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Construction of an RFLP map of barley

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Summary. In order to construct an RFLP map of barley, two populations were analyzed using 251 genomic and cDNA markers: one population comprised 71 F_1 anther-derived double haploid (DH) individuals of an intraspecific cross (IGRI × FRANKA), and the other 135 individuals of an interspecific F_2/F_3 progeny (VADA × *H. spontaneum*). The distribution of nonrepetitive clones over the seven barley chromosomes revealed a maximum for chromosome 2H and a minimum for 6H. The polymorphism of the interspecific progeny (76%) clearly exceeded that of the intraspecific progeny (26%) although, based on their pedigrees, IGRI and FRANKA are only distantly related. The contribution of individual chromosomes of the DH parents to the overall polymorphism varied between 8% and 50%. A significant portion (44% versus 10% of the interspecific progeny) of the markers mapped on the DH offspring showed distorted segregation, caused mainly by the prevalence of variants originating from the parent that better responded to in vitro culture (IGRI). In contrast to the interspecific map, probes displaying skewed segregation were clustered on the DH map on discrete segments. The colinear arrangement of both maps covers a distance of 1,453 cM and identifies regions of varying map distances.

Key words: Restriction fragment length polymorphism – Linkage map – *Hordeum vulgare* – Double haploids

Introduction

Their environmental stability and nearly unlimited availability have made RFLP (restriction fragment length polymorphism) markers an ideal tool for plant breeding. Already 10 years after their first application in the human genome, RFLP maps have been or are being constructed for the genomes of various major crop plants (Helentjaris et al. 1986; McCouch et al. 1988; Bonierbale et al. 1988; Gebhardt et al. 1989; Slocum et al. 1990), first correlations between RFLP markers and qualitative or quantitative traits have been reported (Paterson et al. 1988; Sarfatti et al. 1989; Barone et al. 1990; Klein-Lankhorst et al. 1991; Yu et al. 1991), and procedures have been devised to maximize the benefits of marker-assisted selection based on RFLPs in long-term breeding programs (Tanksley et al. 1989; Murray et al. 1991). RFLP markers also provide useful tools for both evolutionary studies and the characterization of germplasm stocks (Debener et al. 1990; Miller and Tanksley 1990; Melchinger et al. 1991). Tightly linked RFLP markers may serve in turn as starting points for the characterization of genes without prior knowledge of their products or may render possible the physical characterization of large DNA fragments by pulsed field gel electrophoresis (Ganal et al. 1989; Rommens et al. 1989; Jung et al. 1990; Siedler and Graner 1991).

An indispensable prerequisite for the application of these techniques is the availability of an RFLP map of appropriate resolution. As a diploid inbreeder, barley has been an ideal plant for genetic studies for decades, which is illustrated by its elaborate genetic map. It comprises more than 150 phenotypic markers, which define seven linkage groups corresponding to the haploid chromosome number of barley (von Wettstein-Knowles 1991). Moreover, barley represents one of the first major crop plants that could be regenerated in vitro from androgenic structures (Clapham 1973). Today, refined methods allow haploid regeneration that is efficient enough for genetic studies of both qualitative and quantitative traits (Kuhlmann and Foroughi-Wehr 1989).

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Nevertheless, first results regarding mapped RFLP markers in barley have only emerged recently (Shin et al. 1990). This might be partly due to technical problems resulting from the large genomes shared by most graminaceous species also containing huge amounts of repetitive DNA, and partly to a comparatively low degree of polymorphism (Graner et al. 1990), which leads to a considerable increase in labor intensity of the mapping process.

This paper describes attempts to develop an RFLP map for barley pursuing two strategies: (i) a population consisting of 71 anther-derived F_n plants of an intraspecific cross was used in order to construct a map of highly polymorphic markers, and (ii) a population comprising 135 F_2 individuals of an interspecific cross between *Hordeum vulgare* and *Hordeum spontaneum* was analyzed in order to map a maximum number of markers, which is a prerequisite for the construction of a high-density map.

Materials and methods

Plant material

For RFLP mapping, a population of 71 F_1 anther-derived double haploid (DH) plants of the winter barley cultivars IGRI (two-rowed) and FRANKA (six-rowed) was used. In vitro regeneration of anthers was conducted as described by Foroughi-Wehr and Friedt (1984). The cultivar IGRI contains the *Mla8* and FRANKA the *Mla6* resistance gene against powdery mildew. Segregation of the *Mla6* gene was evaluated in two replications according to Jahoor and Fischbeck (1987a), using the powdery mildew (*Erysiphe graminis* DC f. sp. *hordei* Marchal) isolates 184/21 and Ru/3, both virulent against *Mla8* and avirulent against *Mla6*.

A second mapping population was formed by 135 F_2 individuals/F_3 families of an interspecific cross between the spring barley cultivar VADA and the *H. spontaneum* line 1B-87. This line contains an absolute resistance gene against powdery mildew, which is effective against all European isolates but whose chromosomal location remains yet to be determined (Jahoor and Fischbeck 1987b).

A set of wheat-barley addition lines (Islam et al. 1981) containing disomic additions of entire chromosomes (except of chromosome 1H) or of telosomes was used for the chromosomal assignment of RFLP probes. Individual barley chromosomes have been designated according to their homoeology with wheat chromosomes (Jahoor et al. 1991).

RFLP probes

Two sources of anonymous RFLP probes were used: (i) various independent *Pst*I libraries of genomic barley DNA, which were constructed as described previously (Graner et al. 1990), and (ii) a cDNA library, which was prepared from total RNA of 10-day-old barley leaves (MacDonald et al. 1987). The cDNAs were prepared from poly A+ RNA (Maniatis et al. 1982) according to the instructions of the manufacturer (cDNA synthesis kit, Boehringer, Mannheim). Both types of libraries were cloned into the plasmid vector "Bluescript" (Stratagene, San Diego) and maintained in *E. coli* DH5α. Recombinant plasmids containing inserts larger than 500 bp (cDNAs 400 bp) which generated single-or low-copy signals on Southern blots were used for further analysis. RFLP probes were designated by a code, with the first three letters ("MWG") referring to the institutes of the authors, followed by a numerical designation. Multiple loci detected by one probe were distinguished by lowercase letters at the end of the code, e.g., "MWG561a." A lowercase "c" at the beginning of the code identifies cDNA probes, e.g., "cMWG655."

Probes from cloned genes and isoenzyme analysis

A cDNA clone of the waxy gene from barley (pWx27) was kindly provided by Dr. W. Rohde (Max Planck Institute, Cologne). *Hor1* (pc612) and *Hor2* (pchor2–4) specific probes, which were a gift from Dr. A. Brandt (Carlsberg Laboratory, Copenhagen), were trimmed and subcloned into "Bluescript" to give pBSC5 and pBSC4 (Siedler and Graner 1991). Analysis of esterases from 14-day-old leaves was essentially done according to Deimling et al. (1988). Identification of individual esterase bands was performed according to the scheme of Kahler and Allard (1970).

DNA techniques

Isolation of genomic DNA, Southern analysis, and probe labelling were performed essentially as described (Graner et al. 1990), except that a solution containing 0.4 *M* NaOH and 0.6 *M* NaCl was used for the transfer of DNA onto Nylon membranes (Pall, Portsmouth). Five restriction endonucleases (*Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII, *Xba*I) were chosen for RFLP mapping in both populations; in addition, a sixth enzyme, *Sac*I, was used for the analysis of the DH population.

Data analysis

Linkage analysis in both populations was performed on a Sun work station run under a Unix operating system using "Mapmaker" computer software (Lander et al. 1987). The following parameters were preset (values for DH population are given between brackets): two-point analysis LOD 3.0 (2.7), multipoint analysis LOD 3.0 (2.7), maximum recombination value 0.35 (0.3). Recombination values were converted into map distances (centiMorgan, cM) by applying the Kosambi function (Kosambi 1944).

Results

Genomic distribution of clones

Over 450 recombinant barley genomic or cDNA clones were screened to identify polymorphism within the two mapping populations. Genomic clones were selected from several independently constructed *Pst*I libraries for those that hybridized to polymorphic single- or low-copy number sequences in the parents. RFLP clones were derived from all seven barley chromosomes as judged from hybridization with the wheat-barley addition lines. The distribution of 320 such probes across the chromosomes is illustrated in Fig. 1. The combination of the individual data sets for each chromosome illustrates an overall accumulation of clones on chromosome 2H and a reduced number for chromosome 6H. Some variation in the distribution of clones derived from different *Pst*I libraries was noted for chromosome 4H (hatched bar versus black bar).

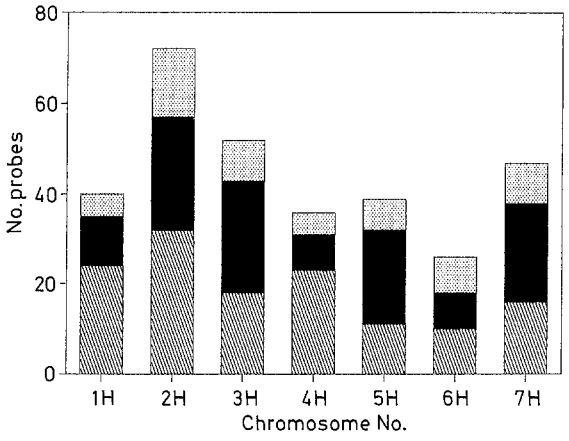

Fig. 1. Distribution of DNA markers from different sources across the barley genome. *Hatched* and *black bars:* genomic *Pst*I clones isolated in two independent experimental series. *Stipled bars:* cDNA clones

Polymorphism within mapping populations

Approximately 76% of the clones tested were polymorphic between the parents of the interspecific cross between *H. vulgare* and *H. spontaneum*, but only 28% were polymorphic between those of the intraspecific DH progeny. The percentage of informative single-copy clones for the latter population is listed in Table 1 for each individual chromosome. Homologous chromosomes of the two parents differ considerably in their individual contribution to the overall polymorphism. Chromosomes 5H, 6H, and 7H display a substantially higher diversity between the two parents than the remaining chromosomes.

Map construction

Intraspecific DH progeny. The map derived from linkage data of the DH progeny comprises 88 loci, which are identified by 114 RFLP markers. It covers approx. 870 cM of the barley genome. Based on the segregation data, the markers can be divided into three groups (Table 2). The first group, which comprises approx. 56% of the markers, shows the expected 1:1 ratio of IGRI and FRANKA variants. The second and third display a prevalence of IGRI (30%) or FRANKA variants (14%). Taken together significantly more IGRI than FRANKA variants were detected. Loci showing distorted segregation, however, were not scattered throughout the genome but confined to a few distinct segments on chromosomes 2H, 3H, 5H, and 7H (Fig. 2).

Complementary to RFLP markers, two phenotypic (*hex-v, Mla6*) and two isoenzyme markers (*Est1*, *Est5*), as well as three cloned genes (*Hor1, Hor2, wx*) were included in the DH map. Of these, *Mla6* is known to be located on the short arm of chromosome 1H, where it is flanked by the *Hor1* and *Hor2* loci, respectively. The *hex-v* locus

Table 1. Contribution of individual chromosomes to the polymorphism of the DH mapping population. The number of single-copy clones, determined by hybridization on wheat-barley addition lines, is shown in column 2. The fraction of polymorphic probes between both parents (IGRI, FRANKA) is given in columns 3 and 4

Chromosome	Addition-lines	Polymorphic	%
1H	17	3	18
2H	37	8	22
3H	30	5	14
4H	12	1	8
5H	26	11	42
6H	12	6	50
7H	29	13	45

Table 2. (I) Distribution of RFLP markers showing normal or distorted single-locus segregation in DH progeny ($P > 95\%$; I > F: prevalence of IGRI variants, F > I: prevalence of FRANKA variants). (II) Distribution of IGRI and FRANKA variants for individual chromosomes. IGRI variants prevail on chromosomes 3H, 5H, 6H, and 7H, FRANKA variants on chromosome 2H. Summed values show a strong deviation from 1:1 segregation ($P > 99.95\%$)

(I)				(II)		
Chromosome	Undistorted	I > F	F > I	Chromosome	IGRI	FRANKA
1H	10	2	0	1H	177	160
2H	6	0	15	2H	559	901
3H	7	10	0	3H	958	515
4H	4	0	0	4H	35	36
5H	15	9	0	5H	761	579
6H	10	0	0	6H	390	296
7H	11	13	0	7H	838	636
%	56	30	14,	Σ	3,718	3,123

(six-row) maps on chromosome 2H; the starch synthase locus, which is marked by pWx27, identifies the short arm of chromosome 7H. Furthermore, on chromosome 7H, the *Est5* locus has been determined to map 1.4 cM distally to the *wx* locus. At the end of the long arm of chromosome 3H the *Est1* locus has been included on the map.

Interspecific progeny. The map deduced from the interspecific cross comprises more than 160 markers covering 1,408 cM. The majority of the loci (86%) were evaluated on the basis of a 1:2:1 segreagtion. The fraction of markers that displayed skewed segregation was considerably smaller (10%) in this progeny than in the DH progeny. Furthermore, these probes were in general more evenly distributed over the genome. Due to the relatively large number of markers, a better genome cov-

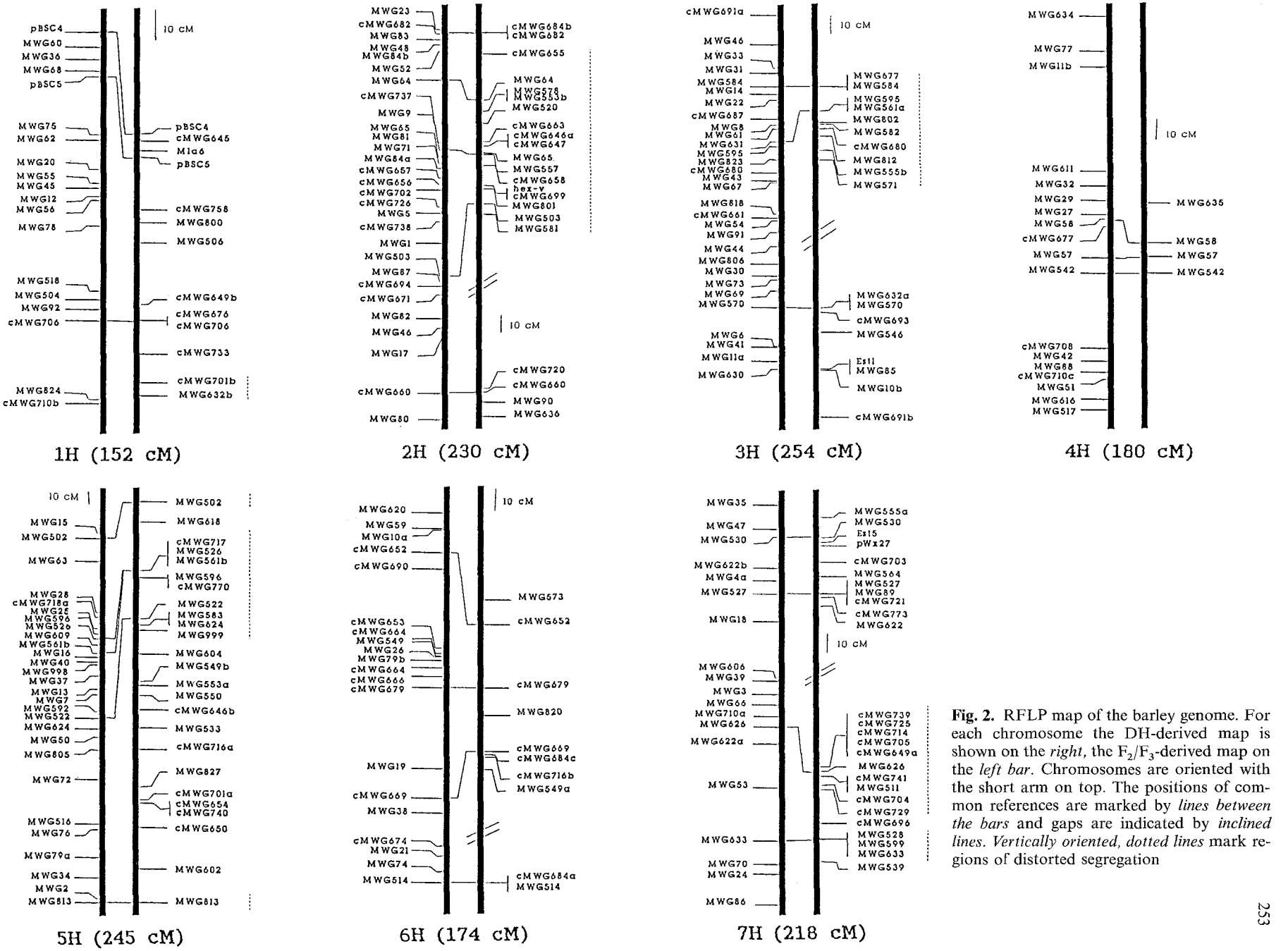

Fig. 2. RFLP map of the barley genome. For each chromosome the DH-derived map is shown on the *right*, the F_2/F_3-derived map on the *left bar*. Chromosomes are oriented with the short arm on top. The positions of common references are marked by *lines between the bars* and gaps are indicated by *inclined lines*. *Vertically oriented, dotted lines* mark regions of distorted segregation

erage could be achieved by mapping with the interspecific progeny.

Alignment of both RFLP maps. In an attempt to align the two linkage maps, a common set of RFLP markers for each offspring and chromosome was included. It provides contact sites between both maps and allows their colinear arrangement. The integrated map covers a total of 1,453 cM and comprises 226 loci, which are detected by 251 markers (Fig. 2).

The orientation of individual chromosome arms was determined by hybridization of selected markers to ditelosomic wheat-barley addition lines or, in the case of chromosome 1H, by incorporation of previously mapped genes (*Hor1, Hor2*).

A comparison of both maps identifies regions of varying map distances. In most cases the DH progeny shows reduced intervals between common markers, which is accompanied by an apparent accumulation of RFLP probes showing no recombination. Despite the lower number of mapped markers, the DH map contains a higher portion of intervals smaller than 5 cM (38% versus 32%). Nevertheless, the linear order of markers is generally conserved on both maps.

Discussion

In the present study, more than 200 RFLP probes were used to construct a molecular map of the barley genome. Hybridization on wheat-barley addition lines disclosed a slightly uneven distribution of single- and low-copy markers between the chromosomes, with a maximum of probes extracted from chromosome 2H. The number of markers per chromosome is certainly a function of chromosome size; on the other hand our findings show that chromosome 1H, which is cytologically the smallest, carries more markers than three other chromosomes (6H, 7H, 4H) and chromosome 3H carries fewer markers than 2H, altough both are of similar size. Disregarding the presence of satellites on chromosomes 5H and 6H, this might indicate an uneven distribution of repetitive DNA in the genome.

In order to map RFLP markers in barley, two different genetic systems can be used: F_2/F_3 progenies, which are easily obtained from a wide range of genotypes but provide only a limited source of plant material to be scored for phenotypic traits. In contrast, the generation of DH progenies either by the *bulbosum* technique (Kasha and Kao 1970), by anther or isolated microspore culture (Kuhlmann and Foroughi-Wehr 1989) is more complex and time-consuming. Segregating populations of the latter kind, however, provide a good basis to record small phenotypic differences, which is often a prerequisite if quantitatively inherited traits are to be considered. The present IGRI × FRANKA DH population has been chosen for analysis, since it segregates both for mildew resistance and resistance to barley yellow mosaic virus (BaYMV), a trait which is difficult to score on single plants.

As expected, the degree of polymorphism of the interspecific progeny (76%) clearly exceeded the diversity of the intraspecific progeny, in which only 28% of the single-copy probes could be mapped. Taking into account the fact that cDNA probes reveal less polymorphism than genomic clones (unpublished results), this value is in accordance with data from a previous study (Graner et al. 1990). Detailed analysis of DNA polymorphism between IGRI and FRANKA revealed clear differences in the diversity of homologous chromosomes. Chromosomes 5H, 6H, and 7H exhibit diversity of more than 40%, whereas the remaining chromosomes showed less than 25%, implying that it might become difficult to fill gaps or to saturate the map for these chromosomes even after extensive analysis of the progeny. This is especially true for chromosome 4H, where only a very limited number of markers showed segregation to date.

These RFLP data are opposed by a low coefficient of parentage between IGRI and FRANKA (0.05), which means that, at least based on their pedigree data, both cultivars represent quite distantly related genotypes within the gene pool of winter barley. Together with previous results (Graner et al. 1990) this suggests that variation, at least within the German gene pool of winter barley, might be insufficient to construct a saturated RFLP map based on the analysis of a single intraspecific cross.

The colinear arrangement of both maps shows regions in which map distances differ. In interspecific crosses, reduced crossover frequencies may in regions of limited homology. However, reduced recombination in DH progenies has also been reported by Powell et al. (1986), findings that are consistent with our observation of reduced map distances in the DH map in regions, which are delimited by common markers. This map also includes a large number of loci marked by more than one RFLP probe. Further data are needed, however, to decide whether these phenomena are the result of a general reduction of recombination in the male gametophyte.

Forty-three percent of the probes mapped in the DH population showed skewed segregation ($P <= 0.05$). These probes are not distributed randomly throughout the genome but confined to distinct regions. A preponderance of FRANKA variants was only observed for a large region of chromosome 2H, whereas segments showing prevalence of IGRI variants were found in discrete regions on chromosomes 1H, 3H, 5H, and 7H. Distorted segregation in the same order of magnitude has been observed in serveral DH progenies of barley (Powell et al. 1986; Thompson et al. 1991). The differential trans-

mission of alleles might be the result of selection during the in vitro phase. This assumption is in accordance with the overall accumulation of alleles from IGRI, the parent that better responds to in vitro culture.

The objective of the present study was to construct an RFLP map, providing the basis for marker-assisted selection. Although recombination frequencies of the DH-derived map are slightly reduced on several chromosomes, the colinearity of both maps underlines the usefulness of anther-derived DH progenies for further genetic studies. In order to combine the advantages of both mapping populations used in this study, the generation of a DH population derived from an interspecific cross is currently underway. Together with the construction of chromosome-specific DNA libraries (Lüdecke et al. 1990), this should facilitate the generation of a high-density map, which is a prerequisite to further investigate the barley genome at the molecular level.

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