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Genetic linkage map of a wheat × spelt cross

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Abstract We constructed a genetic map of a cross between the Swiss winter wheat (*Triticum aestivum* L.) variety Forno and the Swiss winter spelt (*Triticum spelta* L.) variety Oberkulmer. For the linkage analysis,176 polymorphic RFLP probes and nine microsatellites were tested on 204 F_5 recombinant inbred lines (RILs) of Forno × Oberkulmer revealing 242 segregating marker loci. Thirty five percent of these loci showed significant ($P > 0.05$) deviation from a 1 : 1 segregation, and the percentage of Forno alleles ranged from 21% to 83% for individual marker loci. Linkage analysis was performed with the program MAPMAKER using the Haldane mapping function. Using a LOD threshold of 10, we obtained 37 linkage groups. After finding the best order of marker loci within linkage groups by multi-point analysis we assembled the linkage groups into 23 larger units by lowering the LOD threshold. All except one of the 23 new linkage groups could be assigned to physical chromosomes or chromosome arms according to hybridisation patterns of nullitetrasomic lines of Chinese Spring and published wheat maps. This resulted in a genetic map comprising 230 marker loci and spanning 2469 cM. Since the analysed population is segregating for a wide range of agronomically important traits, this genetic map is an ideal basis for the identification of quantitative trait loci (QTLs) for these traits.

Key words *Triticum aestivum* · *Triticum spelta* · RFLP · Genetic map · QTL

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Introduction

Genetic markers offer the possibility to analyze the inheritance of parental alleles at a single locus. RFLP and PCR-based markers were successfully used to identify monogenically inherited traits as well as for the dissection of quantitative traits into Mendelian factors of inheritance, so called quantitative trait loci (QTLs) (Michelmore 1995). A pre-requisite for QTL analysis is the construction of genetic maps that cover most of the genome. To achieve a good coverage of the wheat genome with molecular markers is more difficult than in other crops because of its hexaploid structure, the low degree of polymorphism at the DNA level (Liu and Tsunewaki 1991) and the huge genome size (1.45×10^{10} bp per haploid nucleus, Bennet et al. 1982). For these reasons, the first QTL studies in wheat were conducted on partial-genome assays. QTLs for ear emergence time, plant height, tiller weight, yield and 50-grain weight were analysed by Hyne et al. (1994) concentrating on chromosomes 6B, 7A, 7B, and 7D. Single-chromosome substitution lines for 5A have been used to locate genes for drought-induced abscisic acid production (Quarrie et al. 1994), vernalization requirement and frost resistance of wheat (Galiba et al. 1995). Anderson et al. (1993) analysed two populations of recombinant inbred lines for QTLs involved in preharvest sprouting with 37 and 27 polymorphic RFLP markers, respectively.

Since these initial studies, genetic maps of wheat have been consistently improved by using wide crosses

to increase the level of polymorphism and also by the development of additional markers (RFLP probes, wheat microsatellites, AFLPs) to obtain a better marker density. Liu and Tsunewaki (1991) used 66 F_2 progeny from a cross between wheat (variety Chinese Spring) and spelt (var. *duhamelianum*) to construct a genetic map of about 1800 cM covering all 21 chromosomes with 185 RFLP marker loci, whereas Gale et al. (1995) analysed 120 F_2 progeny of an interspecific cross between the spring wheat variety Chinese Spring and synthetic wheat (the amphiploid of *Triticum turgidum* × *Triticum tauschii*) covering about 2600 cM with RFLP markers. Another RFLP map of wheat with about 3500 cM was based on 114 F_7 lines of a cross between synthetic wheat and the spring wheat variety Opata (Nelson et al. 1995 a,b,c; Van Deynze et al. 1995; Marino et al. 1996). In contrast, Cadalen et al. (1997) analysed an intervarietal cross, which is of special interest for QTL mapping and marker-assisted selection. Their genetic map, with a length of about 1800 cM, was based on 106 androgenically derived double-haploid lines obtained from F_1s between monosomics of the two wheat varieties Chinese Spring and Courtot.

For our mapping population we used a cross between winter wheat (*Triticum aestivum* L.) and winter spelt (*Triticum spelta* L.), both originating from Switzerland. Spelt was chosen as a crossing parent because of the higher level of genetic diversity expected between wheat and spelt than within wheat itself (Liu et al. 1990; Siedler et al. 1994) and because of our interest in spelt-specific traits for breeding purposes. Wheat and spelt are both hexaploid species with three genomes (AABBDD) and seven homoeologous chromosome groups. Both crops are autogamous but can be crossed with each other. In comparison with wheat, spelt is taller (150 up to 200 cm), has long lax ears (15–20 cm), a brittle rachis, tight glumes and a better tillering capacity (Winzeler et al. 1994). In addition, spelt varieties have less kernels per ear but a higher thousand-kernel weight (47–52 g) and very different rheological properties (Ranhotra et al. 1995). Spelt has been a major crop in Switzerland since the beginning of this century (Winzeler and Rüegger 1990) but was then replaced by wheat, which out-yielded spelt by 10–20% under high-input conditions. However, in marginal growing regions with heavy soils, high precipitation (600–1200 mm), a cold winter and high altitudes (up to 1000 m), spelt showed a higher yield stability than wheat and remained in this niche (Winzeler 1988). Today, spelt is grown on about 2000 ha in Switzerland and mainly used for human consumption as bread and speciality bakery. Wheat × spelt crosses are commonly made in spelt breeding programs to increase the genetic diversity for lodging resistance and disease resistance (Schmid and Winzeler 1990; Schmid et al. 1994).

The aim of the present project was to construct a genetic map of the hexaploid wheat and spelt genomes as a prerequisite for QTL mapping. Linkage analysis resulted in a genetic map of 2469 cM covering all 21 chromosomes. This map has been used for the QTL mapping of disease resistance genes (manuscript in preparation), lodging resistance genes (Keller et al. 1999) and genes responsible for rheological characteristics (manuscript in preparation).

Materials and methods

Plant material

For the construction of the genetic map we used a cross between the winter wheat variety Forno and the winter spelt variety Oberkulmer Rotkorn, both originating from the breeding programs of the Swiss Federal Research Station for Agroecology and Agriculture (FAL, Zürich-Reckenholz, Switzerland). These parents were chosen because both have been grown on a considerable acreage in Switzerland and, besides the distinctive morphological and rheological characteristics of wheat and spelt, differ for many agronomically important traits. The wheat parent Forno has proven durable disease resistance against leaf rust, shows medium resistance against powdery mildew but is highly susceptible to leaf and glume blotch caused by *Septoria nodorum*. The spelt parent Oberkulmer has poor resistance against leaf rust and powdery mildew but a high level of resistance against *S. nodorum*. The progeny of the cross Forno × Oberkulmer was propagated as a bulk until the F_5 generation, from which 226 single plants were randomly chosen. Three ears of each F_5 plant were harvested in 1993 and threshed by hand. The 226 F_5 recombinant inbred lines (RILs) were propagated as a one-row plot in the field in 1994 and threshed and de-hulled for further experiments. Twenty kernels of each RIL were grown in the greenhouse to produce the plant material for DNA extraction and genetic mapping. The remaining seed was used as stock for QTL analysis in field trials.

Parental screening for polymorphism with RFLP markers

Genomic DNA was extracted from 0.5 g of freeze-dried leaf material. DNA isolation, restriction enzyme digestion, agarose-gel electrophoresis, alkaline Southern blotting to nylon membranes, and hybridisation methods were all performed as described by Graner et al. (1990). RFLP probes were labelled with [^{32}P]dCTP using the random primer labelling method (Feinberg and Vogelstein 1983). Non-incorporated nucleotides were removed by filtration through Sephadex G-50 columns. Most of the RFLP probes were genomic DNA clones. The wheat clones PSR were kindly provided by Dr. M. Gale (John Innes Centre, Norwich, England) and Dr. P. Gay (Ciba Seeds, Basel, Switzerland). The wheat clones pTAG (*Xglk* locus) from Dr. K. Tsunewaki and Dr. Y.G. Liu, Kyoto, Japan (Liu and Tsunewaki 1991), were provided by the Australian Triticeae Mapping Initiative (ATMI). The barley clones MWG were provided by Dr. A. Graner (BBA, Grünbach, Gatersleben, Germany). The rice clone C970 was provided by Dr. T. Sasaki (Rice Genome Research Program, Kannondai, Japan). The pWIR232 clone coding for a wheat thaumatin (Rebmann et al. 1991) was provided by Dr. R. Dudler (University of Zürich, Switzerland). The Lrk10 clone, provided by Dr. C. Feuillet (University of Zürich) codes for a wheat protein kinase (Feuillet et al. 1997) and the PL_AP clone, provided by Dr. A. Penger (University of Zürich), encodes a P-loop protein in wheat (personal communication). The genomic DNA of the parental lines Forno and Oberkulmer was digested with seven restriction enzymes (*Eco*RI, *Hin*dIII, *Eco*RV, *Xba*I, *Dra*I, *Bam*HI, *Bgl*II) and tested for polymorphism with 310 DNA probes (191 PSR clones,

95 pTAG clones, 19 WG clones, one KSU clone, three α-amylase clones, one PWIR clone). The autoradiographies of the RFLP analysis were visually scored on a light box.

Parental screening for polymorphism with wheat microsatellite markers

The same DNA source as for RFLP analysis was used for wheat microsatellite analysis. We tested 24 GWM wheat microsatellites (Plaschke et al. 1995; Röder et al. 1995), two wheat microsatellites in the coding region of the low-molecular-weight glutenin (P1P2) on 1AS and of a γ-gliadin pseudogene (R1F2) on 1BS (Devos et al. 1995 a), as well as three microsatellites (AC22, AC29, AG10) described by Ma et al. (1996). PCR-amplifications of microsatellites were performed as described by Röder et al. (1995) and Ma et al. (1996) with minor modifications. The gels were re-used up to four times. Fragment sizes were calculated using the computer program AlleleLinksTM 1.0 (Pharmacia Biotech) by comparison with internal size standards. The resolution was 1–2 bp.

Genotypic assessment of the RILs of Forno × Oberkulmer with molecular markers

One hundred and seventy six of the polymorphic RFLP probes and nine microsatellites were tested on the 226 RILs of Forno × Oberkulmer using the same methods as described for the parental screening. Whenever possible, dominant RFLP loci were scored as co-dominant markers based on differences in band intensity. For each of 226 RILs of Forno × Oberkulmer the percentage of heterozygous marker loci, the percentage of marker bands deviating from the parents, as well as the percentage of the two parental genomes, was determined based on the total of 242 polymorphic marker loci. Twenty two RILs were discarded because of the high degree of heterozygous bands ($>10\%$) or because of the detection of bands ($>1\%$) that were not present in the parental lines. The remaining 204 RILs were used for linkage analysis. The individual marker loci were tested for distorted segregation using the sequential rejective Bonferoni procedure (Holm 1979).

Linkage analysis

Linkage analysis of the 242 marker loci was performed with the computer program MAPMAKER (Lander et al. 1987) for selfed recombinant inbred lines. As a control we also used the program JoinMap (Stam and Van Ooijen 1995) which takes the selfing generation into account and allows for the calculation of heterozygous loci. The results obtained were similar (data not shown). The linkage analysis was performed as an iterative procedure, considering first only the undistorted marker loci and adding the distorted markers in a second step. This should help to detect artefactual linkage groups caused by strong distortion. In the first step linkage groups were determined with a likelihood odds (LOD) ratio of 10 as a threshold. We used the mapping function of Haldane (1919) because of the independent cross-over events in different meiotic phases during the development from the F_1 to the F_5 generation. Two-point, three-point and multi-point analyses were used in order to determine the best order of marker loci within the linkage groups. The relatively small linkage groups were assigned to the 21 wheat chromosomes according to published wheat and spelt maps (McGuire and Qualset 1997) and RFLP patterns of nulli-tetrasomic lines of Chinese Spring kindly provided by Dr. M. Gale (John Innes Centre, Norwich, England). Marker loci and linkage groups that were more than 20 cM apart were not significantly linked. In order to join the linkage groups and to add unlinked markers to these linkage groups the LOD threshold was lowered ($3.0 < \text{LOD} > 10.0$) and all possible combinations were tested. In case of more than one possible arrangement of linkage groups within homoeologous chromosome groups we chose the one with the smallest genetic distance between the adjacent marker loci of the linkage groups to construct the genetic map. For the drawing of the map we used the computer program DrawMap Version 1.1 developed by J. Van Ooijen (Centre for Plant Breeding and Reproduction Research CPRO-DLO, Wageningen, The Netherlands).

Results

Parental screening

Out of the 310 RFLP probes tested for polymorphism on the parental lines 26 showed no clear hybridisation pattern. Of the remaining probes, 183 (64%) were polymorphic between Forno and Oberkulmer with at least one restriction enzyme. Most probes produced RFLP patterns with three, six or even nine bands per genotype. However, for most probes only one of these bands showed a polymorphism between the parental lines. Five of twenty nine tested microsatellites showed no clear bands, five were monomorphic, and 19 showed polymorphic amplification products between Forno and Oberkulmer differing in only a few (2–10) base pairs. Although wheat microsatellite markers are in general genome-specific, the primer pairs GWM6 and GWM111 amplified two and three polymorphic bands, respectively.

Segregation of marker loci

The polymorphic marker loci detected by the 176 RFLP probes and nine microsatellites were analysed on the 226 RILs resulting in 230 and 12 segregating loci, respectively, with an average of 1.3 polymorphic loci per probe or microsatellite. In cases where more than one locus was detected per probe or microsatellite, the different loci were indicated by small letters at the end of the clone name (a,b,c,d). Of the 242 segregating loci 168 (70%) showed co-dominant inheritance, while 43 were dominant for the Forno band and 30 for the Oberkulmer band. Averaged over all loci and all 226 RILs of Forno × Oberkulmer the level of heterozygosity was 5.2%, which is very close to the expected 6.25% in an F_5 generation. However, 13 genotypes had an extremely high level of heterozygosity (11–35%), indicating a mixture of seed, and nine genotypes showed 1–12% bands that did not occur in the pattern of the parental lines, indicating outcrossing events during line development. Therefore, these lines were excluded from further analysis to avoid errors due to heterogeneous material. On average the 204 RILs inherited 49.2% of their marker alleles from the female wheat parent Forno, with a range of 31–67% for individual RILs. For an individual locus the percentage of Forno alleles ranged from 21% (*Xglk165*) to 83% (*Xglk317d*).

Altogether 84 (35%) of the marker loci showed significantly ($P < 0.05$) distorted segregation favouring either the marker alleles of Forno (31) or Oberkulmer (53).

Genetic map

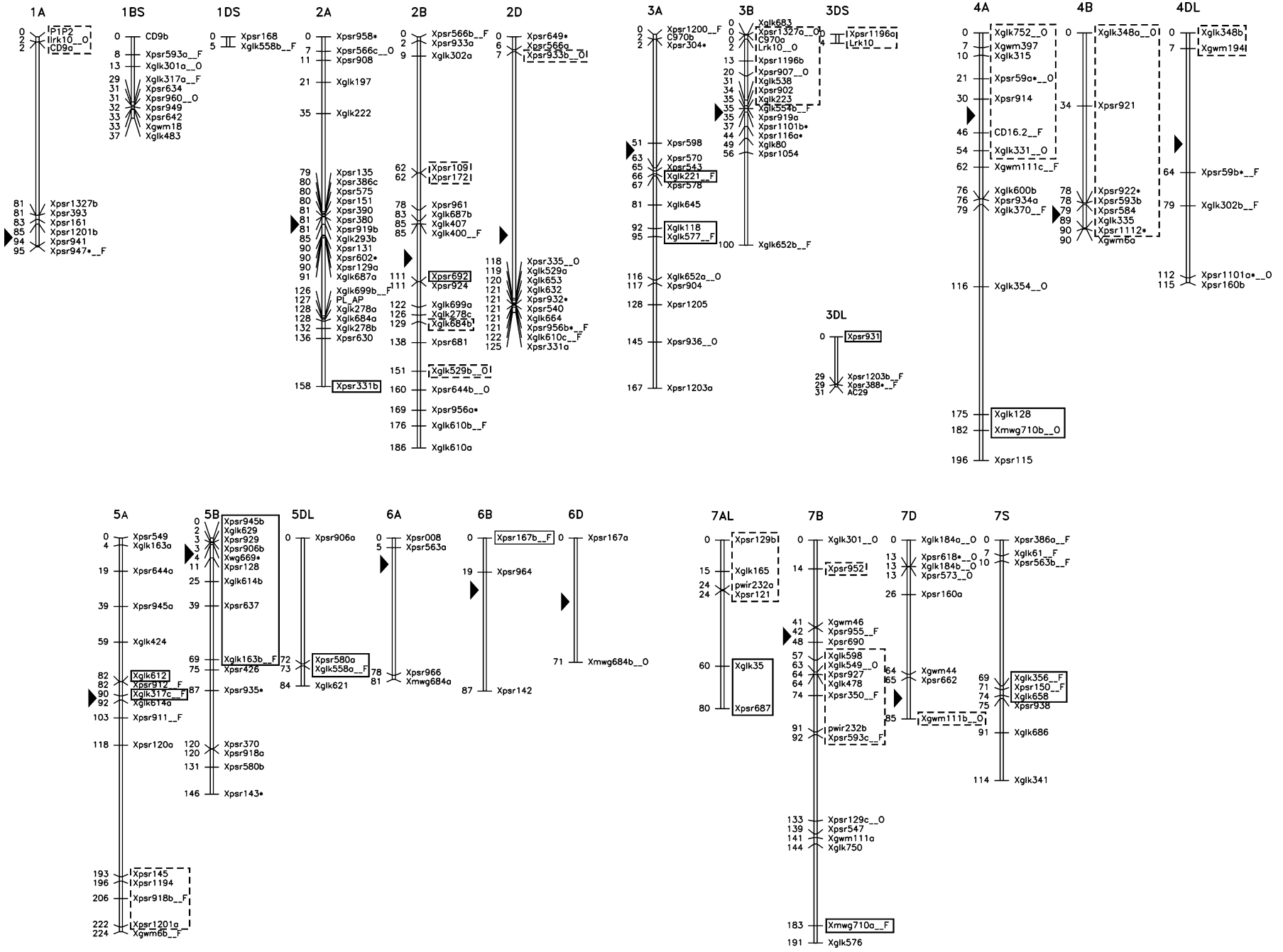
For the mapping of the 242 marker loci into linkage groups we tried several LOD thresholds. Starting from a LOD of 3.0, which is usually applied for linkage analysis, one large linkage group containing almost all marker loci was obtained. Linkage analysis with a LOD of 5.0 and 8.0 still resulted in large linkage groups, consisting of up to 199 and 50 loci, respectively. This was also the case if we used only the subset of 158 undistorted marker loci. We gradually increased the LOD threshold for significant linkage until no more *Xpsr* loci, which belong to different physical chromosomes according to the RFLP patterns of nulli-tetrasomic lines of Chinese Spring provided by M. Gale (personal communication) were linked together. Only at a LOD of 10.0 were linkage groups obtained which in all cases were in agreement with nulli-tetrasomic data. However, markers with a genetic distance of more than 20 cM were no longer significantly linked at this threshold. The mapping of the 242 marker loci with a LOD of 10.0 resulted in 37 distinct linkage groups consisting of 2–28 loci and 20 unlinked loci. After finding the best order of markers within these linkage groups, we assembled them into larger units by lowering the threshold. This resulted in a genetic map with 23 linkage groups comprising 230 loci and spanning 2469 cM (Fig. 1). These linkage groups could be assigned to the 21 wheat chromosomes based on published maps and the available nulli-tetrasomic data, except for one linkage group which belongs to homoeologous chromosome group 7 but could not be assigned to the A, B, or D genome. Some RFLP probes showed different hybridisation patterns from those indicated by the provider of the probes. These might be different from the original probe and map to a different chromosome location. Such probes are marked by stars in Fig. 1. The 84 marker loci with distorted segregation were not randomly distributed, but clustered on certain chromosome regions, i.e. chromosome segments of 3A, 5B, and 7A showed an excess of Forno alleles (solid boxes in Fig. 1), while Oberkulmer alleles were more frequent (dashed boxes) on chromosomes 1BS, 3B, 3DS, 4A, 4B, 7A, and 7B. The distorted segregation of the marker loci was not correlated with the mode of inheritance (co-dominant vs dominant). An accumulation of dominant marker loci was observed for chromosomes 4A, 4DL, 7B, 7D and 7S, where about half of the loci showed dominant inheritance in the Forno × Oberkulmer cross. For none of the linkage groups did we observe exclusively dominant markers. However, for chromosomes 5A and 7S all the dominant loci were inherited from the female wheat parent Forno, whereas for chromosome 7D all the dominant loci were inherited from the male spelt parent Oberkulmer (Fig. 1). For the 44 probes and two microsatellites with more than one polymorphic fragment, the respective loci were in 26 cases located on homoeologous chromosomes (e.g. *Xpsr566a* on 2DS; *Xpsr566b* on 2BS; *Xpsr566c* on 2AS), whereas in 17 cases these loci belong to non-homoeologous chromosomes (e.g. *Xpsr593a* on 1BS, *Xpsr593b* on 4BS; *Xpsr593c* on 7BL) and in three cases different loci of the same marker were located on the same chromosome arm (*Xglk278a/b* on 2AS; *Xglk610a/b* on 2BL; *Xglk184a/b* on 7DS). The average marker density of our map was 10.7 cM. However, the marker loci were not evenly distributed across the genome but were clustered at certain chromosomal regions (Fig. 1). As a consequence the coverage with markers varied considerably within and between chromosomes, with a minimum of two marker loci per chromosome.

Discussion

Polymorphism between wheat and spelt

Since wheat is one of the most important crops worldwide, the identification of agronomically important genes in wheat and related species is of great interest to breeders. Although genetic maps of a few mapping populations of wheat are available (McGuire and Qualset 1997) breeders are primarily interested in the genetic characterisation of their own breeding material. For QTL mapping we decided to use a wheat × spelt mapping population because of the higher degree of polymorphism we found between Forno and Oberkulmer (64%) as compared to 48% of polymorphic loci between the two Swiss winter wheat varieties Arina and Forno. The level of polymorphism between wheat and spelt was also higher than the one (28%) found by Liu et al. (1990). This can be explained by the fact that we tested each RFLP probe with seven, instead of four, different restriction enzymes and mainly used probes that had already been mapped in wide crosses (PSR clones: Gale et al. 1995; pTAG clones: Liu and Tsunewaki 1991). Cadalen et al. (1997) employed an

Fig. 1 Linkage map of 230 marker loci analysed on the 204 RILs of the cross Forno × Oberkulmer using the mapping function of Haldane. Marker loci with a *star* indicate a different RFLP pattern than that indicated by the provider of this probe. Marker loci ending with __F and __O indicate that the polymorphism was based on a locus present in Forno but absent in Oberkulmer and vice versa. Marker loci in *solid and dashed boxes* indicate a significantly ($P < 0.05$) distorted segregation in favour of the Forno alleles and Oberkulmer alleles, respectively. The approximate position of the centromeres, indicated by *arrowheads*, were deduced from published wheat maps (McGuire and Qualset 1997)


1A
1BS
1DS
2A
2B
2D
3A
3B
3DS
3DL
4A
4B
4DL
5A
5B
5DL
6A
6B
6D
7AL
7B
7D
7S

inter-varietal cross between the French variety Courtot and the variety Chinese Spring (derived from a Chinese land race) and found 58% polymorphism between the parental lines that were tested with 391 RFLP clones and seven different restriction enzymes. These results are in agreement with the hypothesis that the genetic distance between Swiss wheat and spelt germplasm is greater than within the wheat germplasm, and that wheat germplasm derived from different origins yields a higher level of polymorphism than varieties derived from the same breeding program.

Coverage of the genome

The 242 polymorphic marker loci analysed did not cover the whole genome because of clustering of the marker loci, especially on chromosomes 1BS, 2A, 2D and 3B. Clustering of marker loci in centromeric regions is a common phenomenon in wheat linkage maps (Gale et al. 1995; Cadalen et al. 1997). To obtain an improved coverage of the telomeric regions of all chromosomes a large number of additional markers would be needed. According to extended linkage map data from integrated genetic maps of wheat (McGuire and Qualset 1997) the average chromosome length varied between 150 and 200 cM, which would result in a total genome length of 3200 to 4200 cM. This corresponds to the three-fold genome length of an integrated genetic map of diploid barley (1060 cM; Qi et al. 1996). With our map spanning 2469 cM, we probably cover 2/3 of the whole genome of wheat and spelt. The distribution of marker loci across the 21 chromosomes was very similar to the wheat × spelt linkage map of Liu and Tsunewaki (1991) with a good coverage of chromosomes 2A, 2B, 4A, 5A, 5B, 7B but a generally low marker density on the D genome. This lack of polymorphism found for the D genome was also evident in the inter-varietal marker map of Cadalen et al. (1997) and is therefore not specific for wheat × spelt crosses. Despite the low marker-density on certain chromosomes, our map contributes towards a more comprehensive integration of the genetic maps of wheat collected and published by the International Triticeae Mapping Initiative (McGuire and Qualset 1997) since both *Xpsr* and *Xglk* marker loci were mapped in one population.

Chromosomal assignment

The genetic map was not readily obtained by standard software but the most likely assignment of the 37 linkage groups to the 21 physical chromosomes was achieved by an iterative process considering all possible alignments and the known map positions of some probes. One reason for the difficulty in obtaining larger linkage groups was the use of recombinant inbred lines (RILs) as a mapping population instead of F_2 plants or doubled-haploid (DH) lines. Since effective recombination events can occur in every meiotic phase until the plants are completely homozygous, the recombination frequencies between two markers are largely increased during line development from the F_1 to the F_5 generation compared to F_2 or DH populations. However, genetic distances are given in cM considering the crossover events per single meiotic phase. For example, markers with an exchange rate of 20% at the F_2 level would show an exchange rate of about 40% at the homozygous level of the RILs, both corresponding to a map distance of 20 cM. Therefore, we could not assign markers or whole linkage groups with more than a 20 cM distance without the help of nulli-tetrasomic data of Chinese Spring and published maps.

Another peculiarity of our wheat × spelt population was the high percentage of dominant marker loci (30%), which was almost twice as large as for diploid cereals (e.g. *T. tauschii*: 16%, Gill et al. 1991; barley: 14%, Graner et al. 1991; rye: 24%, Loarce et al. 1996). Liu and Tsunewaki (1991) in their wheat × spelt F_2 population also found 37% dominant RFLP marker loci, which are indicative of loci with null alleles in one of the parental lines. Such null alleles may result from insertion/deletion events or chromosome rearrangements. In an earlier study on different wheat accessions, Liu et al. (1990) reported a larger number of RFLP bands in the wheat variety Chinese Spring (66 bands) than in the spelt var. *duhamelianum* (62 bands). In our population we obtained the same ratio of polymorphic bands for the wheat variety Forno (212 bands) versus the spelt variety Oberkulmer (199 bands). If large chromosome insertions/deletions or translocations occurred between wheat and spelt one would expect the dominant marker loci to be clustered together. A continuous accumulation of dominant marker loci could only be observed for the telomers of chromosome 7DS (dominant for Oberkulmer) and for the linkage group 7S (dominant for Forno), while on all other chromosomes the dominant bands for Forno or Oberkulmer were randomly distributed and alternated with co-dominant marker loci (Fig. 1). This supports the hypothesis of several independent and small insertion/deletion events in wheat and spelt. Since an equally high degree of dominant RFLP marker loci (27%) was found in a wheat × wheat cross (Cadalen et al. 1997), one might conclude that hexaploid species in general can tolerate more chromosomal rearrangements than diploid species.

An additional problem in the assignment of linkage groups to physical chromosomes was the high proportion (37%) of heterologous marker loci, i.e. markers detecting 2–4 polymorphic loci, which do not map to the corresponding chromosomes of the A, B or D genomes, but map to different chromosome groups. Heterologous marker loci provide evidence for chromosomal rearrangements between the A, B or D genomes in wheat and/or spelt. While most of the

homoeologous marker loci (which mapped according to expectation to the homoeologous chromosomes of the A, B, or D genomes) belong to chromosome groups 2, 3 and 5, several heterologous marker loci map to groups 4 and 7 (*Xmwg710a/b*; *Xpsr593b/c*; *Xpsr160a/b*; *Xgwm111a/b/c*) or to groups 1 and 5 (*Xpsr1201a/b*; *Xglk317a/c*; *Xglk558a/b*). As a consequence, the assignment of the linkage groups to these chromosomes (1A, 4A, 4D, 5A and 7) was rather difficult. Strikingly, the same chromosomes also showed a large proportion of dominant markers (Fig. 1). Chromosome rearrangements in modern wheat have been studied in detail by Devos et al. (1995 b). They analyzed the collinearity between the wheat chromosomes 4A, 5A, and 7B and concluded that chromosome 4A of *T. aestivum* has evolved by a 4AL/5AL reciprocal translocation, followed by a 4A pericentric inversion and a 4AL/7BS reciprocal translocation and another 4AL paracentric inversion. While the 4AL/5AL translocation breakpoint was found in a range of *Triticeae* species, the other rearrangements were not found in *Triticum monococcum*, and probably arose in the tetraploid progenitor of wheat because no different arrangements were found between Chinese Spring and the synthetic wheat (Devos et al. 1995 b). These chromosomal rearrangements among homoeologous groups 4, 5 and 7 were confirmed by the mapping data of F_7 lines in a cross between synthetic wheat and variety Opata (Nelson et al. 1995 c). However, different chromosome rearrangements in the spelt parent Oberkulmer compared to the wheat parent Forno can not be excluded and might disturb the construction of the genetic map.

Genetic basis for QTL mapping

The primary goal of our mapping efforts was the construction of a genetic map that is useful to identify QTLs for disease resistance genes that are present in the Swiss wheat and spelt breeding material. Since the mapping population originates from the relevant breeding material, many positive alleles were accumulated in the parental lines that will allow the identification of QTLs for a wide range of agronomically important traits. Such QTLs can be directly exploited in practical breeding programs by marker-assisted selection. The detection of QTLs in genomic regions with a high marker density opens up the possibility of the map-based cloning of genes for quantitative resistance. On the other hand, this map is of great value for the localisation of isolated candidate resistance genes, like the *lrk10* (Gallego et al. 1998), or the *pwir232* gene coding for a thaumatin-like protein (Rebmann et al. 1991), in order to check coincidences with detected QTLs.

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References

Anderson JA, Sorrells ME, Tanksely SD (1993) RFLP analysis of genomic regions associated with resistance to preharvest sprouting resistance. Crop Sci 33:453–459

Bennet MD, Smith JB, Heslop-Harrison JS (1982) Nuclear DNA amounts in angiosperms. Proc R Soc Lond 216:179–199

Cadalen T, Boeuf C, Bernard S, Bernard M (1997) An intervarietal molecular marker map in *Triticum aestivum* L. Em Thell. and comparison with a map from a wide cross. Theor Appl Genet 94:367–377

Devos KM, Bryan GJ, Collins AJ, Stephenson P, Gale MD (1995 a) Application of two microsatellite sequences in wheat storage proteins as molecular markers. Theor Appl Genet 90:247–252

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

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Feuillet C, Schachermayr G, Keller B (1997) Molecular cloning of a new receptor-like kinase gene encoded at the *Lr10* disease resistance locus of wheat. Plant Jour 11:45–52

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 (eds) Proc 8th Int Wheat Genet Symp, China Agricultural Scientech Press, Beeijing, China, pp 29–40

Galiba G, Quarrie SA, Sutka J, Morgounov A, Snape JW (1995) RFLP mapping of the vernalization (*Vrn1*) and frost resistance (*Fr1*) genes on chromosome 5A of wheat. Theor Appl Genet 90:1174–1179

Gallego F, Feuillet C, Messmer M, Penger A, Graner A, Yano M, Sasaki T, Keller B (1998) Comparative mapping of the two wheat leaf rust resistance loci *Lr1* and *Lr10* in rice and barley. Genome 41:328–336

Gill KS, Lubbers EL, Gill BS, Raupp WJ, Cox TS (1991) A genetic linkage map of *Triticum tauschii* (DD) and its relationship to the D genome of bread wheat (AABBDD). Genome 34:362–374

Graner A, Siedler H, Jahoor A, Herrmann RG, Wenzel G (1990) Assessment of the degree and the type of restriction fragment length polymorphism in barley (*Hordeum vulgare*). Theor Appl Genet 80:826–832

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Herrmann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Haldane JBS (1919) The combination of linkage values, and the calculation of distance between loci of linked factors. J Genet 8 : 299–309

Holm S (1979) A simple sequentially rejective multiple test procedure. Scan J Statist 6 : 65–70

Hyne V, Kearsey MJ, Martinez O, Gang W, Snape JW (1994) A partial genome assay for quantitative trait loci in wheat (*Triticum aestivum*) using different analytical techniques. Theor Appl Genet 89 : 735–741

Keller M, Karutz Ch, Schmid JE, Stamp P, Winzeler M, Keller B, Messmer MM (1999) Quantitative trait loci for lodging resistance in a segregating wheat × spelt population. Theor Appl Genet 98 : 1171–1182

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

Liu YG, 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

Liu YG, Mori N, Tsunewaki K (1990) Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat. Jpn J Genet 65 : 367–380

Loarce Y, Hueros G, Ferrer E (1996) A molecular linkage map of rye. Theor Appl Genet 93 : 1112–1118

Ma ZQ, Röder M, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri- and tetra-nucleotide microsatellites in wheat. Genome 39 : 123–130

Marino CL, Nelson JC, Lu YH, Sorrells ME, Leroy P, Tuleen NA, Lopes CR, Hart GE (1996) Molecular genetic maps of the group-6 chromosomes of hexaploid wheat (*Triticum aestivum* L. em. Thell). Genome 39 : 359–366

McGuire PE, Qualset CO (1997) Progress in genome mapping of wheat and related species. Joint Proc 5th and 6th Public Wrkshps Int Triticeae Mapping Initiative, 1–3 Sept 1995, Norwich UK, and 30–31 Aug 1996, Sydney Australia. Report No 18. University of California Genetic Resources Conservation Program, Davis, California, USA

Michelmore R (1995) Molecular approaches to manipulation of disease resistance genes. Annu Rev Phytopathol 15 : 393–427

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Merlino M, Atkinson M, Leroy P (1995 a) Molecular mapping of wheat homoeologous group 2. Genome 38 : 516–524

Nelson JC, Van Deynze AE, Autrique E, Sorrells ME, Lu YH, Negre S, Bernard M, Leroy P (1995 b) Molecular mapping of wheat homoeologous group 3. Genome 38 : 525–533

Nelson JC, Sorrells ME, Van Deynze AE, Lu YH, Atkinson M, Bernard M, Leroy P, Faris JD, Anderson JA (1995 c) Molecular mapping of wheat. Major genes and rearrangements in homoeologous groups 4,5, and 7. Genetics 141 : 721–731

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

Qi X, Stam P, Lindhout P (1996) Comparison and integration of four barley genetic maps. Genome 39 : 379–394

Quarrie SA, Gulli M, Calestani C, Steed A, Marmiroli N (1994) Location of a gene regulating drought-induced abscisic acid production on the long arm of chromosome 5A of wheat. Theor Appl Genet 89 : 794–800

Ranhotra GS, Gelroth JA, Glaser BK, Lorenz KJ (1995) Baking and nutritional qualities of a spelt-wheat sample. Lebensm-Wiss u-Technol 28 : 118–122

Rebmann G, Mauch F, Dudler R (1991) Sequence of a wheat cDNA encoding a pathogen-induced thaumatin-like protein. Plant Mol Biol 17 : 282–285

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

Schmid JE, Winzeler H (1990) Genetic studies of crosses between common wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). J Genet Breed 44 : 75–80

Schmid JE, Winzeler M, Winzeler H (1994) Analyis of disease resistance and quality characters of F_1 hybrids of crosses between wheat (*Triticum aestivum*) and spelt (*Triticum spelta*). Euphytica 75 : 105–110

Siedler H, Messmer MM, Schachermayr GM, Winzeler H, Winzeler M, Keller B (1994) Genetic diversity in European wheat and spelt breeding material based on RFLP data. Theor Appl Genet 88 : 994–1003

Stam P, Van Ooijen JW (1995) JoinMap (tm) version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Van Deynze AE, Dubcovsky J, Gill KS, Nelson JC, Sorrells ME, Dvorak J, Gill BS, Lagudah ES, McCouch SR, Appels R (1995) Molecular-genetic maps for group-1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genetics 38 : 45–59

Winzeler H (1988) Dinkelanbau und Dinkelforschung in der Schweiz. 1. Dinkelsymposium in der Universität Hohenheim, 29 July, 1988, pp 51–59

Winzeler H, Rüegger A (1990) Dinkel: Renaissance einer alten Getreideart. Landwirtschaft Schweiz 9 : 503–511

Winzeler H, Schmid JE, Winzeler M (1994) Analysis of the yield potential and yield components of F_1 and F_2 hybrids of crosses between wheat (*Triticum aestivum* L.) and spelt (*Triticum spelta* L.). Euphytica 74 : 211–218