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Genetic linkage map of ISSR and RAPD markers in Einkorn wheat in relation to that of RFLP markers

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Abstract The potential of PCR-based markers for construction of a genetic linkage map in Einkorn wheat was investigated. From a comparison of polymorphisms between two Einkorn wheats, *Triticum monococcum* (Mn) and *T. boeoticum* (Bt), we obtained 49 polymorphic bands produced by 33 primers for inter-simple sequence repeat (ISSR) and 36 polymorphic bands shown by 25 combinations of random amplified polymorphic DNA (RAPD) primers for mapping in 66 individuals in the F_2 population. Although 44 ISSR fragments and 29 RAPD fragments statistically showed a 3:1 segregation ratio in the F_2 population, only 9 markers each of the ISSR and RAPD bands were able to be mapped on the RFLP linkage map of Einkorn wheat. ISSR markers were distributed throughout the chromosomes. The mapped positions of the ISSR markers seemed to be similar to those obtained by the RFLP markers. On the other hand, 4 of the 9 RAPD markers could map the RFLP marker-poor region on the short arm of $3A^m$, suggesting a potential to map novel regions containing repetitive sequences. Comparisons of the genetic linkage map of Einkorn wheat to the linkage map and cytological map of common wheat revealed that the marker orders between the two maps of Einkorn wheat and common wheat coincided except for 4A, which harbors chromosome rearrangements specific for polyploid wheats, indicating a conservatism between the two genomes. Recombinations in Einkorn wheat chromosomes took place more frequently around the centromere and less at the distal part of chromosomes in comparison to those in common wheat. Nevertheless, recombinations even in Einkorn wheat chromosomes were strongly suppressed around the centromere. In fact, the markers located within 1 cM of the centromere were located almost in the central part of the chromosome arm.

Key words Microsatellite · RAPD · PCR · Linkage map · Wheats

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Introduction

In recent years it has become well recognized that PCR (polymerase chain reaction) -based DNA markers are powerful tools for genetic analysis as well as for breeding programs because of their simplicity and ease of handling. The RAPD (randomly amplified polymorphic DNA) marker (Williams et al. 1990) has been proposed as a new source of genetic markers for mapping studies (Martin et al. 1991; Monna et al. 1994). This system overcomes many technical limitations recognized in restriction fragment length polymorphism (RFLP) analysis. For example, RFLP markers can not efficiently map repetitive sequences because most RFLP probes are derived from single-copy or low-copy sequences, constituting gene-rich regions (Gill et al. 1996a, b); but RAPD markers are possibly mapped in these regions. Actually, RAPD markers have been able to map novel chromosome regions (Monna et al. 1994).

Simple sequence repeats (SSRs), also called microsatellites such as $(GA)_n$ or $(CT)_n$, are interspersed in plant genomes (Wu and Tanksley 1993). A high level of polymorphism with SSRs has been observed in plants (Akkaya et al. 1992; Morgante and Olivieri 1993; Plaschke et al. 1995), and microsatellite markers are expected to become additional tools for constructing

linkage maps in plants. However, the time and costs necessary to identify the sequence containing SSRs and to design flanking primers have prevented the broad use of microsatellites in plants (Beckmann and Soller 1990; Roeder et al. 1995).

ISSRs (inter-simple sequence repeat polymorphisms) are detected by the use of repeat-anchored primers that amplify between SSRs. ISSR markers are also useful for detecting genetic polymorphisms among accessions (Zietkiewicz et al. 1994; Tsumura et al. 1996; Nagaoka and Ogihara 1997). ISSR analyses are easier than SSR analyses in some steps, as there is no need of any prior knowledge of the target sequences of the SSR (Tsumura et al. 1996). But to our knowledge, ISSR markers have not yet been mapped on plant chromosomes.

In wheats, which have a complex genome containing a large number of repetitive DNAs, RFLP markers have been applied for the construction of genetic maps (Chao et al. 1989; Kam-Morgan and Gill 1989; Liu et al. 1991; Dubcovsky et al. 1996). However, linkage maps of wheat chromosomes prepared with RAPD or ISSR markers have not been reported, probably due to their great genome size (Bennet and Smith 1976). We report herein mapping data on the ISSR and RAPD markers located on the chromosomes of Einkorn wheat and disclose the nature and applicability of these PCR-based markers for mapping studies of wheat chromosomes.

Materials and methods

Plant materials

Cultivated Einkorn wheat, *Triticum monococcum* Early mutant, abbreviated as Mn (AA as genome formula and KT3-5 as collection no. of KIBR), and its wild relative, *T. boeoticum* ssp. *boeoticum*, abbreviated as Bt (AA and KT1-1), were used in the present investigation. Sixty-six individuals in the F_2 population derived from a cross between these parents were self-pollinated so as to produce F_3 seeds. Total DNA was extracted from these 66 individual lines, each containing more than 50 plants.

Genetic mapping of RFLP markers

Wheat total DNAs were extracted from green seedlings (ca. 14 days old) according to the CTAB method (Murray and Thompson 1980). To check polymorphisms between the parental lines, we digested 10 μg of total DNAs of Mn and Bt with the following four restriction endonucleases, *Dra*I, *Eco*RI, *Eco*RV and *Hin*dIII. When no polymorphisms between the parental lines were detected, Four additional restriction enzymes, *Bam*HI, *Bgl*II, *Xba*I and *Xho*I, were tested. Methods for southern hybridization were as previously described (Ogihara et al. 1994). Genomic and cDNA clones of wheat, goat grass, oat, and barley developed at the John Innes Centre, UK (PSR clones), Kansas State University, USA (KSU clones), Cornell University (CDO clones), and North American Barley Genome Project (ABG clones) were used as the probes for Southern hybridization. The genetic map was constructed with the aid of the computer program MAPMAKER/EXP 3.0 (Lander et al. 1987) using the Kosambi function (Kosambi 1944).

PCR amplification of ISSR

Of the 100 SSR primers obtained from the University of British Columbia Biotechnology Laboratory (UBCBL) primer set no. 9, 33 gave distinct polymorphic bands between Mn and Bt (Nagaoka and Ogihara 1997). These Primers were used to study segregation patterns of the F_2 population. Amplifications were performed in 10 m*M* TRIS-HCl (pH 9.0), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.1 m*M* dNTPs. 0.1% Triton x-100, 2% formamide, 200 n*M* primer, 1 unit of *Taq* polymerase (Promega Co), and 5 ng of wheat total DNA, in a 25-μl reaction mixture. The PCR cycles were as follows: initial denaturation was at 94°C, for 7 min, followed by 45 cycles of 30 s at 94°C, 45 s at 52°C, 2 min at 72°C, and a final extension at 72°C, for 7 min.

RAPD amplification

Two hundred combinations of two 10-mer oligonucleotide primers were randomly selected from UBCBL primer sets nos. 1 and 2 to check RAPD in wheat examined for the amplifications of RAPD sequences (Nagaoka and Ogihara 1997). Twenty-five combinations of primers that showed clear polymorphic bands between Mn and Bt were used to study linkage analysis. PCR amplifications were performed in 10 m*M* TRIS-HCl (pH 9.0), 50 m*M* KCl, 2.5 m*M* $MgCl_2$, 0.1 m*M* dNTPs, 0.1% Triton X-100, 200 n*M* of each primer, one unit of *Taq* polymerase (Promega Co), and 50 ng of total DNA per 25-μl reaction. Initial denaturation was at 94°C for 3 min, followed by 45 cycles of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C, and a final 7-min extension at 72°C. PCR reactions were carried out in a PTC-100 thermal cycler (MJ Research). PCR products were electrophoresed on 2% agarose gels and detected by staining with ethidium bromide.

Linkage analysis of ISSR and RAPD markers

The ISSR and RAPD primers showing polymorphisms between Mn and Bt were used for the checking of segregation patterns in the F_2 population. After the PCR reactions, the genotypes of each F_2 line were scored as the presence or absence of amplified fragments. Unstable or weak bands were omitted from the scoring. The observed segregation ratio was tested by Chi-square analysis (3 : 1 expectation; $\chi^2 = 3.841$; $P < 0.005$). These polymorphic ISSR and RAPD data were used for the construction of the linkage map of Einkorn wheat.

Results

RFLP markers

Parental lines, *T. monococcum* Early mutant (Mn) and *T. boeoticum* ssp. *boeoticum* (Bt) were highly polymorphic. Out of 67 DNA clones screened for RFLPs, 63 (94%) revealed polymorphisms between them for at least one of the eight restriction endonucleases. Thus, 63 RFLP markers, in which DNA markers located around the distal end of each chromosome were included, were mapped in this population. Four markers, namely PSR129, PSR169, PSR303, and PSR578, showed a segregation distortion that was significantly different from the 3 : 1 ratio at the 1% level. Marker orders were the same as that observed in diploid wheat (Dubcovsky et al. 1996) and similar to that

of common wheat (Gale et al. 1995) with few exceptions (see below).

ISSR loci

Out of 100 primers tested, 33 gave clear polymorphic bands between *T. monococcum* (Mn) and *T. boeoticum* (Bt) (Nagaoka and Ogihara 1997). Forty-nine ISSR fragments produced from 33 primers were scored for segregation. Typical ISSR segregating band patterns of the F_2 population obtained between Mn and Bt are presented in Fig. 1A. Fitness of the segregation pattern to the 3:1 ratio was statistically analyzed for these 49 ISSR fragments: 44 (90%) showed the normal 3:1 segregation pattern, whereas the remaining 5 fragments (10%) revealed significant deviation from the normal ratio. Only 9 (19%) of the 49 ISSR markers were able to be mapped on the RFLP linkage map of Einkorn wheat, as depicted in Fig. 2. These 9 ISSR loci were distributed over five chromosomes. Three ISSR loci were located on chromosome $1A^m$ (IS835B, IS848C, and IS848B), 1 on $2A^m$ (IS849B), 2 on $3A^m$ (IS824C and IS826), 1 on $4A^m$ (IS811C), and 2 on chromosome $7A^m$ (IS855 and IS856). The other fragments were unsuccessfully assigned a chromosome location. Consequently, 9 ISSR primers gave 9 ISSR loci (Table 1). All 9 ISSR primers contained dinucleotide repeats, but they did not contain tri-, tetra-, or penta-nucleotide motifs. Out of the 9 ISSR primers, 4 had the $(AC)_8$ motif and the remaining 5 harbored $(AG)_8$, $(GA)_8$, $(CA)_8$, $(GT)_8$ and $(TC)_8$ motifs, respectively.

RAPD loci

Two hundred combinations of a pair of 10-mer primers were tested in the wheat species. Out of 88 polymorphic bands, 36 fragments produced from 25 RAPD primer combinations were checked for segregation in the F_2 population. Typical segregating band patterns are shown in Fig. 1B. The χ^2-test was carried out to check the segregation patterns for fitness of the 3:1 ratio. Twenty-nine fragments were fitted to the 3:1 segregation ratio, but 7 (19%) revealed significant deviation from the normal segregation pattern. Of 36 RAPD markers 9 (25%) could be mapped on the RFLP linkage map of Einkorn wheat (Fig. 2). The 9 markers were located in three chromosomes: 1 locus (R39/40) was mapped on the $2A^m$ chromosome, 4 (R141/191C, R50/100A, R75/76, and R73/74) on the $3A^m$, and 4 (R117/118, R189/190, R13/63A, and R40/90A) on the $6A^m$. The nucleotide sequences of these primers are presented in Table 2. There was a tendency for the RAPD markers to be clustered in certain regions of the chromosomes, thereby showing deviation from the random distribution (Fig. 2).

Einkorn wheat map

Eighteen PCR markers, 9 ISSR markers and 9 RAPD markers, were mapped onto the RFLP linkage map of Einkorn wheat. A total of 81 DNA markers were constructed into seven linkage groups. The map distances

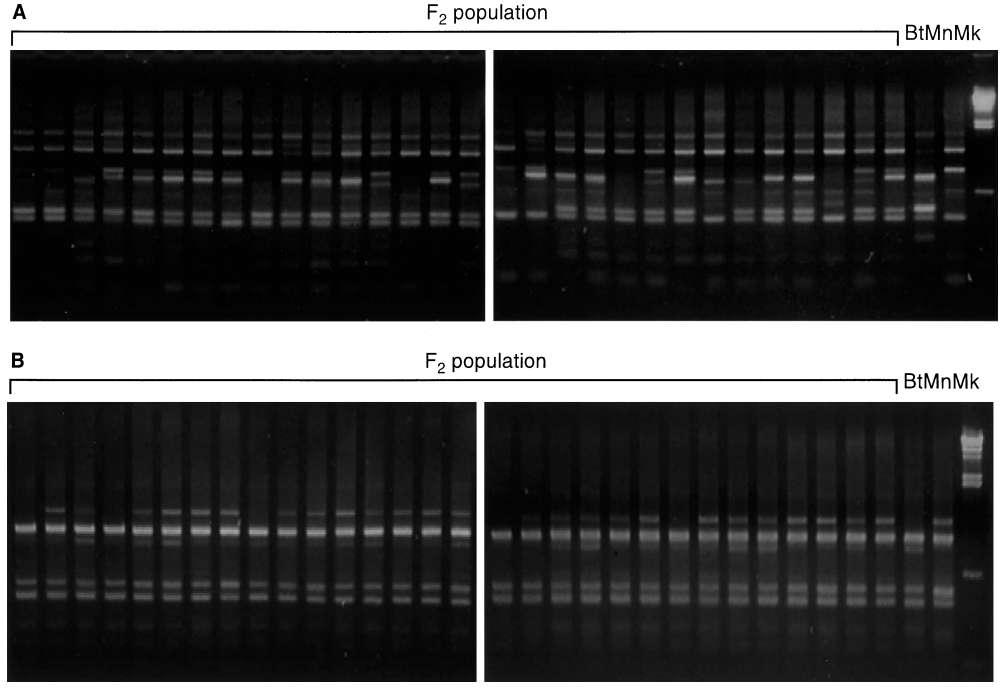

Fig. 1A, B Segregation patterns of ISSR (**A**) and RAPD (**B**) markers in the F_2 population obtained from the cross between Mn and Bt. Bands were generated by PCR primed with UBC811 (**A**), and the combination of UBC136 and 186 (**B**). *Mk* molecular size marker, *Mn* and *Bt* parental patterns, respectively

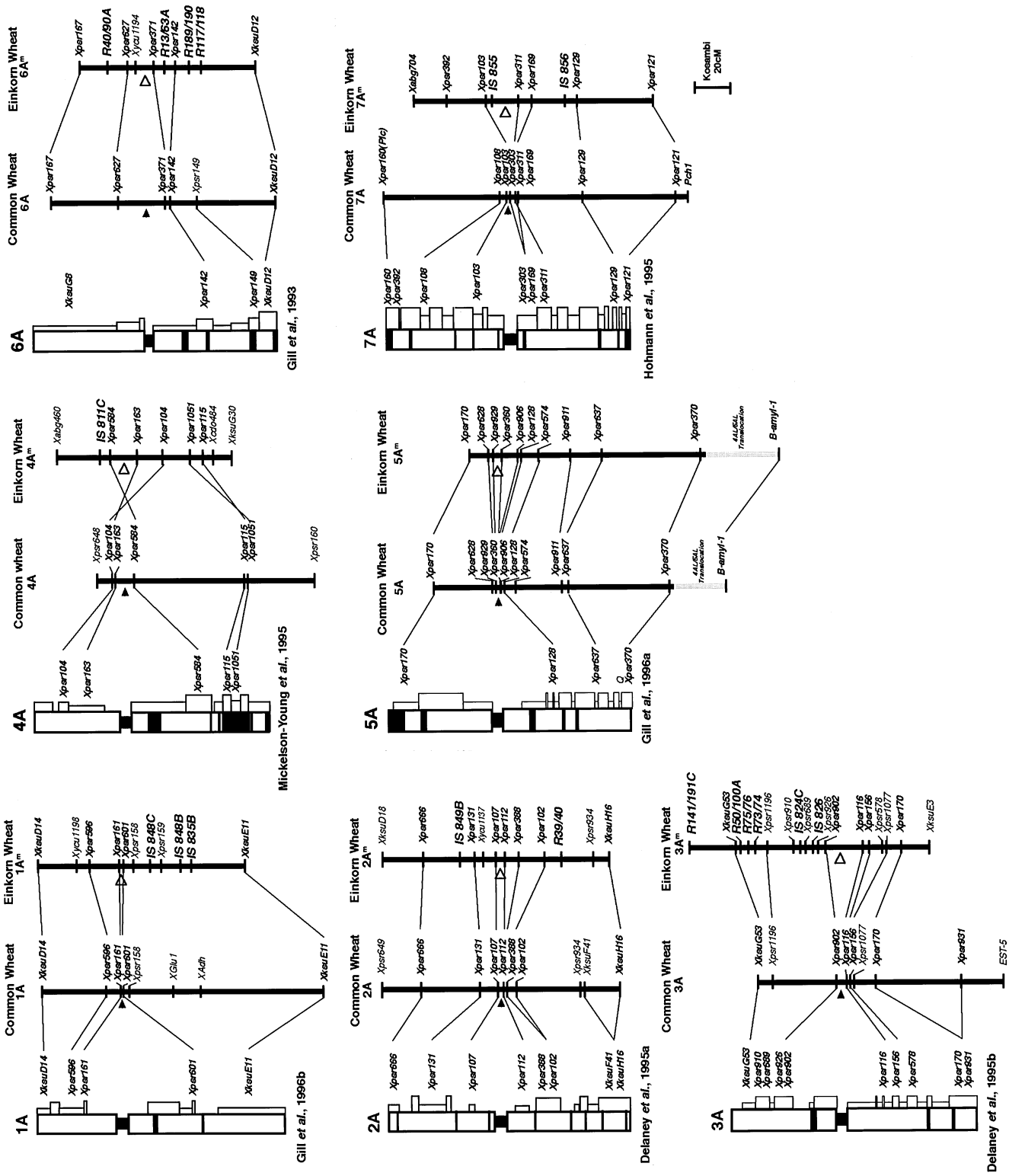

Fig. 2 Comparison of the genetic linkage map of Einkorn wheat (*right*) constructed by RFLP, ISSR (IS- -), and RAPD (R- -) markers to the linkage map (*center*) and cytological map (*left*) of common wheat. ISSR and RAPD markers are in *bold print*. Corresponding loci are connected by *lines*. Idiograms of the karyotype of each chromosome are cited from references listed *below* the chromosome. *Filled arrowheads* indicate the position of the centromere (Gale et al. 1995), and the putative position of the centromere in Einkorn wheat chromosomes is shown by an *open triangle*. The translocated segment in 5AL is *shaded*

Table 1 ISSR primers that gave distinguishable bands between Mn and Bt

Primer no.[a]	Sequence (5′ → 3′)[b]
811	GAGAGAGAGAGAGAGAC
824	TCTCTCTCTCTCTCTCG
826	ACACACACACACACACC
827	ACACACACACACACACG
835	AGAGAGAGAGAGAGAGYC
848	CACACACACACACACARG
849	GTGTGTGTGTGTGTGTYA
855	ACACACACACACACACYT
856	ACACACACACACACACYA

[a] Primers were obtained from the University of British Columbia set no. 9
[b] Y, Pyrimidine; R, purine

Table 2 Combinations of RAPD primers that gave distinguishable bands between Mn and Bt

Primer combinations[a]	Sequence (5′ → 3′)
39/40	TTAACCGGGC/TTACCTGGGC
73/74	GGGCACGCGA/GAGCACCTGA
75/76	GAGGTCCAGA/GAGCACCAGT
13/63	CCTGGGTGGA/TTCCCCGCCC
40/90	TTACCTGGGC/GGGGGTTAGG
50/100	TTCCCCGCGC/ATCGGGTCCG
117/118	TTAGCGGTCT/CCCGTTTTGT
189/190	TGCTAGCCTC/AGAATCCGCC
141/191	ATCCTGTTCG/CGATGGCTTT

[a] A pair of primers obtained from the University of British Columbia set nos. 1 and 2 were put in one tube for the PCR reaction

of chromosome $1A^m$–$7A^m$ were 118.3, 120.6, 148.6, 105.5, 142.7, 98.8, and 127.7 cM, respectively. These markers covered 862.2 cM of Einkorn wheat chromosomes with an average interval of 10.6 cM. The total length of the linkage map was similar to that of diploid wheat, 1067 cM (Dubcovsky et al. 1996) and that of common wheat, 1047.6 cM (Gale et al. 1995), suggesting that the present map covers almost all of the chromosome regions of diploid wheat. Sixty-three RFLP markers gave 818.8 cM of the genetic map, and PCR markers contributed the additional 43.4 cM (Table 3).

Comparison of Einkorn wheat map to common wheat map

In order to grasp the nature of the genetic map of Einkorn wheat, the genetic linkage map of Einkorn wheat was compared with the linkage map as well as with the cytological map of common wheat. Characteristic features of each chromosome are described below.

Chromosome 1A^m

The genetic linkage map of the $1A^m$ chromosome of Einkorn wheat was compared with that of common wheat (Gale et al. 1995). The marker order of $1A^m$ was colinear to that of common wheat 1A, showing the conservation of gene synteny. The map distances of corresponding markers were not distorted between the Einkorn and common wheats, although the distance of the proximal region in Einkorn $1A^m$ tended to be longer and that of the distal region shorter than those regions of common wheat 1A. Two ISSR primers gave 3 ISSR loci on the $1A^m$ chromosome. The primers having (CA) repeats gave 2 loci (IS848B and IS848C), and the primer with (AG) made 1 locus (IS835B), as shown in Fig. 2. The region mapped by ISSR markers seemed not to be specific since the 3 ISSR markers did not contribute to a drastic expansion of the map distance (Table 3). A comparison of marker positions in the genetic map and cytological map clearly indicates the strong suppression of recombination around the centromere; the map distance of *Xpsr161* and *Xpsr601* was 0.4 cM and 1.4 cM, respectively. Whereas, both markers took positions at the central part of both chromosome arms in the cytological map (Fig. 2).

Chromosome 2A^m

Although the marker orders of linkage maps between Einkorn and common wheats were colinear, a significant distortion of map distances was found between the two maps: map distances of Einkorn wheat around the centromere were larger than those of common wheat, whereas marker distances in the distal part of the chromosome were shortened. The markers closest to the centromere (2 cM and 0.6 cM, respectively) were cytologically located in the central part of each chromosome arm (Fig. 2). An ISSR marker (IS849B) and a RAPD marker (R39/40) were mapped between the 2 adjacent RFLP markers. They contributed 5.4 cM to the elongation of the linkage map (Table 3).

Chromosome 3A^m

This chromosome also showed expansion of the genetic distance around the centromere and a decrease in it at the distal region of the chromosome, in comparison to common wheat. Two ISSR markers (IS824C and IS826) and 4 RAPD markers (R141/191C, R50/100A, R75/76, R73/74) were mapped on the short arm of $3A^m$. Although the 2 ISSR markers were located close to the adjacent RFLP markers and did not contribute to the expansion of the linkage map, the 4 RAPD markers, 3 of which were clustered in the distal part of $3A^mS$ and the remaining 1 located in the most distant part of $3A^mS$, significantly expanded the map length of $3A^mS$

Table 3 Number of PCR markers mapped on the seven chromosomes of Einkorn wheat and map distances to which RFLP markers and PCR markers contributed

Source of DNA markers	Homologous chromosome							
	$1A^m$	$2A^m$	$3A^m$	$4A^m$	$5A^m$	$6A^m$	$7A^m$	Total
Number of DNA markers mapped								
RFLP	8	10	12	8	11	6	8	63
ISSR	3	1	2	1	0	0	2	9
RAPD	0	1	4	0	0	4	0	9
Total	11	12	18	9	11	10	10	81
Map distance (cM)								
RFLP	116.3	115.2	119.2	104.2	142.7	97.2	124.0	818.8
ISSR/RAPD	2.0	5.4	29.4	1.3	0	1.6	3.7	43.4
Total	118.3	120.6	148.6	105.5	142.7	98.8	127.7	862.2

(about 30 cM, Table 3). This suggests that the RAPD markers could map a novel chromosome region(s) that RFLP markers were unable to map.

Chromosome $4A^m$

Two inversions of marker order were detected in our system between the $4A^m$ and 4A chromosomes because the 4A chromosome of common wheat carries high rearrangements such as translocations, and para- and peri-centric inversions (Devos et al. 1995). One ISSR marker (IS811C) was mapped 7.6 cM distant from *Xpsr584*.

Chromosome $5A^m$

The long arm of this chromosome has been extensively analyzed (Kojima and Ogihara 1997). The chromosome of Einkorn wheat harbors a 4AL-5AL reciprocal translocation as does common wheat (Dubcovsky et al. 1996). The expansion of map length around the centromere in $5A^m$S was also observed. No ISSR or RAPD markers were mapped on this chromosome.

Chromosome $6A^m$

Similar relationships between Einkorn wheat and common wheat chromosomes were also found in this chromosome. Four RAPD (R40/90A, R13/63A, R189/190, R117/118) markers were mapped on this chromosome. Their contribution, however, to the expansion of map length was only 1.6 cM (1.6%), as presented in Table 3. Therefore, RAPD markers on this chromosome did not map any novel region different from that mapped by the RFLP markers.

Chromosome $7A^m$

A similar tendency of map distances between Einkorn wheat and common wheat was also observed in this chromosome. One ISSR marker (IS856) was mapped on the central part of $7A^m$L, 7.6 cM distant from *Xpsr129*. The addition of IS856 to the map caused a 3.7-cM elongation of the linkage map.

Discussion

Characterization of PCR-mediated markers revealed by segregation patterns in the F_2 population of Einkorn wheat

It is well known that PCR-mediated markers have some advantages and disadvantages in comparison with RFLP markers. The advantages of PCR markers are the following: (1) easy handling; (2) no need for information on template DNAs or the synthesis of specific primers except for SSR (simple sequence repeat) markers; (3) generation of novel markers that can mark regions where other marking systems cannot map, such as repetitive sequences. On the other hand, there are some disadvantages, such as: (1) poor reproducibility of amplified bands, and (2) low accuracy for construction of a linkage map, probably because of their dominant nature. In this context, we examined ISSR and RAPD markers for their use in mapping Einkorn wheat chromosomes.

Two hundred and twenty-four ISSR polymorphic bands have been detected between Mn and Bt (Nagaoka and Ogihara 1997). Among these polymorphic bands, only 9 fragments (4.0%) could be mapped on the Einkorn wheat chromosomes (Fig. 2), though 44 fragments significantly showed a 3 : 1 segregation pattern in the F_2 population. This poor mapping situation

is probably due to the following. (1) A poor resolution of the ISSR fragments fractionated by agarose gel electrophoresis. Non-denaturing polyacrylamide gel electrophoresis might solve this problem (Zietkiewicz et al. 1994). (2) The complex genome of wheat, which contains a large fraction of repetitive DNA.

We have screened 88 polymorphic RAPD bands obtained with 200 primer combinations (Nagaoka and Ogihara 1997). Out of these 88 bands, 29 fragments revealed a significant 3:1 segregation ratio, but only 9 (10.2%) were able to be mapped on the Einkorn wheat chromosomes (Fig. 2). This poor mapping situation has also been reported in rice (Monna et al. 1994), in which over 1400 fragments were scored, but only 102 (7.3%) RAPD markers could be subsequently mapped on the 12 rice chromosomes. It can be concluded, therefore, that approximately 10% or less of polymorphic RAPD bands are possibly mapped on diploid plant chromosomes. On the other hand, RAPD mapping in the polyploid species such as common wheat is very difficult (Devos and Gale 1992).

Consequently, we conclude that only a low percentage of the polymorphic bands mediated by PCR can be mapped on diploid wheat chromosomes.

Einkorn wheat map with ISSR and RAPD markers

In this study, 9 ISSR loci and 9 RAPD loci were mapped on five and three of the seven Einkorn wheat chromosomes, respectively (Fig. 2). The ISSR markers tended to be distributed throughout the chromosomes, whereas the RAPD markers were gathered into some regions to form clusters. Since SSRs or microsatellites are distributed throughout the genome in both mammalian (Dietrich et al. 1992; Hazan et al. 1992; Weissenbach et al. 1992) and plant genomes (Wang et al. 1994), the present data, which represent the first mapping of ISSR markers in plants, support the widespread distribution of SSRs in the genome. ISSR markers fell into the regions mapped by the RFLP markers (Fig. 2) and did not substantially contribute to the expansion of the linkage map distance (Table 3). Furthermore, the variability of ISSR markers was similar to that of RFLPs (Nagaoka and Ogihara 1997). These lines of evidence strongly suggest that the ISSR markers map on or around the same region mapped by RFLP markers. Four ISSR markers obtained by priming with (AC) repeats [three $(AC)_8$ and one $(CA)_8$] could be mapped on the Einkorn wheat chromosomes. In vertebrates, (AC) repeats are very prevalent and useful in constructing genetic maps of mammalian genomes (Dietrich et al. 1992; Hazan et al. 1992; Weissenbach et al. 1992). Although (AT) repeats are the most abundant repeats in plant genomes (Wang et al. 1994), our data clearly shows that clusters of (AC) repeats are exclusively useful for mapping wheat chromosomes.

On the other hand, out of the 9 RAPD primers, 4 were gathered into a specific region of Einkorn wheat chromosomes, namely, the short arm of 3A chromosome ($3A^mS$). This region is barely marked by the RFLP markers (Gale et al. 1995; Dubcovsky et al. 1996), strongly suggesting that the RAPD markers identify regions distinctly different from those detected by RFLP markers. Actually, Southern hybridization analysis by probing with RAPD fragments produced smear bands, suggesting the repetitive nature of RAPD products (Devos and Gale 1992).

Comparison of the genetic map of Einkorn wheat to the genetic and cytological maps of common wheat

The genetic linkage map of Einkorn wheat was compared with that of the A genome of common wheat (Fig. 2; Gale et al. 1995). The marker orders of each chromosome in Einkorn wheat coincided with those of common wheat except for 4A chromosome, which harbors translocations and inversions (Devos et al. 1995), indicating conservatism between them. Therefore, the construction of a high-density map of diploid wheat with PCR markers in combination with RFLP markers became possible and applicable to an analysis of the polyploid wheat genome (Gill and Gill 1994). A stronger suppression of recombination around the centromere of common wheat chromosomes was disclosed in the present study. On the other hand, recombination at the distal part of chromosome arms more frequently took place in the chromosomes of common wheat, giving longer genetic map distances at the distal part of common wheat chromosomes (Fig. 2). Although we consider our map to be unsaturated (81% of total length to Dubcovskys' map), most PCR markers, except for the 4 RAPD markers, did not significantly contribute to the expansion of the genetic map (Table 3). These 4 RAPD markers, all of which were located on the distal part of $3A^mS$ (Fig. 2), contributed about 30 cM to the extension of the genetic map. Accordingly, these RAPD markers might be mapped on a novel chromosome region differing from the region marked by the RFLP markers.

The cytological map shows the position of C-bands, deletion break-points, and the distribution of markers (Fig. 2). Comparision of the genetic map to the cytological one showed that the relative distance between commonly mapped markers provides information on genetic linkage and cytological distance within a chromosome region (Werner et al. 1992). It is striking that markers mapped in the region close to the centromere in the genetic map (less than 1 cM) were located cytologically almost in the central part of chromosome arms (Fig. 2). This clearly indicates that recombinations around the centromere were strongly suppressed. Suppression of recombination in the centromeric region occurs irrespective of the amount of

heterochromatin (Gill et al. 1996a,b). Moreover, recombination does not take place randomly on the chromosomes (Steinmetz et al. 1987; Bollag et al. 1989; Ganal et al. 1989; Werner et al. 1992; Lichten and Goldman 1995) but should occur in a discrete region, namely, a gene-rich region, where many cDNA markers as well as genomic clones have been cloned from such regions (Gill et al. 1996a,b). Since ISSR markers were linked to the RFLP markers, and SSRs are frequently a part of gene structure (Becker and Heun 1995), ISSR markers might provide new sources for marking gene-rich regions. The facts that RAPD markers could map a novel chromosome region and gave Southern hybridization profiles showing repetitive sequences (Devos and Gale 1992) strongly suggest that RAPD markers provide a new source for landmarks of chromosomes such as gene-poor regions.

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References

Akkaya MS, Bhagwat AA, Cregan PB (1992) Length polymorphisms of simple sequence repeat DNA in soybean. Genetics 132:1131–1139

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 21:607–614

Beckmann JS, Soller M (1990) Toward a unified approach to genetic mapping of eukaryotes based on sequenced tagged microsatellite sites. Bio/technology 8:930–932

Bennet MD, Smith JB (1976) Nuclear DNA amounts in angiosperms. Philos Trans R Soc London Ser B 274:227–274

Bollag RJ, Waldman AS, Liskay RM (1989) Homologous recombination in mammalian cells. Ann Rev Genet 23:199–255

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl Genet 78:495–504

Delaney DE, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995a) Cytologically based physical maps of the group-2 chromosomes of wheat. Theor Appl Genet 91:568–573

Delaney DE, Nasuda S, Endo TR, Gill BS, Hulbert SH (1995b) Cytologically based physical maps of the group-3 chromosomes of wheat. Theor Appl Genet 91:780–782

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Devos KM, Dubcovsky J, Dvorak J, Chinoy CN, Gale MD (1995) Structural evolution of wheat chromosomes 4A, 5A and 7B and its impact on recombination. Theor Appl Genet 91:282–288

Dietrich W, Katz H, Lincoln SE, Shin H-S, Friedman J, Dracopoli NC, Lander ES (1992) A genetic map of the mouse suitable for typing intraspecific crosses. Genetics 131:423–447

Dubcovsky J, Luo M-C, Zhong G-Y, Bransteitter R, Desai A, Kikian A, Kleinhofs A, Dvorak J (1996) Genetic map of diploid wheat, *Triticum monococcum* L., and its comparison with maps of *Hordeum vulgare* L. Genetics 143:983–999

Gale MD, Atkinson MD, Chinoy CN, Harcourt RL, Jia J, Li QY, Devos KM (1995) Genetic maps of hexaploid wheat. In: Li ZS, Xin ZY (ed) Proc 8th Int Wheat Genet Symp. China Agricultural Scientech Press, Beijing, pp 29–40

Ganal MW, Young ND, Tanksley SD (1989) Pulsed field gel electrophoresis in the *Tm-2a* region of chromosome 9 in tomato. Mol Gen Genet 215:395–400

Gill KS, Gill BS (1994) Mapping in the realm of polyploidy: the wheat model. BioEssays 16:841–846

Gill KS, Gill BS, Endo TR (1993) A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma 102:374–381

Gill KS, Gill BS, Endo TR, Boyko EV (1996a) Identification and high-density mapping of gene-rich regions in chromosome group 5 of wheat. Genetics 143:1001–1012

Gill KS, Gill BS, Endo TR, Taylor T (1996b) Identification and high-density mapping of gene-rich regions in chromosome group 1 of wheat. Genetics 144:1883–1891

Hazan J, Dubay C, Pankowiak MC, Becuwe N, Weissenbach J (1992) A genetic linkage map of human chromosome 20 composed entirely of microsatellite markers. Genomics 12:183–189

Hohmann U, Endo TR, Herrmann RG, Gill BS (1995) Characterization of deletions in common wheat induced by an *Aegilops cylindrica* chromosome: detection of multiple chromosome rearrangements. Theor Appl Genet 91:611–617

Kam-Morgan LNW, Gill BS (1989) DNA restriction fragment length polymorphisms: a strategy for genetic mapping of D genome of wheat. Genome 32:724–732

Kojima T, Ogihara Y (1997) Common wheat chromosome 5AL: high-resolution genetic mapping, its comparison to cytological map, genetic map of diploid wheat and synteny among cereals. Genes Genet Syst (in press)

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly JM, Lincoln SE, Newberg L (1987) MAPMARKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Lichten M, Goldman ASH (1995) Meiotic recombination hotspots. Annu Rev Genet 29:423–444

Liu Y-G, Mori N, Tsunewaki K (1991) Restriction fragment length polymorphism (RFLP) analysis in wheat. II. Linkage maps of the RFLP sites in common wheat. Jpn J Genet 66:617–633

Martin GB, Williams JGK, Tanksley SD (1991) Rapid identification of markers linked to a pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Mickelson-Young L, Endo TR, Gill BS (1995) A cytogenetic ladder map of the wheat homoeologous group-4 chromosomes. Theor Appl Genet 90:1007–1011

Monna L, Miyao A, Inoue T, Fukuoka S, Yamazaki M, Sun Zhong H, Sasaki T, Monobe Y (1994) Determination of RAPD markers in rice and their conversion into sequence-tagged sites (STSs) and STS-specific primers. DNA Res 1:139–148

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nagaoka T, Ogihara Y (1997) Applicability of inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet 94:597–602

Ogihara Y, Shimidzu H, Hasegawa K, Tsujimoto H, Sasakuma T (1994) Chromosome assignment of four photosynthesis-related genes and their variability in wheat species. Theor Appl Genet 88:383–394

Plaschke J, Ganal MW, Roeder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Roeder MS, Plaschke J, Koenig SU, Borner A, Sorrels ME, Tanksley SD, Ganal MW (1995) Abundance, variability and chromosomal location of microsatellites in wheat. Mol Gen Genet 246 : 327–333

Steinmetz M, Uematsu Y, Fisher LK (1987) Hotspots of homologous recombination in mammalian genomes. Trends Genet 3 : 7–10

Tsumura Y, Ohba K, Strauss SH (1996) Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Theor Appl Genet 92 : 40–45

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88 : 1–6

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vaysseix G, Lathrop M (1992) A second generation linkage map of the human genome. Nature 359 : 794–801

Werner JE, Endo TR, Gill BS (1992) Toward a cytogenetically based physical map of the wheat genome. Proc Natl Acad Sci USA 89 : 11307–11311

Williams JGK, Kubelik ARK, Livak JL, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by random primers are useful as genetic markers. Nucleic Acids Res 18 : 6531–6535

Wu K-S, Tanksley SD (1993) Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol Gen Genet 241 : 225–245

Ziekiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20 : 176–183