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Wheat specific repetitive DNA sequences – construction and characterization of four different genomic clones

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Summary. The construction and molecular analysis of four recombinant clones – pTa1, pTa2, pTa7, and pTa8 – is described. The four clones contain different highly repeated sequences of genomic DNA from *Triticum aestivum* variety 'Chinese Spring'. The wheat specificity has been determined by colony and dot blot hybridization in comparison with total rye DNA (*Secale cereale* variety 'Petka'). The four clones with a variable degree of specificity were compared by sequence analysis after the recloning of wheat DNA inserts into M13 mp8. Within the sequencing data a tendency can be observed that those repeated sequences which show the highest degree of species specificity contain a significantly increased amount of GC residues.

Key words: Repetitive DNA – Wheat – Rye – Relic DNA

Introduction

Cereal genomes contain a very high proportion (> 75%) of repeated sequences and these have been most extensively investigated in the genus *Secale* (Appels et al. 1978; Bedbrook et al. 1980). It has been clearly demonstrated in *Secale* that most of these highly repeated sequences are located in the regions of constitutive telomeric heterochromatin.

It has also been demonstrated that a certain number of repeated sequences are rye specific and absent in wheat (Bedbrook et al. 1980). Differences in the occurrence and distribution of species specific highly repeated sequences are of importance for chromosome pairing and may, therefore, affect the meiotic stability of cereal hybrids. The most common cereal hybrid, *Triticale,* which is formed by crossing wheat and rye (Gustafson 1976), often shows such meiotic disturbances. For this reason the agricultural importance of *Triticale* is still limited. In this paper we describe the cloning of highly repeated sequences from wheat (*Triticum aestivum* variety 'Chinese Spring') and the comparative sequence analysis of four different clones which show different degrees of cross-hybridization to rye DNA (*Secale cereale* variety 'Petka'). The wheat specificity has been determined by colony and dot-blot hybridization in comparison to total rye DNA. To pick up species specific sequences out of total wheat DNA we have used a similar strategy as Bedbrook et al. (1980) used for rye: starting from the isolation of "relic" DNA (for definition see "Material and methods") followed by digestion with a frequently cutting enzyme (in our case Sau3A1) and cloning into the multi-copy vector pEMBL8 (Dente et al. 1983).

Material and methods

Construction of recombinants

Total wheat and rye DNA was isolated from leaf material following the procedure described by Wienand and Feix (1980). The DNA was purified by two successive CsCl gradient centrifugations (Beckman L8-55, rotor 50Ti, 40 krpm, 40 h). DNA was digested to completion with EcoRI, BamHI, HindIII, BglII, PvuII and Sau3A1 using the conditions recommended by the supplier (Boehringer, Mannheim, FRG) and electrophoresed on 1.0 and 1.3% horizontal agarose slab gels (Sigma, USA).

When total cereal DNA is cut by a restriction enzyme with a six nucleotide recognition site a band can be observed on the top of the resulting restriction patterns which is formed by highly repeated DNA without recognition sites for this enzyme (Fig. 1). Because of its high degree of conservation during cereal evolution it is defined as "relic DNA" (Bedbrook et al. 1980). The average length of "relic DNA" is 10^5 bp. For our cloning experiments it was isolated from total BamHI digests of wheat DNA separated on a preparative low-melting point agarose gel (Weislander 1979). The isolated "relic" DNA was digested to completion with Sau3A1 and "shot gun" cloned into the BamHI of the multi-copy vector pEMBL8 (Dente et al. 1983). Plasmid preparations were carried out using rapid alkaline extraction methods (Birnboim and Doly 1979; Kieser 1984).

Colony and dot-blot hybridization

For colony hybridizations, recombinant clones were grown on nitrocellulose filters on YT-plates containing 100 µg/ml ampicillin for 5 h. Colonies were lysed and fixed on the filters following the procedure of Grunstein and Hogness (1975). Hybridization with nick-translated (Maniatis et al. 1975) total wheat and rye DNA was carried out at 65 °C overnight. Dot-blot hybridizations were carried out as described by Raeder and Broda (1984) by blotting 10 µg total wheat and rye DNA each on nitrocellulose and probing with nick-translated recombinant plasmids under the same conditions used in colony hybridizations.

DNA sequence analysis

The wheat Sau3A1 fragments were cut out of the linker sequence of pEMBL8 using the enzymes EcoRI and HindIII which cut in the linker sequence either side of the insert and recloned into EcoRI/HindIII digested M13mp8. (pEMBL8 and M13mp8 have the same linker.) The sequence analyses were carried out following the dideoxy-method (Sanger et al. 1980) using a primer synthesized by S. Minter (U.M.I.S.T, Manchester, UK).

Results

Clone selection

When total wheat and rye DNA is cut with the restriction enzymes EcoRI, BamHI, HindIII and BglII bands can be observed within the resulting patterns which are caused by genomic repeated sequences (Fig. 1). These bands are distributed over the whole pattern but especially on the top of the patterns where a region with higher density is visible. For rye this gel region counts for about 5% of total DNA (Bedbrook et al. 1980). It was shown that this region contains rye specific sequences. Although it is known that the wheat genome contains much less highly repeated DNA than found in rye, on top of the wheat patterns a region with higher DNA content is also visible. We isolated the DNA from this gel region from a preparative BamHI digest of total wheat DNA. After cutting the resulting DNA with Sau3A1 and cloning into the BamHI site of pEMBL8 we were able to isolate about 1,000 recombinants; pTa1 to pTa1000. We screened 350 of these recombinants for wheat specificity in colony hybridizations using nick-translated total wheat and rye DNA, respectively. About 10% of all screened clones showed significant higher hybridization signals to wheat DNA. About 5% of all clones do not cross-hybridize to rye DNA at all. We isolated the plasmids of twelve recombinant clones, pTa1 to pTa12. To estimate the size of the integrated wheat DNA fragments we cut the plasmids with PvuII (results not shown). PvuII cuts the vector plasmid in the positions 1,626 and 1,927. From these digests we got additional 301 bps surrounding the linker region with the inserted Sau3A1 fragments. This enabled us to estimate the size of small inserts just on 1% agarose gels. The insert sizes of the selected clones ranged between 100 to 700 bp. For further analyses we

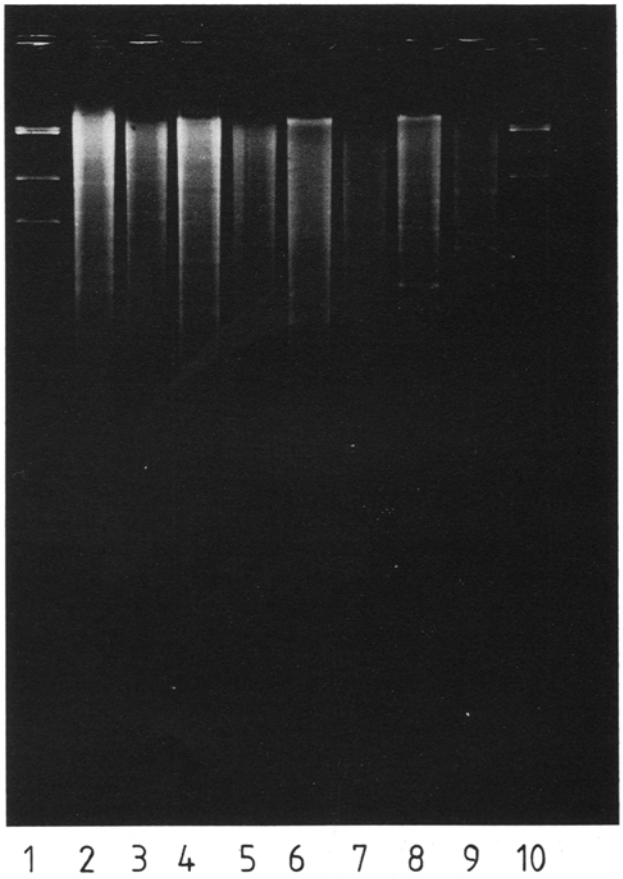

Fig. 1. Restriction enzyme patterns of wheat and rye total DNA. Five µg total DNA of wheat and rye, respectively, were digested and electrophoresed on 1.3% agarose gels: *lane 2* rye DNA/EcoRI, *lane 3* wheat DNA/EcoRI, *lane 4* rye DNA/BamHI, *lane 5* wheat DNA/BamHI, *lane 6* rye DNA/HindIII, *lane 7* wheat DNA/HindIII, *lane 8* rye DNA/BglII, *lane 9* wheat DNA/BglII, *lanes 1 and 10* Lambda DNA/HindIII. On top of all wheat and rye DNA restriction patterns a band containing "relic" DNA can be observed; within the patterns bands due to repeated sequences are visible

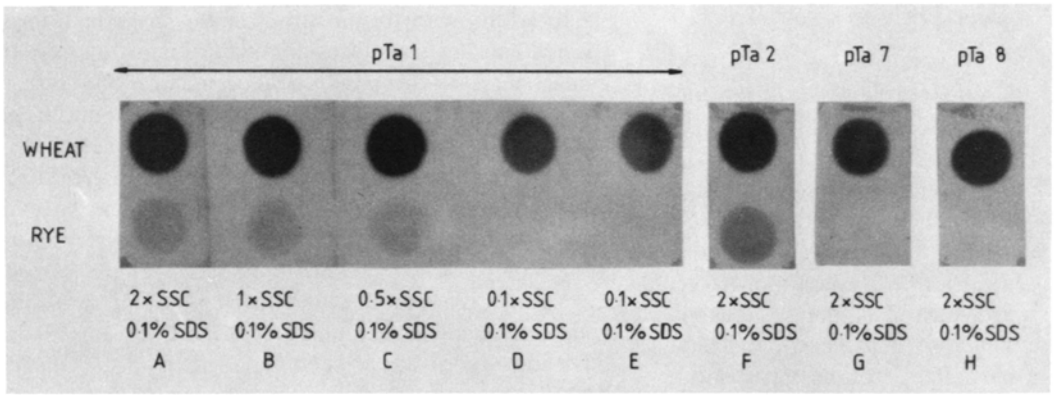

Fig. 2. Dot-blot hybridization of wheat and rye total DNA to labeled wheat specific probes. Ten μg total DNA of wheat and rye, respectively, were blotted onto nitrocellulose filters and hybridized to nick-translated probes. The filters were exposed to X-ray film for 16 h. *Dots A–E:* Hybridization to plasmid pTa1. The filters were washed successively for 15 min at 60 °C under the stated salt conditions. The slight cross-hybridization to rye DNA can be removed by this extensive washing procedure. *Dot F:* Hybridization to plasmid pTa2. A distinct cross-hybridization to rye DNA can be observed. *Dot G:* Hybridization to plasmid pTa7. No cross-hybridization to rye DNA is detectable under the stated hybridization and washing conditions. *Dot H:* Hybridization to plasmid pTa8. No cross-hybridization to rye DNA can be observed

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       1  GATCGAGATTAAAGGTTCGCATGTCAATTGTTAGCTTACGGCCAAGATATCCTACATTGC
(A)   61  ACATGAGTGCATTCAGGATTTCTAGAAGTGATGAATGCTTAATAGTGAAAGATTGAAAAA
     121  TGATGATGTGGCTTTGAATGGTGCATTTTG

       1  GATCAACACCAGAATTCTATGGGTGTTCGATTCATCACAATGTAACTAGATTATTGACTC
(B)   61  TAGTGCAAGTGGGAGACTGTTGGAAATATGCCCTAGAGGCAATAATAAAATGGTTTATTA
     121  TTATATTTCCTTTGTTCATGATAATTGTCC

(C)    1  GATCTCCTCCCATTGTAACCGACTCTGTGTAACCCTAGCCCTCTCCG*CGTCTATATAAAC
(D)       GATCTCCTCCC*TTGTAACCGACTCTGTGTAACCCTAGCCCCCTCCGGTGTCTATATAAAC
      61  TAGAGGGTTTTAGTCCATAGGTCAAACAACAATCCTACCATAGGCTAGCTTCTAGGGTTT
          TGGAGGGTTT*AGTCCGTAGGACAAACAACAATCATACCATAGGCTA*CTTCTAGGGTTT
     121  AGACTCTCTGATC
          AGCCTCTACAGTCTCATGGTAGATC
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Fig. 3. Sequence comparison of the four wheat specific probes. The first 150 nucleotides of pTa1 (*A*) and pTa2 (*B*) have been determined. No homology to each other and to pTa7 (*C*) and pTa8 (*D*) is detectable. pTa7 (133 nucleotides) and pTa8 (143 nucleotides) have been sequenced totally revealing a degree of sequence homology to each other. Deletions or insertions are denoted by *stars*

chose two plasmids, pTa1 and pTa2, which had slightly cross-hybridized with rye DNA in colony hybridizations and two plasmids, pTa7 and pTa8, which had exclusively hybridized with wheat DNA.

Determination of wheat specificity

To determine the degree of wheat specificity of the four selected clones we blotted 10 μg of each total wheat and rye DNA on nitrocellulose filters and hybridized with the labelled plasmids. The results of the dot-blot hybridizations are shown in Fig. 2. After a 15 min washing at 60 °C in 2×SSC, 0.1% SDS we got very strong hybridization signals for total wheat DNA for all four plasmids. The plasmids pTa7 and pTa8 did not show any cross-hybridization to rye DNA, whereas pTa1 showed slight cross-hybridization, and pTa2 cross-hybridized significantly with rye DNA. For the latter two plasmids the cross-hybridization to rye DNA can be removed by extensive washing as described in the legend of Fig. 2 but the hybridization with wheat DNA is preserved mainly.

The results of the hybridization experiments can be summarized as follows:

i) Clones pTa7 and pTa8 contain wheat DNA sequences with a high degree of species specificity which do not occur in rye DNA to a detectable extent.
ii) The clones pTa1 and pTa2 contain wheat DNA sequences with partial homology to rye sequences. The degree of homology must be low as demonstrated by the washing procedure.
iii) The variable degree of specificity of the four clones can be expressed in the series: pTa8 = pTa7 > pTa1 > pTa2.

Comparison of the DNA sequences of the four wheat specific clones

Figure 3 summarizes the sequence analyses of the four clones. The two clones, pTa7 and pTa8, have been sequenced totally and contain 133 bp and 144 bp, respectively. The degree of homology between these two clones is very high. Both sequences are GC-rich, 45.7% and 47.5%, respectively. (The average GC-content of cereal nuclear DNA is 37–38%.) Both contain only very few sites for restriction enzymes with four bp recognition sequences. The sequences of pTa1 and pTa2 have been determined only partially. The total sizes range about 700 bp for pTa1 and 500 bp for pTa2. The sequences of the first 150 bp of both clones show no detectable homology to each other or to pTa7 and pTa8. The GC-content of the sequenced parts of these two clones is significantly less than those of pTa7 and pTa8. pTa1 contains 37.0% GCs, pTa2 only 33.9%. The expression of the GC-content of the four sequenced clones in the series: pTa8 > pTa7 > pTa1 > pTa2 corresponds with the series of the degree of wheat specificity.

Discussion

In this paper we describe the cloning of genomic sequences of wheat which show species specificity. We decided to isolate the sequences from "relic" DNA because it was convincingly demonstrated for rye DNA (Bedbrook et al. 1980) that this DNA fraction contains a certain number of sequence families which are highly repeated and do not cross-hybridize with wheat DNA. Although the amount of "relic" DNA is significantly lower in wheat than in rye we can demonstrate now that 10% of the recombinant clones we isolated from this DNA fraction show wheat specificity. In addition to the band formed by "relic" DNA a large number of repetitive bands can be observed in the restriction patterns of total wheat DNA which are not present in rye DNA patterns. The DNA of these bands can most likely be used for the construction of wheat specific probes. Hutchinson and Lonsdale (1982) reported the cloning of two highly repetitive sequences from hexaploid wheat which cross-hybridized to both rye and barley chromosomes and therefore belong to the larger number of non-wheat-specific highly repetitive sequences.

The preliminary sequence analysis of four selected clones already demonstrates that the sequence divergence of highly repeated sequences seems to be considerable in the wheat genome. The sequences cloned in pTa1, pTa2, and pTa7 do not show any homology at all whereas pTa7 and pTa8 are obviously variants of one basic sequence. Interesting as well seems to be the fact that those sequences which show the highest degree of specificity, pTa7 and pTa8, contain significantly more GC residues.

In addition to the meaning of the investigation of species specific sequences for evolutionary aspects in cereals, the DNA probes are also of practical importance. Because the cloned sequences belong to the large amount of highly repeated DNA they can be used very effectively to mark chromosomes in in situ-hybridizations. By labelling and hybridizing the wheat specific probes to intact metaphase chromosomes they represent a valuable tool in cytogenetic analyses. In the case of the hybrid *Triticale* it would be very useful to isolate and clone sequences which are specific for one of the three introduced wheat genomes, A, B, and D (Gustafson 1976). In situ hybridization experiments using the described clones are in progress.

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