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Mapping of RFLP probes characterized for their polymorphism on wheat

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Abstract Wheat anonymous probes were selected for their efficiency for providing a readable hybridization pattern and revealing RFLP among wheat varieties. We report the mapping of 132 such probes (20 wheat-leaf cDNA, 28 wheat-root cDNA and 84 genomic DNA) on the reference population of the International Triticeae Mapping Initiative (ITMI) derived from the cross W-7984 with Opata85. Each probe has been characterized for its polymorphism information content. The 132 probes allowed us to map 160 loci.

Key words RFLP · Wheat · Genetic mapping

Introduction

Restriction fragment length polymorphism (RFLP) maps have already been constructed for many higher plants. In bread wheat (*Triticum aestivum*), molecular marker maps have been published for the chromosomes of groups 1 (Van Deynze et al. 1995), 2 (Devos et al. 1993; Nelson et al. 1995b), 3 (Devos et al. 1992; Devos and Gale 1993; Nelson et al. 1995c), 4, 5 (Devos et al. 1995; Nelson et al.1995a; Xie et al. 1993), 6 (Jia et al. 1996; Marino et al. 1996) and 7 (Chao et al. 1989; Nelson et al. 1995a). Complete maps have also been published (Liu and Tsunewaki 1991; Gale et al. 1995; Cadalen et al. 1997).

Molecular markers are being used as tools to improve practical plant breeding. However, the use of molecular markers to characterize breeding lines and varieties or to assist selection is hampered in wheat due to the low degree of polymorphism displayed by this crop (Chao et al. 1989; Kam-Morgan et al. 1989; Liu et al. 1990). Moreover, much of the current breeding in wheat involves crosses among lines that share common ancestors. So, it will be important in wheat RFLP studies to identify probes that detect highly polymorphic loci.

The objective of this investigation reported here was to produce RFLP markers useful in selection. Anonymous cDNAs and genomic DNAs were tested for the quality of their hybridization pattern and for their polymorphism on 13 commercial wheat varieties which have been used in Belgian breeding programs. We report the mapping of 160 such markers on the previously published map of the population derived from the cross W-7984 with Opata85 (Van Deynze et al. 1995; Nelson et al. 1995a, b, c; Marino et al. 1996).

Materials and methods

Plant material

The 'Chinese Spring' nullisomic-tetrasomic (NT) stocks produced by E.R. Sears (Sears 1954, 1966) were provided by T.E. Miller (John Innes Centre, Norwich, UK). The 14 wheat varieties used to select the probes were: 'Apollo', 'Camp Remy', 'Sperber', 'Token', 'Odeon', 'Capitaine', 'Castell', 'Soissons', 'Courtot', 'Moulin', 'Genial', 'Pernel', 'Franco' and the reference variety 'Chinese Spring'. Mapping was carried out on the International Triticeae Mapping Initiative (ITMI) population derived by single-seed descent (F_8) from the cross W-7984 (a synthetic wheat) with Opata85 (Nelson et al. 1995a). The seeds were provided by M. Bernard (INRA, Clermont-Ferrand, France) and M. Sorrels (Cornell University, USA).

RFLP probes

Anonymous cDNA probes

Two wheat cDNA libraries were constructed using mRNA isolated from leaves of var 'Escorial' (probes gbx3076 to gbx4986) or

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from roots of 'Odeon' (probes gbxR004 to gbxR958). cDNAs were synthesized using the Amersham cDNA synthesis kit and cloned into the *Eco*RI site of the pUC18 or pEMBL18 plasmid vector.

Anonymous gDNA probes (*probes gbxG034 to gbxG773 and gbxGx46 to gbxGx228*)

A wheat-leaf *Pst*I genomic library was constructed. For this, wheat-leaf DNA from var 'Odeon' was digested with *Pst*I. The fragments were separated on a low-melting-point agarose gel, and the DNA sequences in the size range 500–2500 bp were subsequently isolated and ligated into the *Pst*I site of pUC18 or pEMBL18. Highly repeated sequences were discarded after hybridization with total wheat genomic DNA.

RFLP procedures

Genomic DNA was extracted as described by Sharp et al. (1988). Restriction enzyme digestion and 0.8% agarose gel electrophoresis followed standard procedures. Southern blotting on nylon membranes (Hybond N+, Amersham) was as described by the manufacturer. The probe was [^{32}P]-labeled using the random primed method (Feinberg and Vogelstein 1983). Prehybridization and hybridization were performed as described by Sharp et al. (1988). The membranes were washed at 65°C, twice in 2 × SSC/0.1% SDS, twice in 1 × SSC/0.1% SDS and once in 0.5 × SSC/0.1% SDS for 15 min each. Membranes were exposed to X-ray films with one intensifier screen for 10–15 days.

Informativeness of clones

The polymorphism information content (PIC) described by Botstein et al. (1980) was calculated using the formula $PIC_i = 1 - \Sigma p_{ij}^2$ where p_{ij} is the frequency of the jth RFLP pattern revealed by the probe i (combined with the enzyme giving the most polymorphic pattern).

Mapping

A subset of 71 F_8 recombinant inbred lines derived by single-seed descent from the cross W-7984 with Opata85 (Nelson et al. 1995a) was used for segregation analysis. The RFLP probes were hybridized to the parental DNAs digested by 15 restriction enzymes; the restriction enzyme/probe combination giving the best resolvable polymorphism was selected and applied to the population lines for mapping. Membranes carrying 'Chinese Spring' NT digested with the same enzyme were included in the hybridization. The data were analyzed using MAPMAKER version 2.0 (Lander and Abrahamson 1987). The gbx markers were mapped according to the position of probes placed at regular intervals on the previously published maps (Van Deynze et al. 1995; Nelson et al. 1995a, b, c; Marino et al. 1996); the segregation data for those probes were kindly provided by P. Leroy (INRA, Clermont-Ferrand, France). The 'order' and the 'build' commands were used; markers whose order was established at a log-likelihood ratio (LOD) of 2.0 were assigned to exact positions on the chromosome maps, and the remainder were placed in the interval in which they were best fit by the 'try' command. The genetic distances were calculated with the Kosambi function.

Results

Selection of probes

Four hundred and ninety-nine random wheat cDNA and genomic DNA were tested on a panel of 14 wheat varieties to assess their efficiency for providing a readable hybridization pattern and revealing RFLP. This panel included 13 varieties reflecting pedigrees which have been used in Belgian breeding programs and the reference variety 'Chinese Spring'. DNA was digested with four different restriction enzymes (*Eco*RI, *Hin*dIII, *Eco*RV or *Dra*I). Among the 499 clones, 275 provided a low-copy pattern with strong and easy-to-read signals. The polymorphism information content (PIC) was calculated for each probe; these values are shown in Fig. 1. The average probe PIC was 0.35 with values ranging from 0.00 to 0.88. The highest level of polymorphism (0.88) was shown by the clone gbx3832, a wheat cDNA coding for a thaumatin-like protein (Mingeot and Jacquemin 1997). No difference in the level of polymorphism was found between cDNA and genomic DNA probes.

RFLP between parental varieties

Two hundred and forty-one selected probes (148 genomic DNA and 93 cDNA) were hybridized to restricted genomic DNA from parental lines of the cross Opata85 × W-7984 to identify informative probe/restriction enzyme combinations. Among the 241 probes tested, 174 (72%) exhibited polymorphism between the parents. No significant difference in this proportion was observed according to the library used: 111 genomic probes (75%) and 63 cDNA probes (68%) exhibited polymorphism between the parents.

Mapping

One hundred and thirty-two polymorphic probes (20 wheat-leaf cDNA, 28 wheat-root cDNA and 84 genomic DNA) were hybridized on the progeny of the population derived from the cross W-7984 with Opata85, revealing 171 loci. Linkage maps are shown in Fig. 1. The gbx markers were mapped according to the position of regularly spaced probes on previously published maps (Van Deynze et al. 1995; Nelson et al. 1995a, b, c; Marino et al. 1996); markers that could be ordered at LOD 2.0 are accompanied by tick marks on their respective chromosomes. Out of the 171 loci, 11 remain unlinked. Out of the 132 probes used, 25 detected 2 or more polymorphic loci and in 9 such cases the loci were mapped on non-homoeologous chromosomes.

Five probes detected polymorphism for loci on the three genomes of the same chromosome group: gbxR698

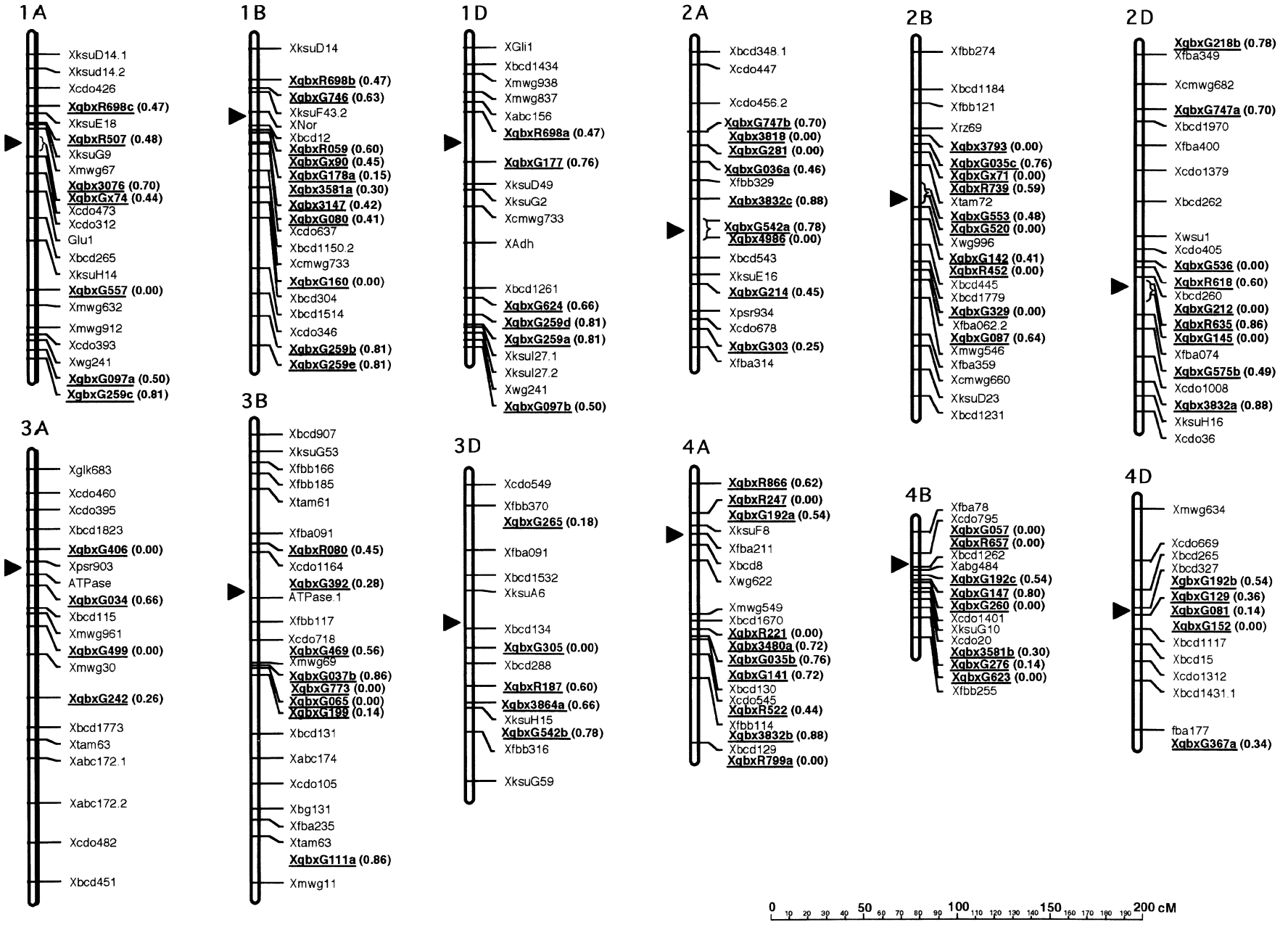

Fig. 1 Continued

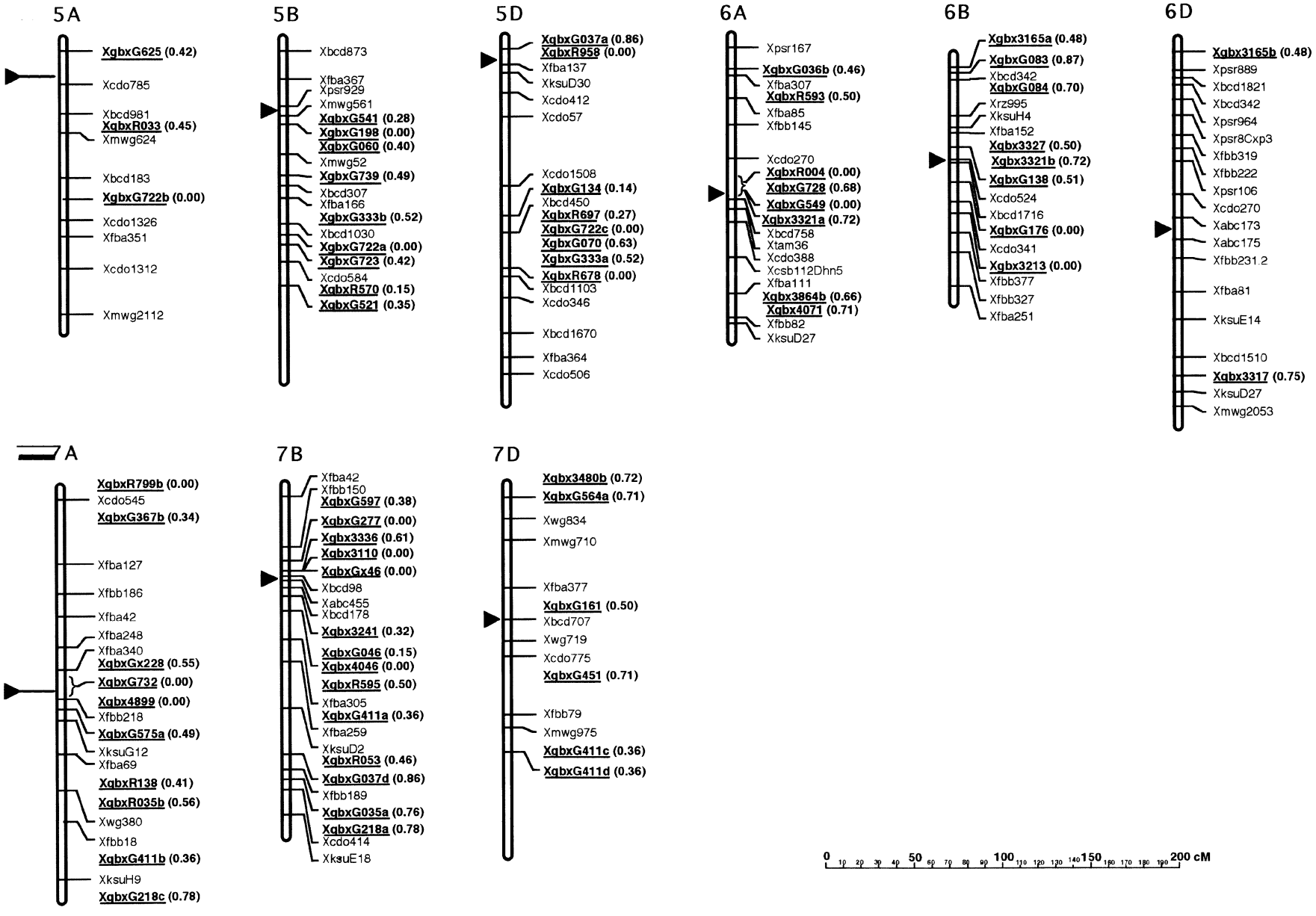

Fig. 1 RFLP map of wheat. The *gbx* markers (in bold and underlined) were mapped according to the position of previously mapped probes (Van Deynze et al. 1995; Nelson et al. 1995a, b, c; Marino et al. 1996); markers that could be ordered at LOD 2.0 are accompanied by *tick marks* on their respective chromosomes. Short arms are at the *top*. *Numbers* in *parenthesis* indicate the PIC of the probe. The position of the centromere is denoted by an *arrowhead*; } indicates that the position of the probe in relation to the centromere is not determined

and gbxG259 on chromosome group 1, gbxG192 on group 4, gbxG722 on group 5 and gbxG411 on group 7. Two probes detected more than 1 locus per chromosome: gbxG411 showed duplicated loci on chromosome 7D, and gbxG259 showed duplicated loci on chromosomes 1B and 1D.

Rearrangements are known to have occurred between chromosomes 4A, 5A and 7B (Naranjo et al. 1987; Devos et al. 1995; Nelson et al. 1995a). In the present study, probe gbxG192 hybridized to the pericentric inversion region of chromosome 4A, detecting loci on chromosomes 4AS, 4BL and 4DL. Probes gbx3480 and gbxR799, detecting loci on chromosomes 4AL and 7DS and chromosomes 4AL and 7AS, respectively, were mapped in the region involved in the translocation among chromosomes 4AL, 5AL and 7BS.

Segregation distortion

Chi-square segregation tests were calculated for each locus. Twenty-four molecular markers (14%) deviated significantly from the 1:1 ratio with χ^2 ranging from 3.92 to 19.60. Eight skewed markers out of the 24 fell in two clusters localized on chromosomes 2A (loci gbxG747b, gbx3818, gbxG281, gbxG036a and gbx3832c) and 4B (gbxG192c, gbxG147 and gbxG260); 17 markers out of the 24, including the 8 clustered loci, showed segregation distortion in favor of Opata85.

Distribution of markers

The assigned loci were not uniformly distributed on all chromosomes. Homoeologous group 3 and 6 chromosomes were the least represented, with 17 and 18 loci, respectively (11% of the 160 loci located); homoeologous group 7 chromosomes are the most represented with 30 loci (19%). Heterogeneity was also detected for the wheat-leaf cDNA library with 33% of the loci assigned to group 6 chromosomes. Only 26% of the loci were mapped on the D genome, while 32% and 42% were located on the A and B genomes, respectively.

Discussion

Among 499 cDNA and genomic DNA probes tested on a panel of 14 wheat varieties, 275 provided a low-copy pattern with strong and easy-to-read signals. The average probe PIC calculated was 0.35 and did not vary according to the library used. This is in contrast with previous observations that genomic DNA displays more polymorphism than cDNA on wheat (Devos et al. 1992), lettuce (Landry et al. 1987), lentil (Havey and Muehlbauer 1989) and tomato (Miller and Tanksley 1990).

One hundred and thirty-two probes (20 wheat-leaf cDNA, 28 wheat-root cDNA and 84 genomic DNA) revealing 160 loci were mapped on the reference population derived from the cross Opata85 × W-7984. Segregation distortion was observed for 24 loci. Among these, 8 are clustered on chromosomes 2A and 4B and are skewed in favor of Opata. This non-random distribution of skewed markers is in accordance with the results of Cadalen et al. (1997) on a wheat doubled haploid population and of Liu et al. (1991) and Devos et al. (1993) on wheat F_2 populations. The assigned loci are not uniformly distributed on all chromosomes: the percentage of loci mapped varies from 11% for homoeologous group 3 and 6 chromosomes to 19% for group 7. Heterogeneity was also detected for the wheat-leaf cDNA library with 33% of the loci assigned to group 6 chromosomes. Anderson et al. (1992) has already pointed out such disparity in probe distribution according to the library used.

Only 26% of the loci mapped to the D genome, while 32% and 42% are located in the A and B genomes, respectively. This lack of polymorphism in the D genome has already been observed (Chao et al. 1989; Kam-Morgan et al. 1989; Liu and Tsunewaki 1991) and echoes the experiments of Van Deynze et al. (1995), Nelson et al. (1995a, c) and Marino et al. (1996) on the same population.

During the last few years, the use of genetic markers based on polymerase chain reaction (PCR) systems has been developed, and the polymorphism level of different kinds of markers has been compared. It was observed that microsatellites markers (SSR) show a much higher degree of polymorphism in wheat than RFLP markers (Plaschke et al. 1995; Röder et al. 1995; Bryan et al. 1997), and a microsatellite map of wheat has been published (Röder et al. 1998). Despite this, RFLP markers remain useful because they are extremely powerful tools for comparative mapping approaches (Ahn et al. 1993; Moore et al. 1995; Yu et al. 1996). Moreover, the published SSR map is not saturated, and the development of microsatellites is extremely time-consuming and expensive. On the other hand, comparing technical facilities, RFLP markers are more laborious to handle, but they can be converted into PCR markers (Sequence Tagged Site). The knowledge of the polymorphism level of RFLP markers should enable the preselection of probes suitable for use in an intraspecific context. We report here the mapping of RFLP markers characterized for their polymorphism; we are currently using this probe set to assess their mapping facilities on intraspecific crosses.

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