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Assessment of the degree and the type of restriction fragment length polymorphism in barley (*Hordeum vulgare*)

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Summary. In order to determine the extent of polymorphism in barley (*Hordeum vulgare*), DNA from 48 varieties was analyzed with 23 genomic, single-copy probes, distributed across all seven chromosomes. Upon hybridization to wheat-barley addition lines, the probes showed different degrees of homology compared to the wheat genome. Polymorphisms were detected in the barley genome at a frequency of 43% after digestion with EcoRI, BamHI, and HindIII. Subgroups of spring and winter barley and of two- and six-rowed types showed less diversity which, in most cases, was due to shifts in allelic frequencies. One probe (MWG1H504) hybridized to an EcoRI restriction fragment exclusively observed in winter barley. A comparison of six different restriction enzymes revealed clear differences with regard to their efficiency in detecting polymorphisms. The respective frequencies were between 13% (HindIII) and 37% (EcoRV). A significant correlation between the efficiency of a restriction enzyme and the mean fragment size detected by the different probes identified insertion/deletion events as the major factor causing polymorphism in barley.

Key words: Barley – Restriction fragment length polymorphism – DNA – Wheat-barley addition lines – Chromosome assignment

Introduction

The application of RFLP techniques to plant breeding may lead to a substantial improvement of selection efficiency in breeding for qualitative and especially for quantitative traits (Beckmann and Soller 1988; Tanksley et al. 1989; Helentjaris et al. 1985). In addition, RFLP markers are potential landmarks for the physical mapping of chromosomal regions that cannot be further resolved by classical genetic methods. As a prerequisite for the general application of this approach to both plant breeding and molecular genetics, RFLP maps are being developed for various plant species, including the major field crops (McCouch et al. 1988; Helentjaris et al. 1986; Bonierbale et al. 1988).

Despite the widespread cultivation of barley and its elaborate genetic map, which comprises morphological as well as isozyme markers (Sogaard and von Wettstein-Knowles 1987), knowledge regarding the application of RFLP markers in this species is limited (Bunce et al. 1986; Blake 1987). On the other hand, in vitro techniques such as anther or microspore culture are well developed in barley. Their combination with marker-assisted selection might accelerate the breeding process considerably. For example, analysis of doubled haploid lines that can be obtained in sufficient numbers from F_1 anthers or microspores (Kuhlmann and Foroughi-Wehr 1989) can provide a sound basis for establishing an RFLP linkage map and be used to dissect quantitative trait loci. The general application of RFLP techniques to plant breeding however, depends on the extent of DNA fragment polymorphism within the population under consideration. It is the objective of the present paper to investigate the degree of genomic variability within *Hordeum vulgare* as a first step towards the mapping of sufficiently large numbers of DNA probes, and to establish linkage relationships between RFLP markers and traits of agronomic interest.

Materials and methods

Plant material

In order to estimate the extent of DNA polymorphism within the species *Hordeum vulgare*, the present study was been based

on data obtained from a collection of 48 varieties with entry dates in the variety list ranging from 1925 to 1988. This collection includes 24 varieties of both spring and winter types (two- and six-rowed), as well as varieties showing high malting performance and/or resistance against various diseases such as powdery mildew or barley yellow mosaic virus. The collection reflects a representative cross section of the barley gene pool, available to any plant breeder in Germany. Wheat-barley addition lines (Islam et al. 1981) were used for the chromosomal assignment of RFLP probes. The presence of the disomic barley chromosome addition (except for chromosome *1H*, which cannot be maintained in a viable addition line) was examined by analysis of metaphase chromosomes in root tips. Barley chromosomes were designated in agreement with the nomenclature of wheat chromosomes, i.e., the former chromosome 5 renamed as *1H*, chromosome 7 as *5H* and chromosome 1 as *7H*.

DNA isolation

DNA for Southern analysis was isolated according to Saghai-Maroof et al. (1984). Genomic DNA (cultivars IGRI and FRANKA) to be cloned into plasmids was prepared as described by Steinmüller and Apel (1986).

Preparation of DNA probes

If not otherwise stated, all protocols used for DNA cloning were performed according to Maniatis et al. (1982). Genomic DNA was digested to completion with PstI, ligated into dephosphorylated, PstI-cut Bluescript vector (Stratagene, San Diego), and transformed into *E. coli* DH5α cells. The ratio of insert DNA to vector DNA was 7 to 1. Plasmid DNA was prepared by the rapid lysis method of Holmes and Quigley (1981). After digestion with PstI, the resulting inserts were separated from vector DNA by electrophoresis using 1.5% low melting agarose. Labeling of total genomic DNA as well as labeling of plasmid inserts was carried out with ^{32}P-dCTP, applying the random-primer method (Feinberg and Vogelstein 1983). Non-incorporated nucleotides were removed by gel filtration through Sephadex G-50 (Pharmacia, Uppsala). Probes were designated by an eight-digit code, with the first three letters representing the acronyms of the institutes of the authors, the following two positions indicating the chromosomal origin of the probe, and the remaining three digits, the laboratory code.

DNA restriction and Southern analysis

Barley DNA was restricted with 3 units enzyme/μg DNA according to the instructions of the manufacturer (Boehringer, Mannheim). Electrophoresis was carried out in 0.8% agarose slab gels at 1.2 V/cm overnight. DNA (10 μg/lane in the case of barley DNA, 30 μg/lane in the case of DNA from wheat-barley addition lines) was transferred onto Biodyne 'B' nylon membranes (Pall, Portsmouth) under alkaline conditions (1.5 *M* NaCl, 0.6 *M* NaOH). Hybridization was performed under standard conditions in a solution containing 5 × SSPE (20 × SSPE = 3.6 *M* NaCl; 200 m*M* NaH_2PO_4, pH 7.4; 20 m*M* EDTA) at 66 °C. After completion of the hybridization, membranes were washed twice for 30 min at 50 °C in 0.5 × SSC, 0.1% SDS (20 × SSC = 3 *M* NaCl, 0.3 *M* Na-Citrate, pH 7.0), once in 0.2 × SSC, 0.1% SDS at 65 °C for 30 min, and exposed at −80 °C to XOMAT AR X-ray film (Eastman Kodak, Rochester), using Quanta III intensifying screens (DuPont, Paris).

Data analysis

Data were analyzed on a PC-AT using an integrated software package (Software Products Intl, Munich). Different bands on the autoradiographs were numbered and entered into the database in a binary fashion, i.e., "1" indicating the presence, and "0", the absence of a band (allele). All possible pairwise comparisons of all barley varieties studied were performed, and the number of informative comparisons was divided by the total number of comparisons (1,128), yielding an index value between zero and one. A probe index was formed by combining the additive information gathered with the restriction endonucleases EcoRI, BamHI, and HindIII. The mean enzyme index was computed from the data of all probes obtained in combination with the respective restriction endonuclease.

Results

Probe selection

Plasmids containing highly repetitive DNA sequences were discarded after dot blot hybridization with total genomic DNA. From the remaining clones, single- or low-copy inserts were identified by analyzing the hybridization patterns on Southern blots. Probes were considered to contain single-copy sequences if the bulk of cultivars showed no more than two bands on Southern blots of HindIII-digested DNA (cv IGRI). This requirement was met by approximately 50% of the clones. Insert sizes ranged from 0.5 to 2.0 kb, with an average of 0.9 kb.

Chromosome assignment

Twenty-three single-copy clones, chosen at random, were mapped to the individual barley chromosomes by hybridization to HindIII-digested DNA of wheat-barley addition lines. All but three probes (MWG6H505, MWG7H510, MWG2H521) showed homology to distinct sequences in the wheat genome, which resulted in up to nine wheat specific bands (Fig. 1). In the case of comigration of wheat and barley bands, a second enzyme (BamHI) was used to achieve a separation of the respective signals.

Polymorphism within the species

A polymorphism is defined by the difference between two individuals or genotypes. Consequently, the probes were characterized by pairwise comparisons of their hybridization patterns. The resulting probe index indicates the likelihood that a given probe can differentiate between two varieties, randomly chosen from the 48 varieties used in this study. In order to allow for the different sources of RFLPs, namely, point mutations and chromosomal rearrangements, the calculation was based on the added information of the three restriction endonucleases EcoRI, BamHI, and HindIII. Nineteen out of the 23 probes tested (83%) detected polymorphism, expressed by up to six different alleles (Table 1, column 2). The average probe index is 0.43, with individual values ranging from 0 to 0.78. The distribution of the probe indices shows that approximately 25% of the tested probes gave

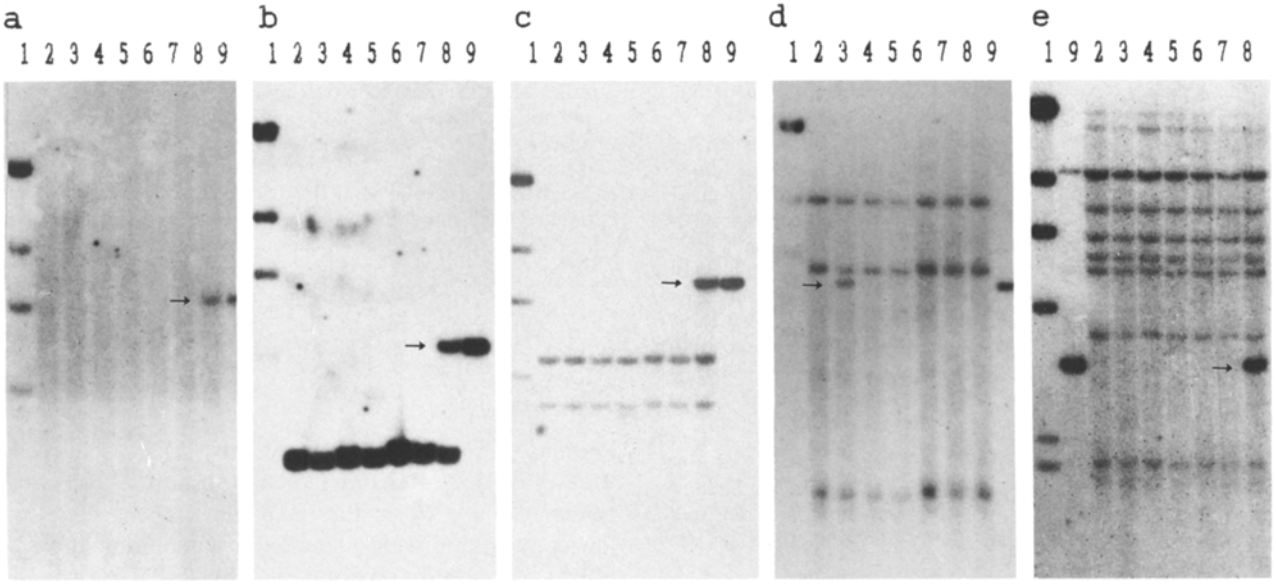

Fig. 1a–e. Examples of different patterns of homology between wheat and barley DNA sequences, as obtained after mapping genomic barley clones to the individual chromosomes by means of wheat-barley addition lines. Southern blots of HindIII-digested DNA of different wheat lines (cv Chinese Spring), each containing a disomic addition of a barley chromosome (cv Betzes), were probed with five different genomic barley clones, representing single-copy sequences in the barley genome (**a–e**). *Lanes* on the respective autoradiographs contain: *1* Lambda HindIII molecular weight marker; *2* DNA of Chinese Spring (CS) + barley chromosome *7H; 3* CS + *2H; 4* CS + *3H; 5* CS + *4H; 6* CS (wheat control); *7* CS + *6H; 8* CS + *5H; 9* Betzes (barley control). Barley bands in addition lines are marked by an *arrow*. Note that wheat lines containing additions of barley chromosome *1H* are not available

Table 1. Compilation of individual and mean index values. Probe indices are based on a data set comprising 48 varieties and the added information contributed by the restriction endonucleases EcoRI, BamHI, and HindIII. Enzyme indices for six different restriction endonucleases are based on comparisons within a subset of 19 varieties

Probe	Probe index	EcoRI	BamHI	HindIII	EcoRV	SacI	XbaI
MWG7H507	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MWG7H510	0.51	0.64	0.00	0.00	0.67	0.00	0.00
MWG7H511	0.15	0.11	0.00	0.00	0.20	0.00	0.00
MWG7H515	0.70	0.76	0.50	0.50	0.54	0.00	0.53
MWG7H523	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MWG7H524	0.69	0.76	0.53	0.53	0.90	0.00	0.45
MWG2H501	0.73	0.00	0.28	0.68	0.67	0.28	0.67
MWG2H520	0.22	0.11	0.11	0.00	0.58	0.11	0.00
MWG2H521	0.61	0.61	0.38	0.11	0.11	0.00	0.11
MWG3H509	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MWG3H512	0.71	0.51	0.43	0.00	0.35	0.39	0.66
MWG4H517	0.71	0.49	0.31	0.30	0.12	0.53	0.20
MWG4H519	0.65	0.64	0.61	0.61	0.75	0.57	0.49
MWG1H504	0.66	0.56	0.20	0.00	0.20	0.77	0.73
MWG1H508	0.00	0.00	0.00	0.00	0.00	0.00	0.00
MWG1H516	0.28	0.22	0.20	0.00	0.49	0.00	0.00
MWG1H518	0.78	0.43	0.41	0.35	0.42	0.45	0.00
MWG1H506	0.04	0.00	0.00	0.00	0.00	0.00	0.53
MWG6H505	0.50	0.00	0.53	0.00	0.53	0.00	0.00
MWG6H514	0.43	0.37	0.37	0.00	0.71	0.84	0.63
MWG5H502	0.47	0.00	0.49	0.00	0.64	0.46	0.52
MWG5H513	0.62	0.00	0.56	0.00	0.56	0.00	0.52
MWG5H522	0.54	0.63	0.00	0.00	0.00	0.00	0.64
Mean	0.43	0.30	0.26	0.13	0.37	0.19	0.29

values below 0.3. On the other hand, a considerable number of probes (ca. 40%) displays values equal or larger than 0.5. It is worth noting that, in most instances, more than 90% of the respective information affecting the probe index is contributed by only one out of the three enzymes mentioned above.

In order to estimate the extent of polymorphism in spring and winter types, comparisons were made within the respective subgroups. In contrast to the entire collection, the respective subgroups showed a marked decrease in polymorphism, reflected by reduced average values (Fig. 2). This reduction is caused by a shift of allelic frequencies, resulting in a reduction of the percentage of informative comparisons within the respective subgroup. Hence, a portion of the overall polymorphism as observed in the entire collection results from alleles that are mainly confined to either spring or winter barley. In this context, eight probes showed decreased levels of diversity, simultaneously within both spring and winter types, indicating that the respective alleles are not evenly distributed across all genotypes, but accumulated within the spring or winter subgroup.

Several probes revealed interesting patterns in showing zero polymorphism confined to a certain subgroup. For example, probe MWG1H506 displayed no polymorphism within winter barley, whereas probe MWG5H522 did not reveal polymorphism within spring barley. Probe MWG6H505 showed no polymorphism within six-rowed winter types. In all three cases, however, the invariant alleles were not restricted exclusively to the respective subgroup. Yet another probe, MWG1H504, was also unable to detect polymorphism in winter barley. In this case, HindIII digests showed an invariant band with both spring and winter types, whereas BamHI digests showed an allele invariant in winter types, but also occurring in spring types, which were polymorphic. EcoRI digests, however, revealed an allele that is restricted exclusively to winter types (Fig. 3). In accordance with locus SH_3 affecting spring/winter habit, this probe maps on chromosome *1H*. Further genetic analysis will be required to substantiate whether this probe is linked to this locus.

Distribution of enzyme indices

Six different restriction endonucleases EcoRI, BamHI, HindIII, EcoRV, XbaI, SacI were compared for their ability to generate polymorphic DNA fragments. To this end, pairwise comparisons within a subset consisting of 19 varieties were performed (Table 1). While four probes did not detect polymorphism with any of the six restriction endonucleases chosen, 14 probes were informative with two or more restriction endonucleases. Mean enzyme indices indicate differences between the different restriction endonucleases with respect to their efficiency

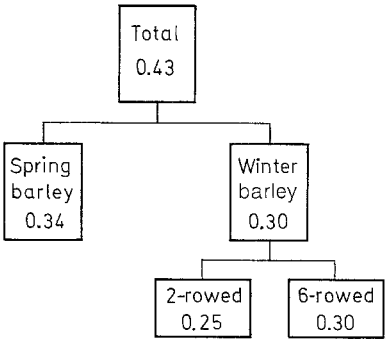

Fig. 2. Mean probe indices as obtained for the subgroups of spring (n=24), winter (n=24), two-rowed winter (n=14), and six-rowed winter types (n=10). The values of the latter group might be slightly overestimated due to the small sample size

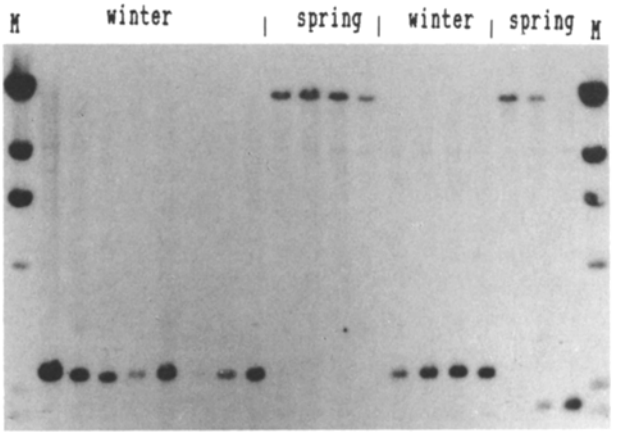

Fig. 3. Hybridization pattern obtained after probing EcoRI-digested DNA of 19 different barley cultivars, including spring and winter types with clone MWG1H504, mapping on chromosome *1H*. Note that the signal at 2.4 kb is restricted to winter types. *M*=molecular weight standard (23.1, 9.4, 6.7, 4.4, 2.3, and 2.0 kb)

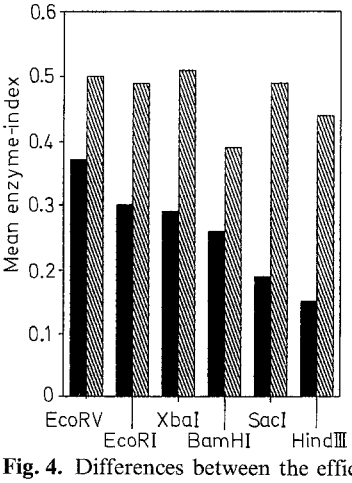

Fig. 4. Differences between the efficiency of six restriction endonucleases to detect polymorphism. The informativity of each restriction endonuclease is illustrated by the mean enzyme index as computed after deletion of noninformative probe/enzyme combinations (*hatched columns*) or based on the whole data set (*black columns*)

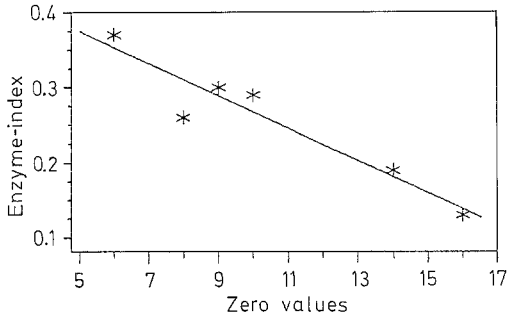

Fig. 5. Plot derived from regressing the average enzyme index of six restriction endonucleases on the number of uninformative probe/enzyme combinations (zero values in Table 1; $r = -0.95^{**}$)

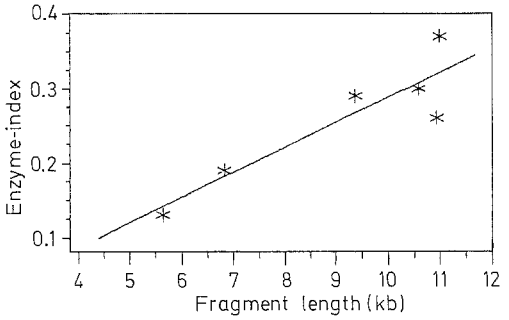

Fig. 6. Plot derived from regressing the average enzyme index on the mean length of hybridizing restriction fragments obtained from 23 probes in combination with 48 barley cultivars. Each asterisk marks the value of one restriction endonuclease ($r = 0.92^{**}$)

in the detection of RFLPs. Figure 4 shows that, if only informative probe/enzyme combinations are taken into consideration, differences between the respective restriction endonucleases are considerably less pronounced. The usefulness of a restriction endonuclease, therefore, is rather a function of the frequency of informative combinations with different probes, as can be deduced if the number of uninformative probe/restriction endonuclease combinations is regressed on the mean enzyme index (Fig. 5). This raises the question as to whether or not the observed differences between restriction endonucleases might be caused by different capabilities concerning the detection of insertion/deletion events. Based on the assumption that the likelihood to be hit by insertion/deletion events increases with the size of a restriction fragment, the mean fragment length, as detected for each restriction endonuclease in combination with all 23 probes, was regressed on the mean enzyme index (Fig. 6). The significant correlation identifies insertion/deletion events as a major factor contributing to RFLPs in barley.

Discussion

The aim of the present study was to answer questions concerning the extent of RFLPs within the species *Hordeum vulgare*, with a view towards directing a systematic approach for the development of an RFLP map and its subsequent utilization in breeding programs. The degree of polymorphism was estimated by analyzing DNA from 48 barley varieties with 23 randomly chosen genomic DNA probes. The assignment of these probes to individual chromosomes has been based on hybridization with wheat-barley addition lines. Due to the lack of a chromosome *1H* addition line, the use of wheat-barley addition lines might result in misinterpretations due to experimental imponderables. However, Chi-square analysis of the chromosomal distribution of 118 genomic single-copy clones, which were assigned in the course of this study, revealed no significant deviation from the expected number of probes for chromosome *1H* ($\chi^2 = 6.1_{6df}$). As expected all probes hybridized to DNA from the barley donor parent (cv BETZES), indicating complete DNA transfer onto the membranes. Most of the pobes showed cross hybridization with the wheat background of the addition lines due to the close relationship of both species. In several cases, the ratio between bands detected in the wheat and barley genome deviated from a 3:1 ratio, probably indicative of deletions in homeologous wheat chromosomes, or of duplication events affecting nonsimultaneously the whole complement of three homeologous chromosomes. Taking advantage of the relationship of both species, a given set of RFLP probes developed for barley could probably be applied simultaneously in wheat.

It is difficult to compare the present results with those obtained by others for different plant species, since varying aproaches, e.g., the analysis of few varieties with a higher number of probes (Landry et al. 1987) and/or restriction enzymes (McCouch et al. 1988), a different assay system (Gebhardt et al. 1989), or the inclusion of different species of a genus (Song et al. 1988) were often used. However, certain trends seem to appear. In the case of barley, the inclusion of wild species might have caused an overestimation of the degree of polymorphism with respect to the application of probes as markers in practical breeding programs, since introgression has been used only rarely as a tool for the introduction of new genes into breeding stocks. Nevertheless, introgression may be of future interest, especially in view of the introduction of new genes for disease resistance (Jahoor and Fischbeck 1987). The results show that the extent of polymorphism in barley is low compared to that observed in maize, potato, and several *Brassica* species (Evola et al. 1986; Gebhardt et al. 1989; Figdore et al. 1988). This might be attributed to the fact that barley is an inbreeding species. Similarly low levels of diversity have been reported for

other inbreeding species like tomato (Helentjaris et al. 1985) and soybean (Apuya et al. 1988).

A comparative study of the diversity of *H. vulgare* and its wild relative, *H. spontaneum*, has disclosed a substantially reduced level of RFLPs at two rDNA loci in the domesticated species (Saghai-Maroof 1984). A further reduction of diversity is observed within the subgroups of spring and winter barley. This is probably a consequence of plant breeding, because (i) crosses are preferentially performed within a subgroup, and (ii) breeders prefer to cross lines of their own companies, both leading to shifts in allelic frequencies and finally to genetic erosion. Since the difference between spring and winter types is of oligogenic origin (Takahashi and Yasuda 1970), the uniform presence of only a few traits in the respective subpopulation would not be expected to result in observable loss of diversity.

Although neither the genetic nor the molecular basis for the ability of probe MWG1H504 to differentiate between spring and winter barley are known, the way in which this probe was selected in a phenotypically well-defined population illustrates an alternative to the analysis of segregating progenies. This strategy might prove useful for the selection of RFLP markers, linked to traits, for which neither near-isogenic lines nor segregating populations are available.

It has been noted that certain restriction endonucleases differ in their capability of detecting polymorphism (Landry et al. 1987). In order to investigate whether or not this applies to varley as well, six restriction endonucleases were compared. Their selection rests on the following criteria: (i) a 6-bp, recognition site, since agarose was used as separation matrix, (ii) the ability to cut genomic DNA to completion, and (iii) a reasonably low price for subsequent use in routine work. Applying these criteria many restriction endonucleases recognizing CpG or CpXpG motifs in their target sequences were not found to be useful because of the generation of partial digests, which resulted in fragments that were too large to be analyzed by conventional gel electrophoresis. This probably is due to C-methylation of CpG dinucleotides and to imbalanced CpG distribution across the genome (Gruenbaum et al. 1981; Bird 1986). Among the six restriction endonucleases tested, clear differences were observed. The general usefulness of a restriction endonuclease to detect RFLPs in barley (shown by the average enzyme index) is strongly correlated with the frequency of detecting polymorphisms (the less useful, the more uninformative probe/enzyme combinations occur). Moreover, the usefulness increased with increasing length of restriction fragments that were picked up by the respective probes. This fact and the observation that additional enzymes do not contribute additional information to the probe index strongly suggest that insertion/deletion events are the major cause of polymorphism in barley. These findings are supported by the fact that, if point mutations are present, a negative correlation between fragment size and informativity is expected. The occurrence of insertion/deletion events in barley DNA is substantiated by the hybridization patterns of four probes, which clearly marked such events, showing identical patterns with different restriction endonucleases (results not shown). Similar results have been reported from maize and rice (Helentjaris 1987; McCouch et al. 1988). In contrast to maize, however, no zero alleles are observed. Despite the reduced level of polymorphism in barley when compared to maize and other outbreeding species, the degree of diversity present in the genome seems to be sufficient for the successful application of RFLP markers in breeding and molecular biology. However, based on the findings of this study, an interspecific cross is proposed, in order to map a maximum number of RFLP markers on a single segregating population.

References

Apuya NR, Frazier BL, Keim P, Jill-Roth E, Lark KG (1988) Restriction fragment length polymorphisms as genetic markers in soybean, *Glycine max* (L.) merrill. Theor Appl Genet 75:889–901

Beckmann JS, Soller M (1988) Detection of linkage between marker loci and loci affecting quantitative traits in crosses between segregating populations. Theor Appl Genet 76:228–236

Bird AP (1986) CpG-rich islands and the function of DNA methylation. Nature 321:209–213

Blake T (1987) Strategies for restriction fragment length polymorphism analysis in barley. In: Yasuda S, Konishi T (eds) Barley genetics V. Sanyo Press, Okayama, pp 503–508

Bonierbale MW, Plaisted RL, Tanksley SD (1988) RFLP maps based on a common set of clones reveal modes of chromosomal evolution in potato and tomato. Genetics 120:1095–1103

Bunce NAC, Forde BG, Kreis M, Shewry PR (1986) DNA restriction fragment length polymorphism at hordein loci: application to identifying and fingerprinting barley cultivars. Seed Sci technol 14:419–429

Evola SV, Burr FA, Burr B (1986) The suitability of restriction fragment length polymorphisms as genetic markers in maize. Theor Appl Genet 71:765–771

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

Figdore SS, Kennard WC, Song KM, Slocum MK, Osborn TC (1988) Assessment of the degree of restriction fragment length polymorphism in *Brassica*. Theor Appl Genet 75:833–840

Gebhardt C, Ritter E, Debener T, Schachtschabel U, Walkemeier B, Uhrig H, Salamini F (1989) RFLP-analysis and linkage mapping in *Solanum tuberosum*. Theor Appl Genet 78:65–75

Gruenbaum Y, Naveh-Many T, Cedar H, Razin A (1981) Sequence specificity of methylation in higher plant DNA. Nature 292:860–862

Helentjaris T (1987) A genetic linkage map for maize based on RFLPs. Trends Genet 3:217–221

Helentjaris T, King G, Slocum M, Siedenstrang C, Wegman S (1985) Restriction fragment length polymorphisms as probes for plant diversity and their development as tools for applied plant breeding. Plant Mol Biol 5:109–118

Helentjaris T, Slocum M, Schaefer A, Nienhuis J (1986) Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor Appl Genet 72:761–769

Holmes DS, Quigley M (1981) A rapid boiling method for the preparation of bacterial plasmids. Anal Biochem 114:193–197

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Jahoor A, Fischbeck G (1987) Sources of resistance to powdery mildew in barley lines derived from *Hordeum spontaneum* collected in Israel. Plant Breed 99:274–281

Kuhlmann U, Foroughi-Wehr B (1989) Production of doubled haploid lines in frequencies sufficient for barley breeding programs. Plant Cell Rep 8:78–81

Landry BS, Kesseli R, Leung H, Michelmore RW (1987) Comparison of restriction endonucleases and sources of probes for their efficiency in detecting restriction fragment length polymorphisms in lettuce (*Lactuca sativa* L.). Theor Appl Genet 74:646–653

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor/NY

McCouch SR, Kochert G, Yu ZH, Wang ZY, Khush GS, Coffman WR, Tanksley SD (1988) Molecular mapping of rice chromosomes. Theor Appl Genet 76:815–829

Saghai-Maroof MA, Soliman KM; Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sogaard B, Wettstein-Knowles P von (1987) Barley: genes and chromosomes. Carlsberg Res Commun 52:123–196

Song KM, Osborn TC, Williams PH (1988) *Brassica* taxonomy based on nuclear restriction fragment length polymorphisms (RFLP's). Theor Appl Genet 75:784–749

Steinmüller K, Apel K (1986) A simple and efficient procedure for isolating plant chromatin which is suitable for studies of DNaseI-sensitive domains and hypersensitive sites. Plant Mol Biol 7:87–94

Takahashi R, Yasuda S (1970) Genetics of earliness and growth habit in barley. In: Barley genetics II. Washington State University Press, pp 388–408

Tanksley SD, Young ND, Paterson AH, Bonierbale MW (1989) RFLP mapping in plant breeding – new tools for an old science. Bio/Technol 7:257–264