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Molecular Mapping of Non-Brittle Rachis Genes *btr1* and *btr2* using STS Markers in Barley

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Abstract

Brittle rachis is an important trait to study the domestication process in barley. Ancient farmers preferred to have non-brittle barley for cultivation, and domesticated barley lost the brittle rachis after a process of human selection. In this study, partial linkage maps in the region of non-brittle rachis genes *btr1* and *btr2* were constructed using two wild × cultivar F_2 populations. STS markers derived from AFLP markers *e09m25-08*, *e30m07-09* and *e50m21-01*, which showed no recombination with the *btr1* and *btr2* loci in recombinant inbred lines (RILs), showed recombination with the *btr1* and *btr2* loci in the two F_2 populations. Though there were few differences in map distances, orders of the markers were highly conserved. OUH602 (wild) × Kanto Nakate Gold (cultivar) was suitable for high-resolution mapping of the *btr1* locus due to unambiguous phenotypes in segregating F_2 plants.

Discipline: Biotechnology

Additional key words: disarticulation, domestication, F_2 mapping populations, recombination, shattering, wild barley

Introduction

Archaeological evidences in the Fertile Crescent showed that barley (*Hordeum vulgare* L.) is one of the founder crops of Old World Agriculture[17]. Brittle rachis is one of the most important traits to study the evolutionary pattern of barley[2,15]. Brittle rachis of wild barley (*H. vulgare* ssp. *spontaneum*) is controlled by two dominant complementary genes *Btr1* and *Btr2*[16]. Since cultivated barley (*H. vulgare* ssp. *vulgare*) has either recessive *btr1* or *btr2*, it has non-brittle rachis. The non-brittle rachis genes were mapped on the short arm of chromosome 3H, being tightly linked mutually, using morphological[16] and amplified fragment length polymorphism (AFLP) markers[6,7]. In the present study a set of STS markers linked with the *btr1* and *btr2* loci[1] were used for comparing the structure of the brittle rachis gene regions.

Materials and methods

'Azumamugi' (AZ) a cultivar of *Btr1Btr1btr2btr2* genotype and 'Kanto Nakate Gold' (KNG) a cultivar of *btr1btr1Btr2Btr2* genotype were obtained from Barley Breeding Laboratory, National Institute of Crop Science, Tsukuba. OUH602 (OUH), a wild barley of *Btr1Btr1 Btr2Btr2* genotype, was obtained from Research Institute for Bioresources, Okayama University, Kurashiki, Japan. Two F_2 populations of OUH × KNG and OUH × AZ consisting of 192 plants from each cross were developed by self-pollination of F_1 plants. We evaluated rachis brittleness in the field after maturation of the plants by gently pulling apart the awns of the two main rows in opposite directions. We scored brittleness quantitatively (%) as 100 × (number of disarticulated spike rachis nodes)/(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. At least three spikes were evaluated for each plant.

Genomic DNAs were extracted from fresh leaf tissues by SDS-method[8]. Seven STS markers linked with the *btr1* and *btr2* loci[1] were taken to analyze the F_2 populations. The PCR mixture (10 μL) contained 20 ng genomic DNA, 300 nM of each primer, 200 μM each of

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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, 1.5 mM to 4.0 mM $MgCl_2$, and 0.25U ExTaq DNA polymerase (Takara, Tokyo). Amplification was done in GeneAmp PCR System 9700 (ABI, Tokyo). The standard PCR condition was denaturing at 94ºC for 5 min followed by 30 cycles of denaturing at 94ºC for 30 s, annealing at 56ºC to 63ºC (depending on primers) for 30 s and extension at 72ºC for 30 s, followed by final incubation at 72ºC for 7 min. Amplified DNAs, after the treatment with restriction enzymes if necessary[8], were separated in a 1.8% (w/v) agarose (Iwai Kagaku, Tokyo) or, for the separation of fragments less than 60 bp, 3% or 4% (w/v) Metaphor agarose (Cambrex, Rockland, USA).

Linkage maps were constructed using the program MAPMAKER 3.0[10,11]. Kosambi's mapping function was applied to estimate map distance[9].

Results

Segregation ratio of F_2 plants for brittle verses non-brittle rachis was 3:1 in both F_2 populations (154 brittle:38 non-brittle in OUH × KNG; 139 brittle:36 non-brittle in OUH × AZ) as shown by chi-square tests (Table 1). Segregation of F_2 plants for all the STS markers fit to the expected 3:1 (dominant) or 1:2:1 (co-dominant) as analyzed by chi-square test (Table 1). Since 17 plants in OUH × AZ showed intermediate values, they were excluded from the analysis.

Orders of the gene and STSs were highly conserved between populations. In the linkage analysis, *e09m25-08STS* and *e50m21-01STS* were separated from the *btr1* locus using OUH × KNG F_2 population, and *e30m07-09STS* and *e50m21-01STS* were separated from the *btr2* locus using OUH × AZ F_2 populations, respectively (Fig. 1, left and right). The three markers did not recombine with the *btr1* and *btr2* loci in the map of AZ × KNG RILs[7] (shown in Fig. 1, center). The distance between *e05m15-09STS* and *e50m21-01STS* was 1.0 cM in the RILs of AZ × KNG and 1.3 cM in the OUH × KNG F_2 population, whereas the distance was increased to 3.1 cM in the OUH × AZ F_2 population. The *e50m21-01STS* was proximal to the *btr1* and *btr2* loci with a distance of 0.52 cM to 1.04 cM (Fig. 1). There were 4 STS markers clustering distal to *btr1* in OUH × KNG, but *e09m25-08STS* is most closely linked to the *btr1* locus as shown in the AZ × KNG RIL map. The *e30m07-09STS* was distal to the *btr2* locus in OUH × AZ.

Discussion

STS markers have many advantages: robustness, cost-effectiveness, and easiness in handling. In this study using STS markers efficiently dissected the recombination break points of the *btr1* and *btr2* loci. The *e09m25-08*, *e30m07-09* and *e50m21-01* showed no recombination with *btr1* and *btr2* in RILs of AZ × KNG map[7] but they were separated from *btr1* and *btr2* in these two F_2 maps.

From this comparative mapping, we confirmed a conservation of marker order across different cross combinations. There was no evidence of chromosomal rearrangement immediately at the *btr1* and *btr2* loci. There are reports about strongly suppressed recombination in wild x cultivar crosses of barley[4,5] whereas an increased recombination rate was noticed in OUH × AZ in this study. Variable recombination ratio was also observed in cultivar × cultivar crosses in barley[14]. Recombination

Table 1. Segregation for rachis brittleness and STS markers in F_2 populations of wild × cultivated barley

F_2 Population	Non-brittle rachis genes and STSs (enzymes)	Observed					Expected	χ^2	Probability
		OUH	OUH or F_1	F_1	Cultivar or F_1	Cultivar			
OUH602 × KNG	e05m15-09STS	–	151	–	–	41	3:1	1.36	0.20–0.30
	e40m19-08STS (*Dra* I)	57	–	94	–	41	1:2:1	2.75	0.20–0.30
	e23m23-07STS (*Acc* II)	57	–	94	–	41	1:2:1	2.75	0.20–0.30
	e09m25-08STS	57	–	–	132	–	1:3	1.56	0.20–0.30
	btr1	–	154	–	–	38	3:1	2.77	0.05–0.10
	e50m21-01STS (*Ava* II)	56	–	94	–	40	1:2:1	2.71	0.20–0.30
	e39m23-11STS	57	–	–	135	–	1:3	1.13	0.20–0.30
OUH602 × AZ	e05m15-09STS (*Apa* I)	46	–	87	–	52	1:2:1	1.26	0.50–0.70
	e23m23-07STS	–	145	–	–	46	3:1	0.03	0.80–0.90
	e30m07-09STS	57	–	–	132	–	1:3	2.69	0.10–0.20
	btr2	–	139	–	–	36	3:1	1.83	0.10–0.20
	e50m21-01STS (*Alu* I)	48	–	85	–	57	1:2:1	2.95	0.20–0.30

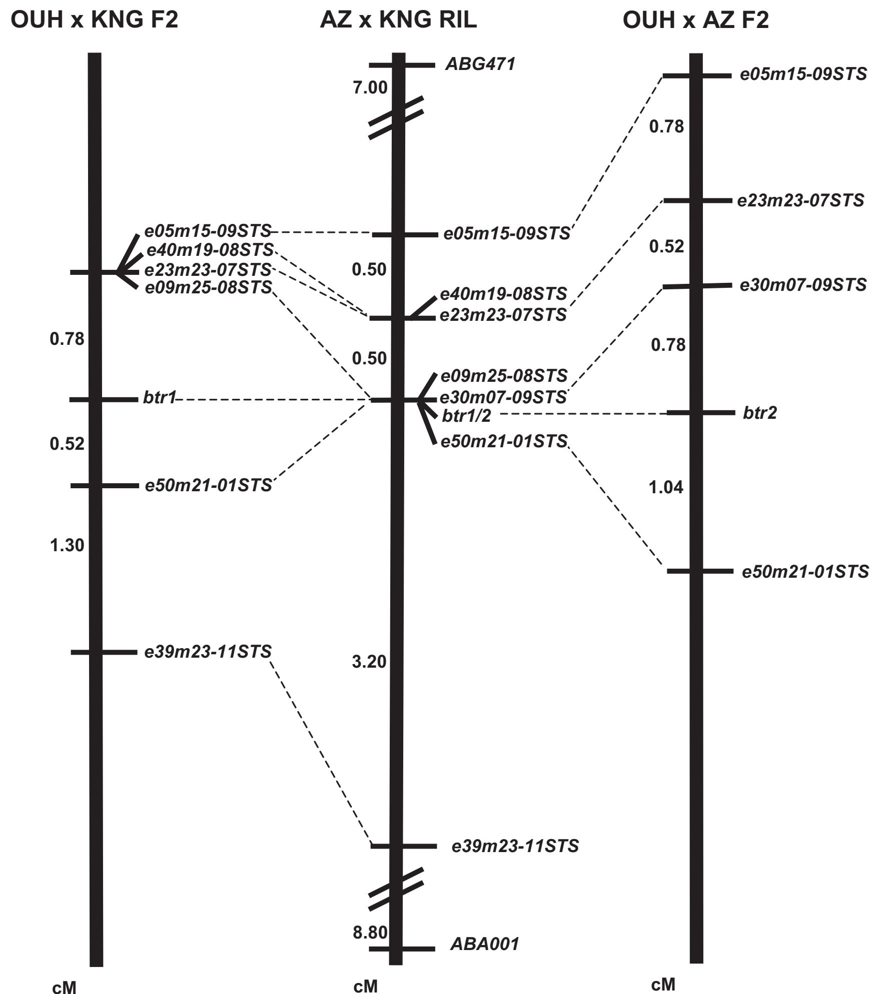

Fig. 1. Mapping of the *btr1* locus in OUH × KNG F_2 population and *btr2* locus in OUH × AZ F_2 population in comparison with AZ × KNG RILs map[7]
The maps show a part of the short arm of chromosome 3H.
Map distances in centi-Morgan (cM).

rate is region-specific in the genome and not only based on the relatedness of parents. Another reason for the slight increase of recombination rates in the two F_2 populations may be due to the population size.

The STS markers flanking the *btr1* and *btr2* loci are valuable for high-resolution mapping of the genes, which is crucial for map-based cloning of the non-brittle rachis genes in barley. OUH × KNG is suitable for the high-resolution mapping, as in this combination it is possible to score brittle and non-brittle spike without ambiguity, however, in OUH × AZ, variation of brittleness was rather quantitative and some F_2 plants were not able to be categorized to either brittle or non-brittle type. A possible explanation is an involvement of modifier genes of AZ[7]. Map-based isolation of genes highly depends on the development of closely linked markers. Development of new markers of the *btr1* locus can be greatly facilitated by synteny between barley chromosome 3H and rice chromosome 1[3,12,13].

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