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Map construction of sequence-tagged sites (STSs) in barley (*Hordeum vulgare* L.)

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Abstract In order to identify sequence-tagged sites (STSs) appropriate for recombinant inbred lines (RILs) of barley cultivars 'Azumamugi' × 'Kanto Nakate Gold', a total of 43 STS primer pairs were generated on the basis of the terminal sequences of barley restriction fragment length polymorphism (RFLP) clones. Forty one of the 43 primer pairs amplified PCR products in Azumamugi, Kanto Nakate Gold, or both. Of these, two showed a length polymorphism and two showed the presence or absence of polymorphism between the parents. PCR products of the remaining 37 primers were digested with 46 restriction endonucleases, and polymorphisms were detected for 15 primers. A 383.6-cM linkage map of RILs of Azumamugi × Kanto Nakate Gold was constructed from the 19 polymorphic STS primer pairs (20 loci) developed in this study, 45 previously developed STS primer pairs (47 loci), and two morphological loci. Linkage analysis and analysis of wheat-barley chromosome addition lines showed that with three exceptions, the chromosome locations of the STS markers were identical with those of the RFLP markers.

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Introduction

Advances in molecular marker technology have led to the development of linkage maps and located genes controlling both quantitative and qualitative agronomic traits. The hybridization-based restriction fragment length polymorphism (RFLP) marker technique has been widely used to construct linkage maps in many cereals (e.g. barley, Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993: rice, McCouch et al. 1988; Saito et al. 1991: and wheat, Chao et al. 1989; Liu and Tsunewaki 1991). However, this technique is slow, costly, and labor-intensive when used in a practical breeding approach, such as marker-assisted selection in large progenies.

Recently, marker technology has been moving from hybridization-based RFLP markers to polymerase chain reaction (PCR)-based markers (Saiki et al. 1985) of sequence-tagged sites (STSs; Olson et al. 1989), random amplified polymorphic DNAs (RAPDs; Williams et al. 1990), or amplified fragment length polymorphisms (AFLPs; Zabeau and Vos 1993). In contrast to hybridization-based techniques, PCR-based markers can be economically and effortlessly handled for large numbers of samples.

An STS is a short unique genomic sequence that is amplified by using allele-specific oligonucleotides as PCR primers. The DNA clones mapped by RFLPs can be used to design PCR-based STS primers (Mullis and Faloona 1987). Standard STS landmarkers have been generated for rice (Inoue et al. 1994) and *Cryptomeria* (Tsumura et al. 1997) to screen yeast artificial chromosome (YAC), or bacterial artificial chromosome (BAC), libraries so as to convert a linkage map to a physical map (Olson et al. 1989), the first step in the map-based cloning of agronomically important genes.

In barley, RFLPs have been converted to STSs to mark specific genes for grain yield (Larson et al. 1996 a) and seed dormancy (Larson et al. 1996 b); for resistance to scald (Graner and Tekauz 1996), net blotch (Graner et al. 1996), spot blotch (Larson et al. 1996 b), and powdery mildew (Mohler and Jahoor 1996); and to construct a physical map by mapping STSs on micro-isolated translocation chromosomes (Künzel and Korzun 1996). In addition, many RFLP markers developed by the North American Barley Genome Mapping Project (NABGMP; Kleinhofs et al. 1993) have been converted to STSs (Blake et al. 1996). These STSs could be useful in developing allele-specific PCR-RFLPs at almost any locus on the barley genome in desirable populations without the need for sequencing or for designing primers.

We screened 117 STS primer pairs developed by Blake et al. (1996) and found that 40 pairs generated 52 polymorphic fragments (52 markers which detected 41 loci) between the parental varieties (Azumamugi and Kanto Nakate Gold) of the recombinant inbred lines (RILs; Sayed-Tabatabaei et al. 1999). However, more markers are needed to generate a linkage map, because of the presence of many unlinked markers in a preliminary map. To detect further polymorphic STS markers for the RILs of Azumamugi × Kanto Nakate Gold, we selected 43 previously mapped RFLP clones (MWG clones), distributed across all seven barley chromosomes in the doubled-haploid lines (DHLs) of an Igri × Franka cross (Graner et al. 1993), for sequencing and for designing STS primers. Here we report on the development of the STS landmarkers and on the construction of a linkage map using both STS markers derived from the MWG clones and an additional 60 markers in the RILs of Azumamugi × Kanto Nakate Gold in barley.

Materials and methods

Plant materials

A total of 99 RILs of Azumamugi × Kanto Nakate Gold, which have been developed to the F_7 generation by the single-seed descent method, were employed. Azumamugi is a six-rowed, Japanese winter-type cultivar of uzu or semi-brachytic growth. Kanto Nakate Gold is a two-rowed, Japanese spring-type cultivar of the non-uzu type.

The RILs were produced to detect and map the quantitative trait loci (QTLs) for shoot differentiation in barley (Komatsuda et al. 1989). The 52 STS markers which identified 41 loci, developed in the study of Blake et al. (1996), were used for segregation of the RILs (Sayed-Tabatabaei et al. 1999). We also used six STS markers (loci) linked to the *vrs1* locus (previous nomenclature *v*) on chromosome 2H (Komatsuda et al. 1996), and two morphological markers, *vrs1* (six-rowed/two-rowed) and *uzu* (previous nomenclature *uz*; uzu or semi-brachytic growth/non-uzu), on chromosomes 2H (Griffee 1925) and 3H (Takahashi and Yamamoto 1951), respectively. Genotypes for six-rowed/two-rowed and uzu/non-uzu plants were determined by the progeny analysis of F_8 lines.

DNA isolation

Template DNA used for PCR analysis was extracted from fresh leaf tissues of parents, F_1 plants, and a single plant of each F_7 line, according to the method of Dellaporta et al. (1985).

Source of clones and primer sequence

Forty three RFLP clones (MWG clones), which were distributed across all seven barley chromosomes (Graner et al. 1993) in the DHLs of an Igri × Franka cross, were selected for sequencing. Cycle sequencing reactions were analyzed on an ABI 373 DNA sequencer (PE Applied Biosystems, Chiba, Japan). Twenty five base-long PCR primers were designed using the software package Oligo 4.0-s (National Biosciences, Plymouth, Minn., USA), and then commercially synthesized (Bex Co., Tokyo, Japan).

PCR amplification

PCR amplification was done in volumes of 10 μl, each containing 0.25 units of *Taq* polymerase (Promega, Madison, Wis., USA); 0.3 μM of primers; 200 μM of dNTPs; 1.0, 1.5, 2.0, or 2.5 mM of $MgCl_2$; 1 PCR buffer [Promega 10: 100 mM Tris · HCl (pH 9.0), 500 mM of KCl, 1% Triton X-100]; and 20 ng of genomic DNA templates. Each reaction mixture was overlaid with mineral oil. The typical PCR amplification condition was 5 min at 95°C, followed by 30 cycles of 1-min DNA denaturation at 95°C, 2-min annealing at 54° or 62°C, and 2-min extension at 72°C, and a final 7-min incubation at 72°C. The PCR was done with a PTC-100™ thermal cycler (MJ Research, Inc., Watertown, Mass., USA). The PCR products were run on 1.8% agarose gels (agarose ME, Iwai Kagaku, Tokyo, Japan) in 0.5 TBE buffer [1 TBE: 89 mM Tris · borate, 2 mM EDTA (pH 8.0)] at 8 V/cm for 80 min and were stained with ethidium bromide. When the yield of PCR products was low, the annealing temperature was decreased, the extension times were increased to 4 min, or the PCR cycles were increased to 35 or 40 cycles. When multiple PCR products were observed, the annealing temperature was increased until a single band remained.

Detection of polymorphisms

The monomorphic PCR products between Azumamugi and Kanto Nakate Gold were subsequently digested with 12 different four- or five-base-cutter endonucleases (*Acc*II, *Alu*I, *Ava*II, *Hae*III, *Hha*I, *Hinf*I, *Msp*I, *Nci*I, *Nde*II, *Rsa*I, *ScrF*I, and *Taq*I; NIPPON GENE, Tokyo, Japan). One unit of each restriction endonuclease was added to 10 μl of PCR products. Digested fragments were electrophoresed in 1.8% agarose or 3% MetaPhor agarose (FMC, Rockland, Me., USA) gels. For the PCR products showing monomorphism, a total of 34 six-base-cutter endonucleases were further tested according to the method of Sayed-Tabatabaei et al. (1999).

PCR fragments showing length polymorphism without enzymatic digestion were scored separately (for presence or absence) and calculated as two different dominant markers, because it was not clear whether this type of polymorphism was caused by insertions or deletions at the same locus or at closely linked loci. Multiple polymorphic fragments in coupling phase were also scored separately and calculated as different markers at the same locus. Length polymorphisms revealed with enzymatic digestion were scored as co-dominant markers.

Map construction

Linkage analysis of the 99 RILs was performed for an F_2 model with Mapmaker 3.0 (Lander et al. 1987) because the RI model of

Mapmaker handled heterozygous types as missing data. Linkage groups were created with a log-likelihood (LOD) score of 6.0 and a recombination fraction of 0.4 because of a doubling of the LOD scores when RI data were analyzed in the F_2 model. The recombination values were converted from the F_2 model to an F_∞ model as described by Haldane and Waddington (1931), and then the recombination values for F_∞ were converted into map distances (cM) by Kosambi's mapping function (Kosambi 1944).

Chromosome assignment of the STS markers

Barley chromosome designations are labelled using the genome symbol 'H' in accordance with the internationally agreed recommendation for barley chromosome nomenclature (Linde-Laursen et al. 1997).

Linkage groups were assigned to barley chromosomes when the orders of STS markers were identical to those of the original RFLP markers (Graner et al. 1993; Kleinhofs et al. 1993). In addition, except for chromosome 1H which was not available, a set of wheat-barley (cv 'Chinese Spring' – cv 'Betzes') chromosome addition lines (CALs) (Islam and Shepherd 1981) were used for the chromosome assignment of STS markers developed in this study and for unlinked NABGMP STS markers. The monomorphic PCR products between Chinese Spring and Betzes were digested with the 12 four- or five-base-cutter endonucleases.

Results

PCR amplification

Table 1 shows the list of STS primers developed in this study and their PCR amplification conditions, the sizes of the amplified products, and their chromosome locations. Sequences of the STS primers have been published by Sayed-Tabatabaei et al. (1998). Forty one of 43 primer pairs amplified PCR products in this study. One STS (G2, MWG832) out of 32 derived from single-copy RFLP clones, and one STS (G3, MWG2310) out of nine derived from low-copy RFLP clones, generated multiple fragments in Azumamugi, Kanto Nakate Gold, or both. Of the 41 primer pairs, two showed length polymorphism (D2, MWG2307; G2, MWG832) and two showed the presence or absence of polymorphism (C1, MWG848; G3, MWG2310) between the parents of the RILs after PCR amplification. The remaining 37 showed a single monomorphic fragment in Azumamugi and Kanto Nakate Gold.

Detection of polymorphisms

In the 37 monomorphic primers, polymorphisms were detected in the PCR products of 14 of them after digestion with at least one of the 12 four- or five-base-cutter endonucleases.

The 650-bp PCR products derived from primer C5 (MWG549) were partially digested with *Hha*I, *Msp*I, *ScrF*I, and *Taq*I (Fig. 1a). When the concentration of endonucleases was increased from 1 unit per reaction mixture to 2.5 units, the same results were observed, indicating that this fragment may consist of multiple PCR products of the same size. Polymorphisms were detected in the PCR products after digestion with four restriction endonucleases, *Hha*I, *Msp*I, *Rsa*I, and *Nde*II (Fig. 1a). As described later, the polymorphic fragments digested with *Hha*I and *Msp*I were located on chromosomes 5H and 6H, respectively, by linkage analysis. Analysis of the wheat-barley CALs assigned the 650-bp fragments to chromosomes 3H, 5H, and 6H (Fig. 1b). These results confirmed that the identical 650-bp PCR products consisted of multiple fragments and could include at least two different markers, named MWG549 (*Hha*I) and MWG549 (*Msp*I). The digested fragments of *Rsa*I and *Nde*II were not tested further because there was no polymorphism in *Rsa*I and *Nde*II at 2.5 units.

The remaining 23 monomorphic PCR products of STSs were digested with 34 additional six-base-cutter endonucleases, and polymorphism was detected in one STS (A4; MWG2077). In all, we detected 19 STS primers (44%, 22 markers) that showed polymorphism between Azumamugi and Kanto Nakate Gold.

Segregation analysis

Of 22 polymorphic MWG STS markers, five – A1 (MWG938), A2 (MWG2261), A4 (MWG2077), E1 (MWG502), and E4 (MWG2230) – showed distorted segregations from the expected 48.7 : 1.6 : 48.7 ratio in the F_7 generation of 99 RILs of Azumamugi × Kanto Nakate Gold. No deviations from the 1 : 1 ratio for the homozygous class were found at these loci. Therefore, the five distorted segregations may be due to high frequencies of heterozygous types in the 99 RILs; that is, the expected frequency of the heterozygous type in the F_7 generation was 1.6, whereas the observed frequencies of the five markers were 5–7.

Map construction

The 22 STS markers and an additional 60 markers were tested for map construction (Fig. 2). Fifteen linkage groups with 66 markers were assigned to all barley chromosomes except 6H, because the orders of STS markers in each linkage group were identical to those of the original RFLP markers (Graner et al. 1993; Kleinhofs et al. 1993). Two linkage groups containing two STS markers (ABG57.4, ABG471) and nine STS markers [F1, MWG2218; MST108 (250, 260, 280 bp); BTA002 (275, 285 bp); ABG458.1; G3, MWG2310 (390 bp); C5, MWG549-*Msp*I], and representing 2.2 cM and 28.7 cM respectively, were identified, but there was a mismatch of the chromosome locations of the original RFLP clones within the linkage groups. These two linkage groups were tentatively assigned to

Table 1 List of STS primers and their PCR amplification conditions, sizes, and chromosome locations

Code no.	Primer name[a]	PCR conditions			$MgCl_2$ (mM)	Size[b] (bp)	Linkage group		
		Annealing temp. (°C)	Extension (min)	Cycle			STS		RFLP[c]
							RILs[d]	CALs[e]	
A1	MWG938	62	4	30	1.0	1500	1H (*Hae*III, *ScrF*I)[f]	1H	1H
A2	MWG2261	54	2	30	1.0	400	1H (*Taq*I)	1H	1H
A3	MWG913	58	2	30	1.5	500	Monomorphic[g]	1H (*Ava*II)	1H
A4	MWG2077	58	2	35	2.0	2000	1H (*Sty*I)	1H	1H
A5	cMWG733	42	4	30	1.5	1600	Monomorphic	1H	1H
A6	MWG912	58	2	30	1.5	580	Monomorphic	1H	1H
B1	cMWG682	62	2	30	2.5	500	Monomorphic	2H	2H
B2	MWG858	58	2	30	1.0	1350	Monomorphic	–[h]	2H
B3	MWG2054	58	2	30	2.5	720	Monomorphic	2H[i]	2H
B4	MWG2123	62	2	30	2.5	2000	Monomorphic	2H	2H
B5	cMWG694	62	4	30	1.5	1500	2H (*Hae*III, *Rsa*I)	2H	2H
B7	MWG532	62	2	30	1.0	870	Monomorphic	2H	2H
B8	MWG2076					ND			2H
C1	MWG848	62	2	30	1.0	ND 700	3H	3H	3H
C3	MWG975	50	2	35	1.0 (2.0)[j]	1500	Monomorphic	7H	3H
C4	MWG973	52	2	40	1.0 (2.0)	1500	– (*HinF*I, *Nde*II, *Taq*I)	3H	3H
C5	MWG549	58	2	30	2.0	650	5H, 6H (*Hha*I, *Msp*I)	3H, 5H, 6H[k]	3H
C6	MWG972	58	2	30	1.5 (2.0)	1350	Monomorphic	1H (*Taq*I)	3H
D1	MWG616					ND			4H
D2	MWG2307	54	4	30	1.0	1000 1250	4H	–	4H
D5	MWG058	54	2	30	1.0	1000	4H (*Ava*II)	4H	4H
D6	MWG2033	54	2	30	1.5	1000	– (*Taq*I)	4H	4H
D7	MWG634	62	2	30	1.5	800	Monomorphic	4H	4H
E1	MWG502	62	4	30	2.5	1050	5H (*Hha*I)	5H	5H
E2	cMWG770	62	2	30	1.5	580	Monomorphic	5H	5H
E3	MWG522	54	2	30	1.0 (2.0)	1300	Monomorphic	5H	5H
E4	MWG2230	62	2	30	2.0	320	5H (*Hae*III)	5H	5H
E5	MWG900	58	2	30	1.5	950	Monomorphic	5H	5H
E6	MWG2193	60	2	30	1.5	1900	5H (*Ava*II)	5H	5H
E7	MWG2249	62	2	30	1.5	500	5H (*Ava*II)	5H	5H
F1	MWG2218	62	2	30	1.5	420	– (*Hae*III, *Nde*II, *Taq*I)	6H	6H
F2	MWG2264	46	2	30	1.0 (2.0)	450	Monomorphic	6H (*Hha*I)	6H
F3	MWG2029	54	2	30	1.0 (2.0)	1000	Monomorphic	6H (*Hae*III)	6H
F4	cMWG669	48	2	35	1.5	1000	Monomorphic	-	6H
F5	MWG897	50	2	30	1.0 (2.0)	1100	Monomorphic	6H (*Hae*III)	6H
F7	MWG620	62	2	30	1.5	620	Monomorphic	6H (*Taq*I)	6H
G1	MWG530	62	2	30	2.0	600	Monomorphic	7H	7H
G2	MWG832	66	2	30	1.0 (2.0)	1580 + 1410 1600 + 1400	7H[l]	7H[m]	7H
G3	MWG2310	62	2	30	1.5	390 + 380 380	6H[n]	5H, 6H, 7H[o] (*Alu*I, *Nci*I)	7H
G4	cMWG704	62	2	30	2.5	400	7H (*Hha*I)	7H (*Hha*I)	7H
G5	MWG2031	54	2	30	1.5	1500	Monomorphic	7H	7H
G6	MWG528	60	2	30	1.0 (2.0)	1360	Monomorphic	3H	7H
G7	MWG2062	54	2	30	2.0	1050	7H (*Taq*I)	7H (*Ava*II, *Taq*I)	7H

[a] Sequences of the STS primers have been published by Sayed-Tabatabaei et al. (1998)
[b] Product sizes were measured by electrophoresis. PCR products showing polymorphisms without digestion indicate two lines, where upper = Azumamugi, lower = Kanto Nakate Gold. ND: not detected
[c] Graner et al. (1993)
[d] Chromosome locations were identified by linkage analysis using RILs of Azumamugi/Kanto Nakate Gold
[e] Chromosome locations were identified using wheat-barley (Chinese Spring-Betzes) CALs
[f] Polymorphic enzymes between Azumamugi and Kanto Nakate Gold (RILs) or between Betzes and Chinese Spring (CALs)
[g] Between Azumamugi and Kanto Nakate Gold
[h] Unknown
[i] A different fragment size (1100 bp) was assigned to chromosome 2H of Betzes
[j] Optimal $MgCl_2$ concentration for Betzes
[k] The PCR product of 650 bp located on chromosome 6H in Betzes consisted of at least two fragments (see Fig. 1c)
[l] The 1600-and 1580-bp fragments were located on chromosome 7H by linkage analysis. The 1410-and 1400-bp fragments were not assigned due to the similarity of the fragment sizes
[m] The 1500-bp fragnent, typical Betzes fragment, and the 1410-bp fragment were assigned to chromosome 7H of Betzes
[n] The 390-bp fragment was located on chromosome 6H by linkage analysis
[o] The 380-bp fragments were located on chromosomes 5H, 6H, and 7H, whereas the location of the 390-bp fragment was unknown due to no amplification in Betzes (Fig. 3)

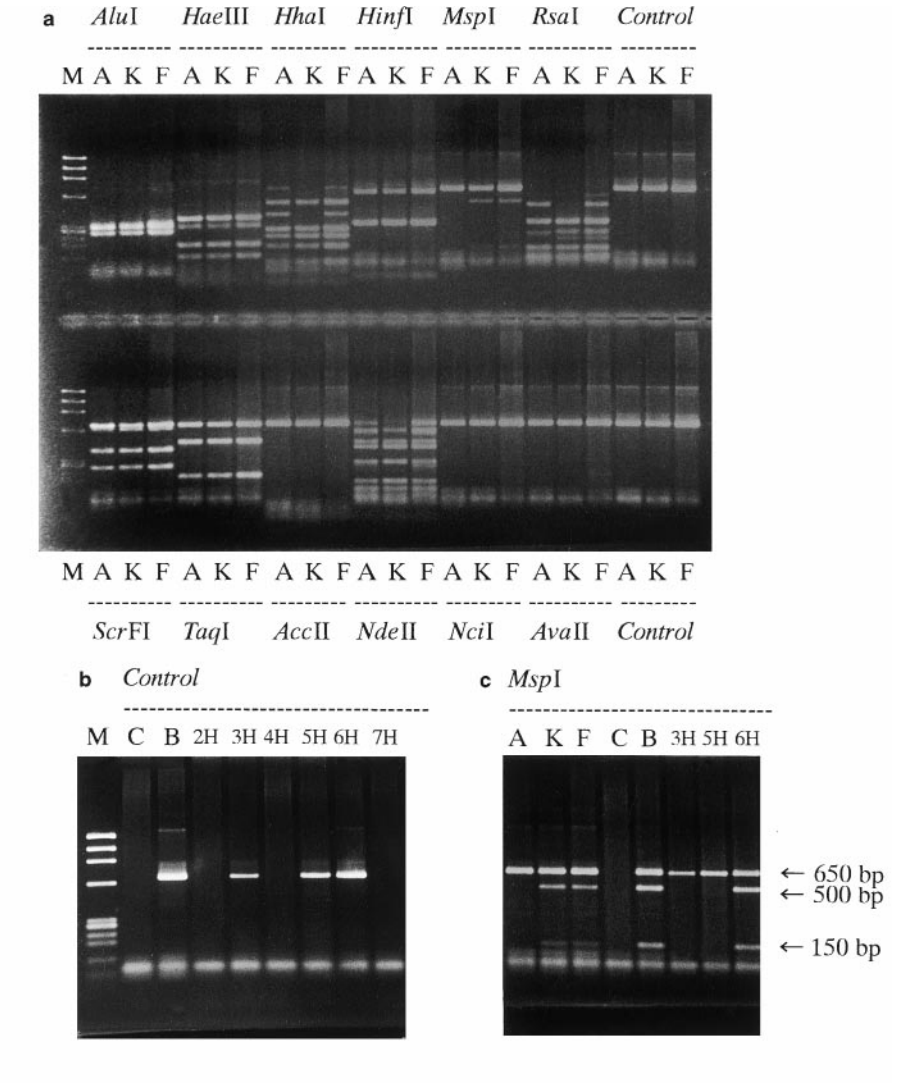

Fig. 1a–c PCR amplification products and their RFLP patterns using primer pair C5 (MWG549). In panel **a**, PCR products of Azumamugi, Kanto Nakate Gold, and their F_1 progeny were digested with *Alu*I, *Hae*III, *Hha*I, *Hinf*I, *Msp*I, *Rsa*I, *ScrF*I, *Taq*I, *Acc*II, *Nde*II, *Nci*I, and *Ava*II. Panels **b** and (**c**) are PCR products of wheat-barley CALs before (**b**) and after **c** digestion with *Msp*I. *Controls* are PCR products without digestion. *Lanes A*, *K*, *and F* are Azumamugi, Kanto Nakate Gold, and their F_1 progeny, respectively. *Lanes C and B* are Chinese Spring wheat and Betzes barley. *Lanes 2H to 7H* are barley 2H, 3H, 4H, 5H, 6H, and 7H CALs, respectively. *Lanes M* DNA size markers (ØX174/*Hae*III digest)

chromosomes 3H (from the result of ABG471) and 6H (F1, MWG2218 and ABG458.1), respectively, using wheat-barley (cv 'Chinese Spring' – cv 'Betzes') CALs. The remaining five markers were unlinked. Three (C4, MWG973; D6, MWG2033; ABG711) were tentatively assigned to chromosomes 3H, 4H, and 6H, respectively, by using CALs, but two [ABG075 and BTA002 (1500 bp)] could not be assigned to any chromosome owing to the lack of amplification in both Chinese Spring and Betzes.

For the PCR products showing length polymorphism between Azumamugi and Kanto Nakate Gold (D2, MWG2307; G2, MWG832), each fragment was scored separately as a dominant marker, and allelic relationships were confirmed by linkage analysis (Fig. 2). The presence of recombination values of 1.1 cM (D2, MWG2307; 1000 bp vs 1250 bp) and 0.5 cM (G2, MWG832; 1600 bp vs 1580 bp) may be due not to a close link between conflicting fragments but to scoring of the heterozygous type as recombinant because of no null type in the RILs. Therefore, although recombination values were observed between PCR products showing length polymorphism, such markers are considered to be co-dominant at the same locus. This criterion applies also to NABGMP primers (Sayed-Tabatabaei et al. 1999).

The linkage map constructed with the 69 loci (identified by 82 markers) consists of 20 STS loci (22 markers) derived from the MWG clones (this study), 41 STS loci (52 markers) of the NABGMP clones (Blake et al. 1996), six STS loci (six markers) linked with the *vrs1* locus on chromosome 2H (Komatsuda et al. 1996), and two morphological loci, *vrs1* and *uzu* (two markers) (Fig. 2). This map covers 383.6 cM, representing 31% of the length of the total barley genome (1250 cM) estimated by Kleinhofs et al. (1993).

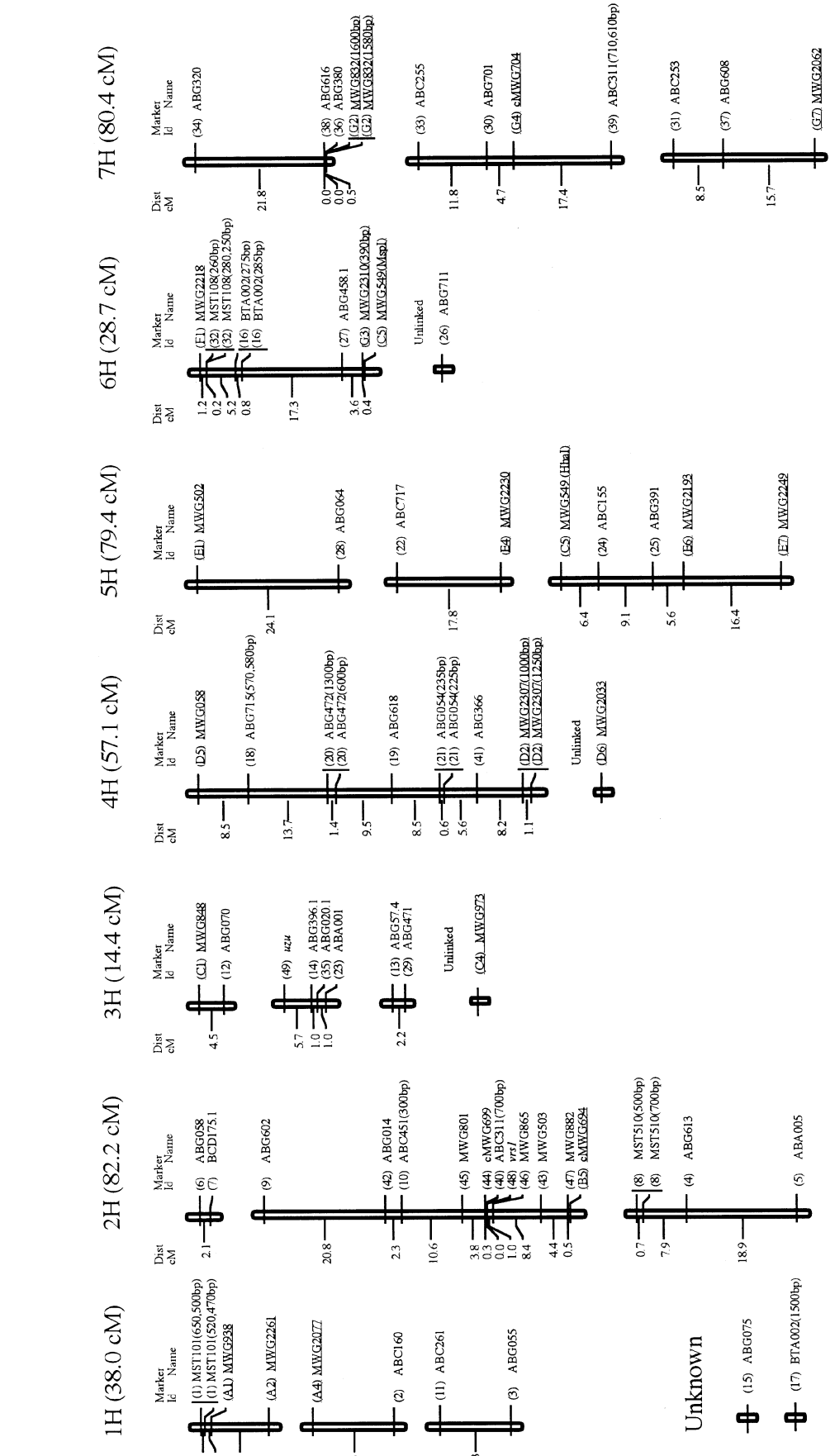

Fig. 2 Linkage map of the barley genome with 67 STS loci (80 markers) and two morphological loci. The chromosome locations of two STS loci are unknown. Chromosomes are oriented with the short arm on top. The order of the three linkage groups on chromosome 3H is unknown. *Underlines* indicate STS markers developed in this study. STS markers combined by *vertical lines* are considered to be one locus

Chromosome assignment of STS markers

Chromosome locations of the 41 STS markers derived from the MWG clones were investigated using wheat-barley CALs. The PCR products of 29 primers (33 loci) could be assigned to chromosome locations, but the remaining 12 could not initially be assigned because of the lack of amplification in Betzes barley under the PCR conditions suitable for Azumamugi and Kanto Nakate Gold. Nine out of these 12 were later assigned to chromosome locations when the $MgCl_2$ concentration was increased from 1.0 or 1.5 mM to 2.0 mM.

Comparison of chromosome locations between STS and RFLP markers

The chromosome locations of the STS markers estimated by linkage analysis in the Azumamugi × Kanto Nakate Gold mapping population were identical to those estimated by using wheat-barley CALs (Table 1). The sizes of the PCR products also corresponded between Azumamugi or Kanto Nakate Gold and Betzes except for B3 (MWG2054). In addition, the chromosome locations of the STS markers estimated by linkage analysis or by using wheat-barley CALs were identical to those of RFLP markers in the Igri × Franka RFLP map (Graner et al. 1993), with exceptions as follows: C5 (MWG549) and G3 (MWG2310), being derived from the low-copy RFLP probes and generating multiple DNA fragments by PCR; and C3 (MWG975), C6 (MWG972), and G6 (MWG528), being derived from the single-copy probes and generating a single DNA fragment by PCR.

The PCR products of C5 (MWG549) at 650-bp were mapped on two different chromosomes, 5H and 6H, using Azumamugi × Kanto Nakate Gold mapping populations (Fig. 2). The 650 bp fragments consisting of multiple fragments were assigned to chromosomes 3H, 5H, and 6H of Betzes barley using wheat-barley CALs (Fig. 1 b). Further, there was a partial digestion of 650-bp products in the barley 6H CAL using *Msp*I (Fig. 1 c), indicating that the 650-bp products on chromosome 6H of Betzes consisted of at least two fragments of the same size.

The STS marker of C5 (MWG549) assigned to chromosome 3H of Betzes (Fig. 1 b) may be allelic to the RFLP marker of MWG549 on chromosome 3H in Igri × Franka (Graner et al. 1993). We found disagreement on chromosome location for the PCR products of C5 (MWG549) between Azumamugi × Kanto Nakate Gold mapping populations (one locus on each of 5H and 6H) and Chinese Spring – Betzes CALs (one locus on each of 3H and 5H, and at least two loci on 6H). This inconsistency can be explained by the fact that the Betzes' PCR products at the locus on chromosome 3H and at one of the loci on 6H did not show polymorphism between Azumamugi and Kanto Nakate Gold.

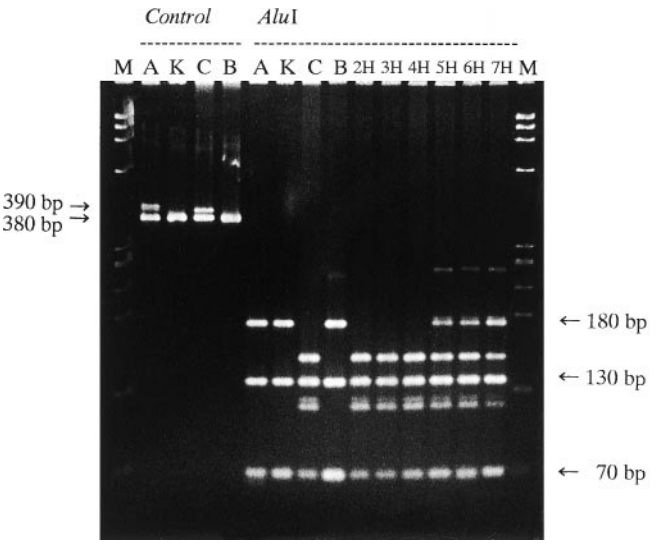

Fig. 3 PCR amplification products and their RFLP patterns using primer pair G3 (MWG2310). *Control* and *Alu*I are PCR products before and after digestion with *Alu*I, respectively. *Lanes A*, *K*, *C*, *and B* are Azumamugi, Kanto Nakate Gold, Chinese Spring wheat, and Betzes barley, respectively. *Lanes* 2*H to* 7*H* are barley 2H, 3H, 4H, 5H, 6H, and 7H CALs, respectively. *Lanes M* DNA size markers (ØX174/*Hae*III digest)

For the primer G3 (MWG2310), the PCR products were detected at 390 bp and 380 bp in Azumamugi, and at 380 bp in Kanto Nakate Gold (Table 1, Fig. 3). The 390-bp fragment was mapped on chromosome 6H by linkage analysis, but the location of this fragment could not be identified using wheat-barley CALs because of the lack of amplification in Betzes. Analysis of wheat-barley CALs assigned the 380-bp fragments to chromosomes 5H, 6H, and 7H of Betzes (Fig. 3). The STS marker on chromosome 7H in Betzes may be allelic to the RFLP marker on chromosome 7H in Igri × Franka (Graner et al. 1993).

Chromosome locations of the STS markers of C3 (MWG975), C6 (MWG972), and G6 (MWG528), estimated by using wheat-barley CALs, which could not be mapped by linkage analysis because of monomorphism between Azumamugi and Kanto Nakate Gold, were not identical to those of RFLP markers in the Igri × Franka map. This may be due to different genetic backgrounds between the Betzes and Igri × Franka mapping populations. These disagreements were also reported in STSs in Betzes and in RFLPs in Steptoe × Morex mapping populations (Blake et al. 1996).

Discussion

Linkage maps containing a large number of RFLP markers throughout the barley genome have been

constructed by many workers (e.g. Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Hayes 1994). However, no STS-marker-based map has been reported for the entire barley genome. In this study, we generated an STS-based linkage map in the RILs of a Japanese barley cross, Azumamugi × Kanto Nakate Gold.

STS markers are useful for tagging barley chromosome segments during their introgression into wheat (Blake et al. 1996). It should be noted that the chromosome locations of the STS markers identified by linkage analysis could differ from those identified by using wheat-barley CALs. In the present study, the chromosome locations of the STS markers were estimated by two different methods, linkage analysis and use of wheat-barley CALs complemented with restriction analysis. STS markers with such information are valuable for reliable wheat-barley introgression.

Integration of several linkage maps is necessary to detect the common functional QTLs controlling agronomically important traits across different genetic backgrounds. Easy to use STS markers will allow different linkage maps to be integrated effortlessly. For example, QTLs for shoot differentiation in barley have been mapped on the long arm of chromosome 2H in three different mapping populations: Azumamugi × Kanto Nakate Gold (Komatsuda et al. 1993, 1995), Steptoe × Morex (Mano et al. 1996), and Harrington × TR306 (Takahashi et al. 1997). The QTL for shoot differentiation in wheat was located on the same homoeologous group, chromosome 2B (Ben Amer et al. 1997). Integration of these Triticeae maps using common STSs, mapped on the long arm of chromosome 2H (Fig. 2), will help in the detection of homoeoallelic QTLs for this trait.

In general, the degree of polymorphism depends on the genetic similarity between parents. In the barley RFLP clones, for example, 76% of more than 450 genomic and cDNA clones tested were polymorphic in an interspecific cross (*H. vulgare* × *H. spontaneum*), but only 28% were polymorphic in an intraspecific one using five restriction endonucleases (Graner et al. 1991). Also within intraspecific crosses, 44% (among Japanese two-rowed cultivars) and 77% (between Japanese two-rowed and Chinese six-rowed cultivars) of 216 clones tested showed polymorphisms using six restriction endonucleases (Miyazaki et al. 1996). The proportions of polymorphic STS primer pairs derived from barley RFLP clones were 44% from the MWG clones (this study) and 38% from the NABGMP clones (Sayed-Tabatabaei et al. 1999) in our mapping populations, comparable to the 44% observed among Japanese two-rowed cultivars. The degree of polymorphisms, 44%, caused some gaps in the linkage map, although a total of 43 STSs, evenly distributed across the barley genome, were applied for map construction of the RILs. Recently, AFLP markers have been developed (Zabeau and Vos 1993; Vos et al. 1995). This technique can detect more polymorphic bands per assay unit than RFLP, RAPD, or simple sequence repeat (SSR) markers (Russell et al. 1997), and has already been developed for genome mapping in barley (e.g. Becker et al. 1995; Waugh et al. 1997; Qi et al. 1998). We have begun to apply AFLP markers to our mapping population. Conversion from AFLPs mapped in the gaps of the linkage map to STSs will help the construction of a complete STS-based map.

Burr and Burr (1991) reported that the levels of heterozygosity in two maize RILs (F_8 generation) were found to be 1.6% and 2.7%, which were 2.0–3.5 times the expected value (0.8%). For the RILs of an F_7 interspecific cross in tomato, a high level of heterozygosity (15%) was observed, which could be the result of unintentional selection against plants with low fertility (Paran et al. 1995). In our study, although there was no fertility barrier between the parents, the average frequencies of heterozygous types of STS markers of MWG (4.6%, this study) and NABGMP (3.6%, Sayed-Tabatabaei et al. 1999) were 2.5–3.0-times the expected 1.6%. One reason may be that the relatively high adaptability of heterozygous types to stresses affected the frequency of genotypes. During the development of the RILs, the first plant in a row (line) was routinely descended to the next generation to avoid selection bias, but first plants were missing from some lines (T. Komatsuda, unpublished).

As well as the STS primer pairs developed by Blake et al. (1996), the 43 STS primers generated in this study will be useful as landmark STSs for different barley populations because they are distributed across all seven barley chromosomes, and their optimal PCR conditions are defined (Table 1). These primers are available from T. Komatsuda upon request.

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