	ATCGACAATACCGGCAGAGAA	AATATACCTCGTCGATGTTTG	62	2.5	213	+	+	+	+	+	+	+	+	+	+	+	+							
e50m12-04STS	AGGATTGCTTGTGTAGTTTCAT	GGTAGGTCATCAAGTATATAA	63	1.5	476	-	+	+	+	+	+	+	+	+	+	+	+							
e50m21-01STS	ACACAATATGCAGCTCGAAC	AGTGAATATTGGGCTTGAGCG	62	1.5	1166	-	-	+	-	-	-	-	-	+	+	+	+	<i>Ava</i> II	A ^b	K ^c	A ^b			
																		<i>Alu</i> I	A ^d	K ^c	K ^e			
e53m62-09STS	CTGATGCAACTAACTACAAGC	TCAAATACAAAGATGCCAAGA	62	4.0	198	-	+	+	+	+	+	+	+	+	+	+	+							
e58m61-09-1STS	CAATAGCTGACAAAAACCAA	ACCTATGCTATGGATGAGATT	60	2.5	166	+	+	+	+	+	+	+	+	+	+	+	+							
e60m22-07STS	<u>CCCGAGAGGCAATAATAAATG</u>	TTCTACTAACATTGTGCCCTC	58	1.5	262	+	+	+	+	+	+	+	+	+	+	+	+							

^aBetween AFLPs and STSs tested using AZ x KNG RILs (no. of identical genotype/no. of RILs tested)

^b588+267+259+52, ^c532+267+259+56+52, ^d561+323+239+29+14, ^e561+323+161+78+29+14

Primers with underlines include repeated sequences

Figure(s)