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Genome specific, highly repeated sequences of *Hordeum vulgare*: cloning, sequencing and squash dot test

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Summary. Highly repeated sequences of nuclear DNA from barley *Hordeum vulgare* (L.) variety 'Erfa' were cloned. Several clones containing barley specific repeated DNA were analysed by sequence analysis and Southern blot hybridization. The investigated repeats differ from each other in their length, sequence and redundancy. Their length ranges from 36 bp to about 180 bp. The repeats are AT-rich and differ widely in their redundancy within the barley genome. Southern analysis showed that the repeats belong to different repetition complexes. The possibility for utilizing these clones as probes for simple and fast genome analysis is demonstrated in squash dot experiments.

Key words: Barley (*Hordeum vulgare*) – Highly repeated sequences – Molecular cloning – Squash dot technique

Introduction

Repeated sequences are a characteristic of eukaryotic nuclear genomes. Recently, the repeated structures of several plant genomes have been investigated (Kato et al. 1985; Viotti et al. 1985; Sakowicz et al. 1986; Leclerc and Siegel 1987) with respect to their structure, function and dispersion. In basic molecular genetical research, and now more recently in applied plant breeding research, the use of such cloned repeated sequences as DNA probes is increasing. These probes are proving to be a valuable tool in genome characterization and the estimation of phyletic relations of plant species.

The genomes of cereals consist of at least 70% repeated sequences, and these have been most intensively investigated in the rye (Appels et al. 1981; Bedbrook et al. 1980) and wheat genomes (Dennis et al. 1980; Metzlaff et al. 1986). Bedbrook et al. (1980) characterized five different families of repeated sequences in rye, *Secale cereale*. Highly repeated sequences of the wheat genome have been cloned by Hutchinson and Londsdale (1982) and Metzlaff et al. (1986). Several repeated sequences from rye and wheat that proved to be genome specific have already been used as DNA probes for analysing hybrid genomes (Appels 1982; Rayburn and Gill 1987). In contrast to the intensive investigations carried out on the rye and wheat genomes, the repeated sequences of the barley genome have barely been looked at. Until now, barley repeated DNA has only been cloned by Dennis et al. (1980) and Ananiev et al. (1986).

In this paper, we describe the cloning and molecular characterization of repeated sequences from barley. Repeated DNA mostly consists of short repetition units (Miklos and Gill 1980). Therefore, we decided to clone short fragments of repeated DNA.

For this we cut 'relic' DNA (for definition see Materials and methods) with the restriction enzyme Sau3A into fragments of about 50–500 bp in a cloning strategy similar to that used previously for wheat repeated DNA (Metzlaff et al. 1986). The fragments were cloned using M13mp8 DNA as a vector (Messing 1983). Barley specific clones were characterized and tested in squash dot experiments.

Material and methods

DNA preparation and cloning

Total DNA from barley (*Hordeum vulgare*), wheat (*Triticum aestivum*) and rye (*Secale cereale*) was isolated as described by Wienand and Feix (1980). DNA digested with EcoRI, HindIII, BglII, HpaI and KpnI was electrophoresed on 1.3% horizontal agarose gels (SeaKem agarose, USA). If total cereal DNA is digested with enzymes that cut six nucleotide recognition sites,

a DNA fraction of low mobility is detectable in agarose electrophoreses. This fraction consists of highly repeated DNA lacking the recognition sites of the used enzyme. This DNA, which has an average length of 10^5 bp, is defined as 'relic' DNA because of its conservative sequence organisation (Bedbrook et al. 1980). Such a 'relic' DNA of barley was isolated after EcoRI digestion by electroelution (Maniatis et al. 1982). The isolated 'relic' DNA was cut with Sau3A and cloned into the BamHI site of M13mp8 DNA. The recombinant DNA was then used to transform competent *E. coli* JM103Y cells as described by Messing (1983). Barley specific repeated sequences were recloned into the pBSM vector. The barley Sau3A fragments were cut out of the linker sequence of M13mp8 using EcoRI and HindIII. This fragment was cloned into EcoRI/HindIII-digested pBSM DNA. The recombinant plasmids were used to transform BMH 7118 *E. coli* cells.

Clone selection

Recombinant phages were selected on X-Gal-containing plates. The template DNA of the recombinant phages was prepared as described by Messing (1983).

Template DNA (0.4 µg) was replica-blotted on three identical nitrocellulose filters (Schleicher and Schüll). These filters were hybridized with nick-translated (Maniatis et al. 1982) wheat, rye and barley total DNA at 65 °C overnight as described in a protocol published by Amersham (1985).

DNA sequencing and Southern blot hybridization

The insertions were sequenced by the dideoxynucleotide chain termination procedure of Sanger et al. (1980) using ^{32}P-dATP (Amersham) and a primer synthesized by S. Minter (U.M.I.S.T. Manchester). Total barley DNA was cut to completion with HindIII, BglII, HapI, KpnI, and transferred onto nitrocellulose filters (Southern 1975). These filters were hybridized with ^{32}P-labelled second strands of recombinant phages, prepared as follows: single-stranded DNA of recombinant phages was used as the template. After primer annealing, as described by Sanger et al. (1980), the second strand was synthesized with Klenow-enzyme, without DNase, under identical reaction conditions as nick-translation (Maniatis et al. 1982), for 40 min at 37 °C. The hybridizations were carried out at 65 °C overnight.

Squash dot hybridization

Squash dot filters with root tips from wheat, rye and barley were prepared as described by Hutchinson et al. (1985). The filters were hybridized with ^{32}P-labelled barley-specific clones (labelling and hybridization as described above).

Results

Cloning and selection of highly repeated sequences

Total DNA from barley was digested with several restriction enzymes (Fig. 1). The restriction patterns show that the amount of 'relic' DNA depends upon the enzyme used. For example, there is a stronger band of 'relic' DNA in the HpaI and KpnI restriction patterns than in the BglII pattern. Comparisons of HindIII restriction patterns from total DNA of barley, wheat and rye (Fig. 1) revealed a lower content of 'relic' DNA in the barley genome. For cloning, we used the 'relic' DNA of barley after it had been EcoRI digested (not shown).

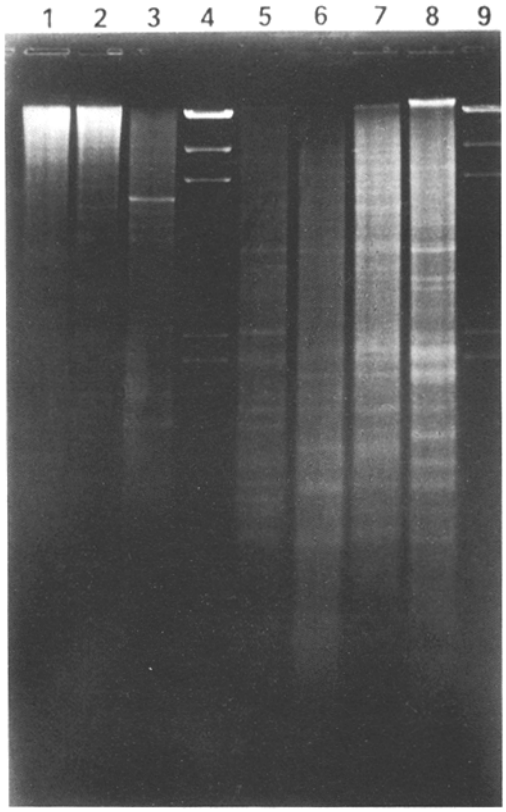

Fig. 1. Restriction enzyme patterns of rye, barley and wheat total DNA. Eight µg total DNA of barley was digested with HpaI (*lane 1*), KpnI (*lane 2*), BglII (*lane 3*) and HindIII (*lane 5*). Nine µg total DNA of wheat-*Triticum aestivum* (*lane 6*), wheat-*Aegilops caudata* (*lane 7*) and rye *Secale cereale* (*lane 8*) were digested with HindIII. λ/HindIII digests were used as size markers (*lanes 4* and *9*). The DNA was electrophoresed on a 1.3% agarose gel

Single-stranded DNA templates were prepared from 137 recombinant phages, MHV1–MHV137 (M13 *Hordeum vulgare*). The barley specificity of the clones was determined by dot blot hybridization. Under the conditions used for selection, 28 clones hybridized exclusively with barley DNA. This does not exclude the fact that the cloned barley sequences exist in rye or wheat genome in low frequencies. Eleven clones cross-hybridized with barley and rye DNA, and 3 clones cross-hybridized with barley and wheat DNA. Barley specific clones were collected for further characterization.

Sequencing and Southern blot analyses

The sequences of four barley-specific MHV clones are shown in Fig. 2. Clones MHV11, MHV16 and MHV56 were sequenced totally; MHV47 was only partially sequenced (180 bp). The lengths and AT-contents of the inserts are summarized in Table 1.

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11

GATCTAGTTG GCCAAACAAA ATCCAAGACT CGGAGGAATC TACAAGGATA

TAAAATCATG CATATAAGAA ATCAGCAAAG ACTCAAATAT ATATCATAGA TAATCT

16

GATCTTTGAT GTTTAACGTT CAAGTAGTTC AATCCAGGTA TTCCTTTGAA

AACTCCTTTC AAATAATCCT GTATGTTTTA CACAAATTCT ACATTACTTC T

47

GATCTTCTTC CGTTCGAGAA AAAATCATTT CGGGGATTTT ATTCCATTTG

GACTCCGTTC CAAAATCATA TCTGAAAAGA GCCAAAAACA CAGAAAAAAC

AAGAACTCGC ACTTGGCACT GAATTAATAA GTTAGTCCCA AAAAAGTTAC

AGGTACATAC ATCCAAGTTG ACAGATACAG

56

GATCTCCATG AAGATCATGG AGAACTTTGT ATTGAA
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Fig. 2. Sequences of four barley specific probes. The clones MHV11 (*11*), MHV16 (*16*) and MHV56 (*56*) were totally sequenced and MHV47 (*47*), only partially. Some examples of the directed repeats are *underlined*

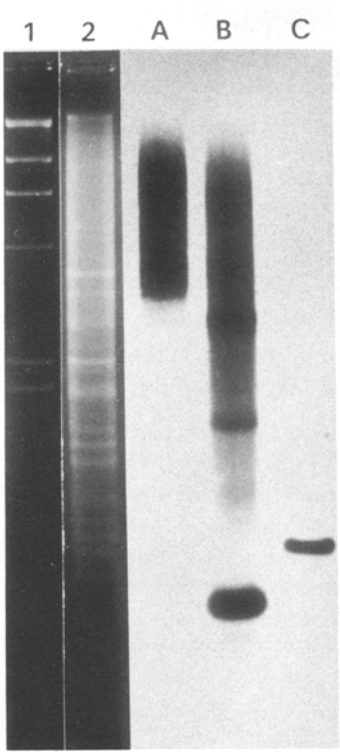

Fig. 3. Southern blot hybridization of HindIII-digested barley DNA with ^{32}P-labelled MHV clones. Digested barley DNA was electrophoresed on a 1.3% agarose gel (*lane 2*) and transferred to nitrocellulose filters. λ/HindIII digests were used as size markers (*lane 1*). These filters were hybridized with radioactively labelled DNA from MHV28 (*filter A*), MHV11 (*filter C*) and MHV47 (*filter B*) overnight at 65 °C. The filters were washed twice for 15 min in 2 × SSC/0.1% SDS-solution at 60 °C

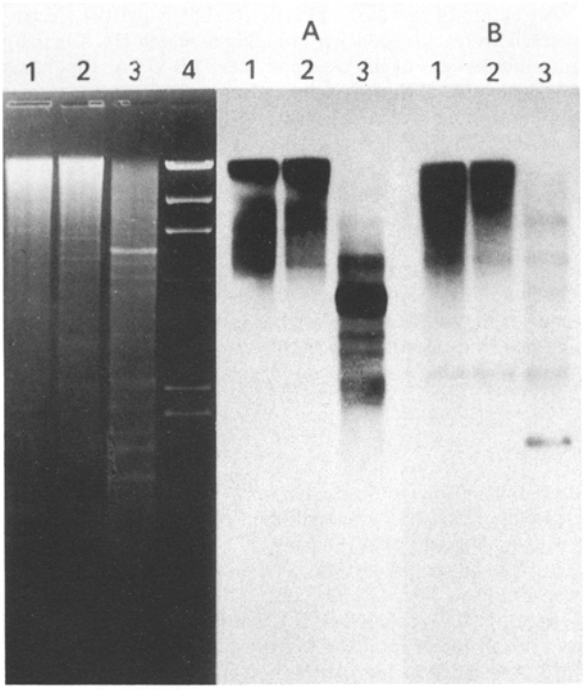

Fig. 4. Southern blot hybridization of digested barley DNA with MHV54 and MHV47 DNA. Barley DNA digested with HpaI (*lane 1*), KpnI (*lane 2*) and BglII (*lane 3*) was electrophoresed on a 1.3% agarose gel and transferred to a nitrocellulose filter. λ/HindIII was used as a size marker (*lane 4*). The filters were hybridized with ^{32}P-labelled DNA from MHV54 (*filter B*) and MHV47 (*filter A*) overnight at 65 °C. They were washed twice for 15 min with 2 × SSC/0.1%SDS-solution at 60 °C

Table 1. Lengths and AT-contents of the cloned inserts

	Length (bp)	AT-content (%)
MHV11	106	68
MHV16	101	64
MHV47	(180)	(63)
MHV56	36	64

The average AT-content of the barley genome is 59% (Chakrabarti and Subrahmanyam 1985). Our sequencing data revealed that the clones contain heterologous sequences lacking tandemly arranged sub-repeats. Computer analyses were unable to detect any homology among these clones. However, an unusual high number of 4–8 bp long directed repeats was detected within the clones. These repeats, however, are not arranged in a repetition structure of a higher order.

Southern blot hybridizations were carried out to clarify the occurrence of the cloned sequences within the genomic repetition complexes (Figs. 3 and 4). We divided the hybridization patterns into three types:

Type 1: the cloned fragment hybridizes only with DNA fragments of a higher molecular weight, especially

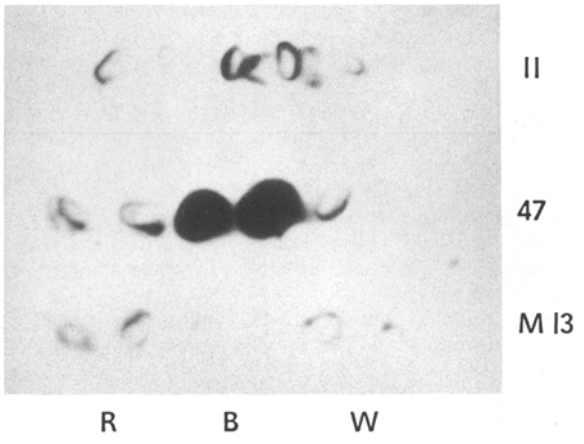

Fig. 5. Squash dot hybridization of root tips from rye, barley and wheat seedlings with MHV clones and M13 DNA. Two root tips (3 mm long) from rye (*R*), barley (*B*) and wheat (*W*) were squashed on nitrocellulose filters. The tips were treated with denaturing and neutralization solution. After baking, the filters were hybridized with ^{32}P-labelled DNA from MHV11 (*filter 11*), MHV47 (*filter 47*) and M13mp8 (*filter M13*) overnight at 65 °C. They were washed once for 10 min with 2 × SSC/0.1%SDS-solution and once for 10 min with 1 × SSC/0.1%SDS-solution

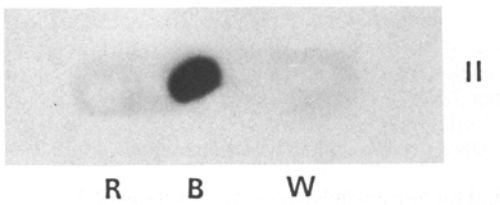

Fig. 6. Squash dot hybridization of root tips from rye, barley and wheat seedlings with pBHV11 DNA. One root tip from rye (*R*), barley (*B*) and wheat (*W*) was squashed on a nitrocellulose filter, and the DNA was fixed. The filter was hybridized with ^{32}P-labelled pBHV11 DNA overnight at 65 °C. It was washed once for 15 min with 2 × SSC/0.1%SDS-solution and once for 10 min with 1 × SSC/0.1%SDS-solution at 60 °C

with 'relic' DNA (Fig. 4, filters A and B, lanes 1 and 2)

Type 2: the cloned fragment hybridizes with several DNA fractions without marking the HindIII or BglII 'relic' DNA (Fig. 4, filters A and B, lane 3, or Fig. 3, filter C)

Type 3: the cloned fragment hybridizes with the whole lane and additional separate DNA (Fig. 3, filters A and B)

When barley DNA was cut with HpaI, KpnI and EcoRI, all analysed clones showed a type 1 hybridization. When barley DNA was cut with BglII and HindIII, clones MHV11, MHV16 and MHV56 showed a type 2 hybridization; clones MHV28 and MHV47, a type 3 hybridization.

The strength of the hybridization signals differed between clones. This is caused by the different redundancies of the cloned fragments. The redundancy can be expressed in the series:

MHV47 > = MHV28 > MHV16 = MHV11 > MHV56

Squash dot hybridization

The clones MHV47 and MHV11 were tested for their practical use in identifying the barley genome with the help of the squash dot technique (Fig. 5). MHV47 and MHV11 show a significant higher marking of barley than wheat and rye root tips. The hybridization signals correlate in strength with the redundancy found in Southern analysis. The slight cross-hybridization with wheat and rye root tips is caused by the vector DNA (M13), and could be prevented by recloning the barley sequences into a BSM vector (Fig. 6).

Discussion

Our cloning of barley repeated sequences is based on the experimental strategies of Bedbrook et al. (1980) and Metzlaff et al. (1986). In their publications, the cloning and characterization of rye-specific and wheat-specific sequences were reported. In our paper, we desribe the cloning and characterization of genome specific repeated DNA of the barley genome. The cloning of highly repeated DNA based on the isolation of 'relic' DNA is a suitable procedure for obtaining a number of different repeated sequences within one cloning experiment. Bedbrook et al. (1980) cloned a telomer well as dispersely located repeated sequences. In our cloning experiment using barley 'relic' DNA, we obtained clones of repeated DNA of varying sizes, sequences and redundancies. The determination of approximately 20% genome specific clones of all 'relic' DNA recombinants correlates well with results published by Flavell et al. (1977). In renaturation studies, Flavell et al. (1977) showed that 28% of the barley genome consists of DNA sequences which do not occur in the wheat and rye genome. Because we found 20% genome specificity in the highly repeated DNA fraction, we can conclude that genome specific DNA exists preferentially in repeated DNA. The sequence analyses show that the clones differ in AT-content and in arrangement and sequence of the internal subrepeats. Similar distributions of directed repeats were found by Sakowicz et al. (1986) in repeated sequences of the *Lupinus luteus* genome. The investigated fragments of repeated DNA are elements of larger repetition complexes.

The Southern analyses revealed that the clones belong to different repetition complexes having different

redundancies. The types of hybridization can be interpreted as follows.

Hybridization patterns of type 1 show that the repetition complexes are hardly even cut by the enzyme used. Type 2 hybridizations are produced from sequences that are organized in repetition complexes of distinct sizes. Hybridization patterns of type 3 indicate a widespread occurrence of the cloned sequences in repetition complexes of different sizes. Kato et al. (1985) determined similar distributions of repeats in several plant genomes, which are homologous to *Vicia faba* BamHI repeats.

Clones of highly repeated sequences can be used very effectively to mark chromosomes in situ or filter-fixed total DNA. Until now, these techniques have been used to investigate wheat and rye genomes and their hybrids (Hutchinson et al. 1985; Rayburn and Gill 1986; Appels 1982). We were able to successfully apply these methods in barley. The squash dot hybridizations we carried out showed that the constructed DNA probes are useful for this technique. The squash dot method is simple and fast.

The cross hybridization of M13mp8 sequences with cereal genomic DNA demonstrates the importance of the vector selection. In a future study, we shall reclone all inserts using BSM vectors which do not cross-hybridize with genomic DNA as the pBHV11 clone shows. Using barley-rye and barley-wheat addition lines, it should be possible to select for chromosome specificity. These experiments are in progress in our laboratory.

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