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 (MWGIH504) 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 F1 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 Steimmler 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 32P-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 x SSPE (20 x SSPE = 3.6 M NaCl; 200 mM NaH2PO4, pH 7.4; 20 mM EDTA) at 66°C. After completion of the hybridization, membranes were washed twice for 30 min at 50°C in 0.5 x SSC, 0.1% SDS (20 x SSC = 3 M NaCl, 0.3 M Na-Citrate, pH 7.0), once in 0.2 x 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 data-base 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