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Dialect-I, species-specific repeated DNA sequence from barley, *Hordeum vulgare*

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Summary. Dialect-1, species-specific repetitive DNA sequence of barley *Hordeum vulgare,* was cloned and analysed by Southern blot and in situ hybridization. Dialect-1 is dispersed through all barley chromosomes with copy number 5,000 per genome. Two DNA fragments related to Dialect-1 were revealed in λ phage library, subcloned and mapped. All three clones are structurally heterogenous and it is suggested that the full-length genomic repeat encompassing Dialect-1 is large in size. The Dialect-1 DNA repeat is represented in the genomes of *H. vulgare* and ssp. *agriocrithon* and *spontaneum* in similar form and copy number; it is present in rearranged form with reduced copy number in the genomes of *H. bulbosum* and *H. murinum,* and it is absent from genomes of several wild barley species as well as from genomes of wheat, rye, oats and maize. Dialect-1 repeat may be used as a molecular marker in taxonomic studies and for identification of barley chromosomes in interspecies hybrids.

Key words: Barley – Repetitive DNA – Species-specific clones

Introduction

The genomes of cereals contain repeated sequences that can account for a considerable portion of the total DNA (75%). The majority of repeated sequences are common to the genomes of barley, wheat and rye, and some of such sequences are species-specific (Flavell et al. 1977). These sequences are of interest in the search for species molecular markers in barley genetics, selection and taxonomy. Investigation of their structure and representation in genome is important for elucidation of cereal DNA organization and evolution. General results in this field were obtained by Flavell and co-workers. In particular, they described rye-specific repeated DNA sequences (Bedbrook et al. 1980). Recently (Metzlaff et al. 1986), large series of wheat-specific DNA clones were obtained with the help of "relic DNA strategy" (Bedbrook et al. 1980). The search for species-specific repeated DNA sequences of cereals, in particular barley, is in progress in other laboratories as well (Chakrabati and Subrahmanyan 1985; Salina et al. 1986). In studies of Ananiev et al. (1986a, b), 114 barley-repeated DNA cloned fragments have been characterized with respect to their homology with wheat DNA by means of Southern blot hybridization. Only few clones showed weak hybridization with wheat DNA. These clones included barley-specific DNA sequences and the aim of present work was to characterize one of such clones, later called Dialect-1: to determine its copy number in barley genome, to describe the type of its distribution in chromosomes and to analyse its representation in a number of barley species.

Materials and methods

Plants

The following *Hordeum* species defined according to Von Bothmer and Jacobsen (1985) were used: *H. vulgare* ssp. *vulgare* varieties Donetski-4, Oksamit, Golozerni (2x), *H. vulgare* ssp. *agriocrithon* (2x), *H. vulgare* ssp, spontaneum (2x), *H. bulbosum* (4x), *H. murinum* ssp. *murinum* (4x), *H. murinum* ssp. *leporinum* (6x), *H. marinum* ssp. *marinum, H. marinum* ssp. *gussoneanum* (*H. geniculatum,* 2x), *H. brevisubulatum* ssp. *violaceum* (2x), *H. jubatum* (4x), and also *Triticum aestivum* var. Omskaya-4, *Secale cereale, Avena sativa, Zea mays.* Five wheat addition lines having individual pairs of barley chromosomes (*H. vulgare* v.

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Betzes) introduced to chromosome complement of hexaploid wheat (*T. aestivum* var. Chinese Spring) were kindly provided by Dr. A. K. R. M. Islam (Waite Agricultural Research Institute, Australia) (Islam et al. 1981).

DNA isolation and analysis

Total DNA was isolated from 5- to 7-day-old seedlings mainly according to the procedure described by Shure et al. (1983). Procedures of plasmid or phage DNA isolation, DNA cloning in plasmid pBR325 or λ EMBL-4 vector, screening of the library, restriction mapping and Southern blot hybridization were performed essentially following Maniatis (Maniatis et al. 1982).

The in situ hybridization was done with metaphase and polytene chromosomes of *Hordeum vulgare* var. nutans. Metaphase chromosomes were prepared from squashed root tips. Polytene chromosomes were prepared from squashed embryo sacs 48–72 h after pollination. The preparations were made and hybridization was carried out in general following the procedure of Pardue et al. (1977): 10 µl of DNA (10^7 cpm/µg, $5 \cdot 10^4$ cpm were applied on each slide).

Results

Selection of barley-specific DNA clones

Screening has been performed earlier (Ananiev et al. 1986b), in which 114 cloned EcoRI fragments from *H. vulgare* DNA partial library in plasmid pBR325 were used as probes for Southern blot hybridization with barley and wheat genomic DNA digested with EcoRI or BamHI restriction enzymes. While 110 clones revealed high homology to DNA of both cereals, 4 clones showed weak hybridization with wheat DNA. One of the latter clones which we called Dialect-1 (D-1) H.v. (Fig. 1) was the subject of the present study.

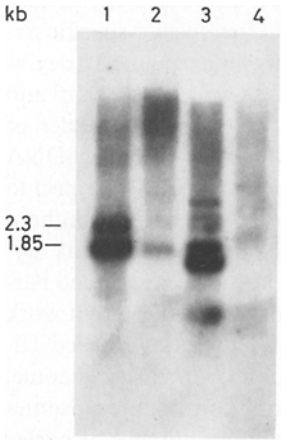

Fig. 1. Southern hybridization analysis of the cloned barley DNA specificity. DNA blot hybridized to ^{32}P-labeled 1.85-kb EcoRI fragment (Dialect-1 H.v.). *Lane 1* – Barley *H. vulgare* DNA digested with EcoRI; *lane 2* – wheat DNA digested with EcoRI; *lane 3* – *H. vulgare* DNA digested with BamHI; *lane 4* – wheat DNA digested with BamHI

To isolate D-1 H.v.-related genomic sequences, we screened the λ EMBL-4 phage library of *H. vulgare* DNA (constructed after partial EcoRI digestion of total DNA). We used D-1 H.v. as a probe and got two different recombinant phage clones, one of which contained inside its insert a 2.3-kb EcoRI fragment homologous to D-1 H.v., and the second a 4.3-kb EcoRI fragment. These fragments were subcloned in pBR 325 and mapped (Fig. 2). The areas of mutual homology of three clones – 1.85 kb (D-1 H.v.), 2.3 kb and 4.3 kb – were determined (Fig. 2). Restriction sites in the homology regions are not coincident, except for the 0.9-kb HindIII fragment, which is apt to be almost identical in the clones. After subcloning in pBR325, the latter was used in some experiments as a D-1 H.v. probe.

Heterogeneity in size and structure of these three cloned sequences indicates that they originate from different sites of barley genome. As will be shown later by genomic blotting, none of them contains a full-length repeat unit together with adjacent sequences.

Copy number

The copy number for the D-1 H.v. repeat (1.85-kb EcoRI fragment) per barley genome was estimated by means of Southern hybridization of this probe to the *H. vulgare* DNA digested by EcoRI endonuclease and to the probe dilutions in parallel (not shown). The copy number for D-1 H.v. repeat was found to amount to approximately 5,000 per genome.

Distribution of DNA sequences homologous to D-1 H.v. repeat in H. vulgare genome

The D-1 H.v. sequence apparently originates from nuclear DNA of *H. vulgare*. Barley total DNA digested with methylation-sensitive restriction enzymes PstI or SalI showed D-1 H.v. hybridization signals only in the

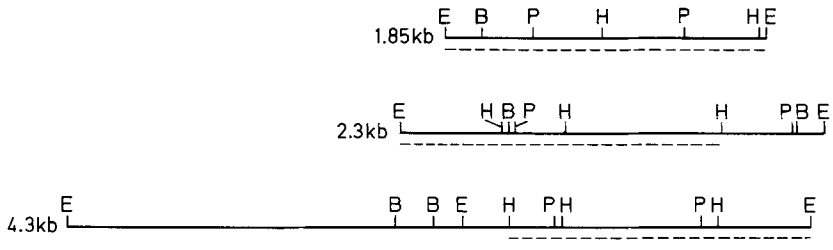

Fig. 2. Restriction maps of three clones composing the D-1 H.v. family. *E* – EcoRI, *B* – BamHI, *H* – HindIII, *P* – PstI, *EV* – EcoRV. The regions of the interclone homology are indicated by *interrupted lines*

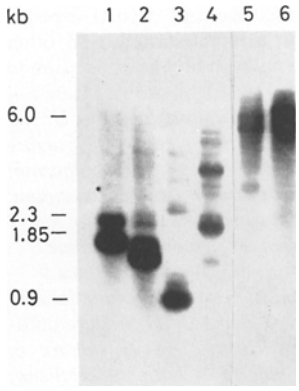

Fig. 3. Southern hybridization analysis of *H. vulgare* DNA. DNA blot hybridized to ^{32}P-labeled D-1 H.v. probe (0.85-kb HindIII fragment). DNA was digested by following restriction endonuclease: *Lane 1* – EcoRI; *lane 2* – BamHI; *lane 3* – HindIII; *lane 4* – HindII; *lane 5* – HpaI; *lane 6* – EcoRV

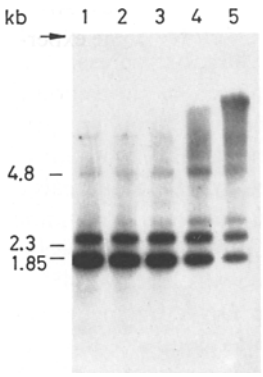

Fig. 4. Southern hybridization of EcoRI-partially digested *H. vulgare* DNA with D-1 H.v. probe (0.85-kb HindIII subclone). The duration of digestion: *Lane 1* – 60 min; *lane 2* – 20 min; *lane 3* – 10 min; *lane 4* – 2 min; *lane 5* – 0.5 min

regions of undigested DNA (nuclear DNA, highly methylated in plants), and no hybridization was revealed in the area of digested chloroplast and mitochondrial DNA (not shown).

Southern blot hybridization of D-1 H.v. probe to *H. vulgare* DNA digested with EcoRI, BamHI, HindIII or other hexanucleotide restriction enzymes (Fig. 3) showed few prominent discrete bands. The absence of a "ladder" on the genomic blots is usual for non-tandem repeat organization. In the case of limited EcoRI digestion of *H. vulgare* DNA and hybridization to D-1 H.v. probe, no multimeres of the basic repeat fragment appeared on genomic blots and the hybridization signals were simply shifted into a high molecular weight zone (Fig. 4).

In situ hybridization of D-1 H.v. probe to *H. vulgare* polytene or metaphase chromosomes resulted in silver grains dispersed throughout all chromosomes (Fig. 5).

We also used DNA from five wheat addition lines having individual pairs of barley chromosomes (see "Materials and methods"). Southern blot hybridization of the D-1 H.v. probe to EcoRI-digested DNAs of barley and wheat addition lines showed that the repeat was evenly distributed in barley genome among different chromosomes.

Taken together, the above data suggested that the D-1 H.v. element is dispersed in the *H. vulgare* genome.

The sizes of discrete hybridization bands on the barley DNA genomic blots (Fig. 3) corresponded to 1.85 kb and 2.3 kb (and a slightly noticeable 4.8-kb band) in the case of EcoRI digest, to 0.9 kb and 2.7 kb in the case of HindIII digest, to approximately 6 kb and 7 kb in the case of HpaI; BglII gave fragments of approximately 5 kb and 6 kb, etc. This provides evidence for a relatively large size of full-length D-1 DNA sequence in barley genome.

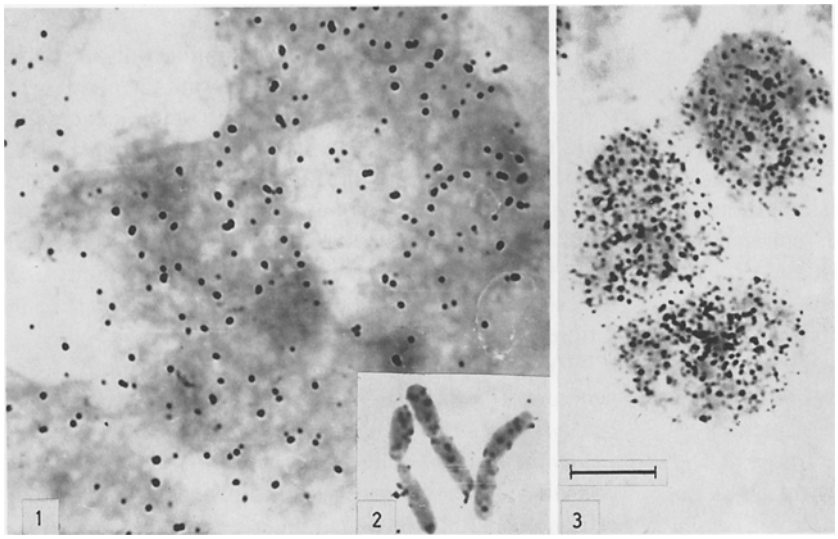

Fig. 5. In situ hybridization of ^{3}H-labeled D-1 H.v. probe to polytene (*1*) and metaphase (*2*) chromosomes and polyploid endosperm nuclei of *H. vulgare* (*3*). *Bar* = 10 μm

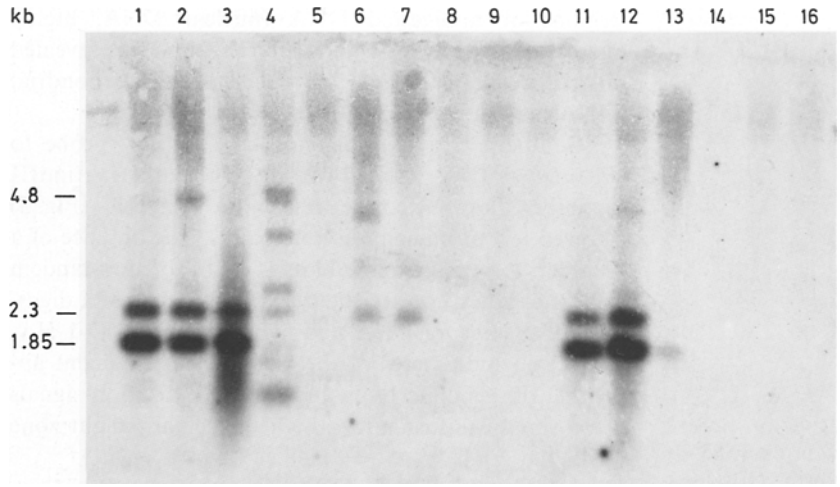

Fig. 6. Comparison of EcoRI-digested DNA from different barleys and other cereals by Southern blot hybridization to ^{32}P-labeled D-1 H.v. probe. *Lane 1 – H. vulgare* ssp. *vulgare; lane 2 – H. vulgare* ssp. *agriocrithon; lane 3 – H. vulgare* ssp. *spontaneum; lane 4 – H. bulbosum; lane 5 – H. marinum* ssp. *gussoneanum; lane 6 – H. murinum* ssp. *murinum; lane 7 – H. murinum* ssp. *leporinum; lane 8 – H. marinum* ssp. *marinum; lane 9 – H. brevisubulatum* ssp. *violaceum; lane 10 – H. jubatum; lane 11 – H. vulgare* cultivar Oksamit; *lane 12 – H. vulgare* c. Golozerni; *lane 13 – Triticum aestivum; lane 14 – Secale cereale; lane 15 – Avena sativa; lane 16 – Zea mays. Numbers* along the margin refer to the linear sizes of DNA fragments in kb

Species-specificity of Dialect-I H.v. fragment

To study the representation of D-1 H.v.-related repeated DNA sequences within the genus *Hordeum*, we have chosen the barley species listed in "Materials and methods". Digestion of the different barley species DNAs with EcoRI followed by fractionation in 0.8% agarose gel and Southern blot hybridization to D-1 H.v. probe (Fig. 6) revealed prominent differences between the species. Within the species *H. vulgare*, no intervarietal polymorphism was observed (Fig. 6, lanes 1, 11, 12). In DNA of ssp. *agriocrithon* (lane 2) and ssp. *spontaneum* (lane 3), the sequences homologous to D-1 H.v. repeat are represented likewise in *H. vulgare* DNA by two prominent bands at 1.85 kb and 2.3 kb, with slight signal in the region of 4.8 kb.

In the case of *H. bulbosum* DNA (Fig. 6, lane 4), about eight hybridization bands are detected in the range from 0.8 to 5.5 kb, the basic repeat of *H. vulgare*, 1.85 kb, being absent. This hybridization pattern shows that DNA sequences homologous to D-1 H.v. are present in *H. bulbosum* genome in rearranged form with reduced copy number. Weak hybridization is noticeable in the region of a 2.3-kb fragment in DNA of *H. murinum* ssp. *leporinum* (Fig. 6, lane 6) and ssp. *murinum* (lane 7). From the genomes of other barley species examined, namely *H. marinum* ssp. *gussoneanum*, *H. marinum* ssp. *marinum*, *H. brevisibulatum* ssp. *violaceum* and *H. jubatum* (Fig. 6, lanes 5, 8–10), the nucleotide sequences exhibiting homology to the D-1 H.v. probe are absent. EcoRI-digested DNAs of wheat, rye, oats and maize were applied into the same gel (Fig. 6, lanes 13–16). They appeared to contain no D-1 H.v.-related sequences either. It must be noted that sometimes the traces of D-1 H.v. sequence are slightly noticeable on wheat DNA genomic blots.

Discussion

The cloned fragments of barley *H. vulgare* DNA obtained earlier (Ananiev et al. 1986a, b) represent repeated nucleotide sequences dispersed in the genome. Many of them were shown to have a homology with DNA of wheat, rye and other cereals. High amount of "common" DNA sequences shared by different cereals is thought to be due to their evolution from a common ancestor (Flavell 1980). The proportion of barley-specific DNA clones in the library we used was less than 4%.

The described species-specific DNA fragment, Dialect-1 H.v., is a moderately repeated sequence member of the family including three types of homologous DNA fragments cut by EcoRI endonuclease to 1.85 kb, 2.3 kb and 4.3 kb (the latter with considerably lower copy number). D-1 H.v.-related sequences are dispersed throughout all chromosomes, they are heterogeneous in restriction maps and the full-length form of Dialect-1 presumably has large size.

The repeated DNA sequences families dispersed in the cereal genome presumably result from the permanent genome rearrangements mainly due to amplification and translocation of individual DNA segments (Flavell 1980). It is suggested that mobile genetic elements take part in most of such genome rearrangements events. The involvement of transposable elements in the described D-1 H.v. repeat is indicated by its species-specificity and by existence of varying forms of related sequences in different barley species.

The D-1 H.v. barley DNA element is absent from genomes of other cereals as well as from genomes of barley species not included in section *Hordeum* (*H. brevisubulatum, H. marinum, H. jubatum*). Amplification of this nucleotide sequence and its propagation through genome, possibly mediated by transposable elements,

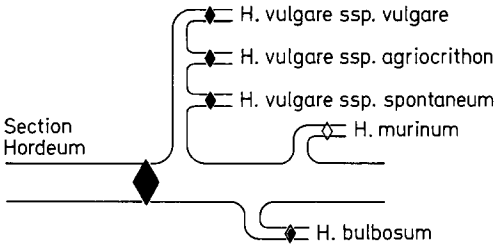

Fig. 7. Hypothetical scheme illustrating the presence of Dialect-1 H.v. DNA repeat (designated as ♦) in the section *Hordeum*. ♦ – the basic form of D-1 H.v.: ◈ and ◊ – rearranged and reduced forms of D-1 H.v.

could occur at the stage following the separation of section *Hordeum* and before the species divergence within this section. The D-1 H.v. repeat is similarly represented in the genomes of cultivated barley *H. vulgare* and its wildly growing subspecies *agriocrithon* and *spontaneum*. These barleys are apparently so closely related that during their evolution after divergence, D-1 H.v. sequence has not been essentially affected in regard to its copy number and distribution in the genome. In DNA of *H. bulbosum*, another member of the section *Hordeum* which has probably diversed later, the D-1 H.v. repeat, is presented in rearranged and reduced form. Only minimal homology with this element has been detected in DNA of *H. murinum*, less closely related to the *H. vulgare* species. The presence of D-1 H.v. DNa repeat in barley species can be illustrated by the scheme in Fig. 7. The scheme is consistent with current phylogenetic schemes based on chromosome pairing and hybrid fertility assay and the isoenzyme spectrum of different barley species (Jorgensen 1986).

The species-specific nucleotide sequence under study which has appeared as the result of the evolutionary events at the molecular level can serve as a DNA marker for barley species of section *Hordeum* and the estimate of their phylogenetic proximity.

Moderately repeated nucleotide sequences are an abundant and rapidly evolving plant genome fraction, but their functions still remain for the most part obscure. Some repeated DNA sequences may provide a contribution to homology between the conjugating chromosomes (*T. aestivum* studies) (Flavell 1982). A similar suggestion can be made about the functional significance of the described DNA repeat.

Experimental results on species-specific repeated DNA sequence combined with data on morphology, cytogenetics and protein biochemistry are sure to find a wide application both for taxonomic purposes and for elucidating phylogenetic relations among barley species, and in interspecific and intergeneric hybridization practice. In particular, the described cloned DNA sequence D-1 H.v. can be used as a molecular marker of *H. vulgare* for chromosome identification in the available barley hybrids with other cereals (Blanco et al. 1987).

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