Theor Appl Genet (1991) 81:397–400

THEORETICAL AND APPLIED GENETICS

A chromosome-specific DNA sequence which reveals a high level of RFLP in wheat

R. L. Harcourt and M. D. Gale

Cambridge Laboratory, Colney Lane, Norwich, NR4 7UJ, UK

Received August 14, 1990; Accepted September 19, 1990
Communicated by J. W. Snape

Summary. An unusual genomic DNA clone, PSR454, was isolated from a partial genomic library of wheat. This sequence is moderately repeated and detects at least 30 related sequences, all located in a tight linkage block on the long arm of chromosome *3B*. When used as a RFLP probe, PSR454 detects a high level of polymorphism between wheat varieties that carry the sequence. There is no detectable hybridisation to sequences in one-third of the varieties tested, providing an "on-off" polymorphism that can be detected on dot blots, rather than the more resource-consuming conventional Southern analysis.

Key words: Hexaploid wheat – Chromosome-specific DNA sequence – RFLP

Introduction

RFLP mapping in bread wheat (*Triticum aestivum*, 2n = 6x = 42) has been hampered by the low levels of intervarietal polymorphism detected with many clones, particularly cDNAs, and by the complexities arising from polyploidy and the relatively large genome size (Gale et al. 1990). The discovery, during the analysis and evaluation of different genomic libraries, of a chromosome-specific DNA sequence that reveals a high level of RFLP, and for which many varieties display a "null" phenotype, is described below.

Materials and methods

Library construction and screening

Five micrograms of wheat leaf DNA, purified by CsCl gradient centrifugation, was digested to completion with 20 units of EcoRI (Gibco-BRL) for 20 h and then ligated with pUC18 at room temperature for 4 h (King and Blakesley 1986). The bacterial strain Bozo 2.7 was transformed with the ligation mixture using the method of Hanahan (1983) and plated out on Xgal/IPTG/ampicillin/LB plates (Maniatis et al. 1982). Clones were picked and grown individually, and plasmid mini-preparations were made. The inserts were found to be in the size range of 0.25 to 2.5 kb.

The DNA from these clones was hybridised to EcoRI-restricted total wheat genomic DNA using a dot blot apparatus (BioRad) to identify those containing highly repeated sequences. Among the clones that hybridised strongly, many were identified as plastid sequences when subsequently probed with wheat chloroplast DNA. The remaining clones were then used as probes to hybridise to Southern blots of HindIII-restricted DNA of the nullisomic-tetrasomic lines of Chinese Spring. The majority of these were found to be moderately to highly repeated, as concluded from the complex patterns of hybridising fragments produced. Only four provided a hybridisation pattern that could be analysed so as to locate hybridising fragments to particular chromosomes. One of these was PSR454, which detected fragments on only a single Chinese Spring chromosome.

Genetic material

The Chinese Spring nullisomic-tetrasomic (NT) and ditelosomic (DT) stocks produced by Prof. E. R. Sears, University of Missouri, Columbia (Sears 1954, 1966) were provided by T. E. Miller and S. M. Reader, Cambridge Laboratory. Other wheat genotypes and segregating populations referred to in the text were provided by A. J. Worland, Cambridge Laboratory. The barley genotypes were provided by J. W. Snape, Cambridge Laboratory, and the two rye genotypes by P. Masojć, Academy of Agriculture, Szczecin, Poland.

RFLP procedures

All methods of DNA extraction, restriction enzyme digestion, agarose gel electrophoresis, alkaline Southern blotting to the nylon membranes Gene Screen Plus (Du Pont, NEN) or Hybond N^+ (Amersham), probe preparation and labelling were as described by Sharp et al. (1988). Prehybridisation was performed in 0.6 *M* NaCl, 20 m*M* PIPES (pH 6.8), 4 m*M* EDTA, 0.2% gelatin, 0.2% Ficoll 400, 0.2% PVP-360, 1% SDS, 0.5% sodium pyrophosphate and 0.5 mg/ml autoclaved salmon testes

DNA for at least 2 h at 65 °C. After removal of this solution, hybridisation was performed with ^{32}P-labelled probe in the same buffer at 65 °C for 16 h. The membranes were washed twice at 65 °C in 2 × SSC, 1% SDS and twice in 0.2 × SSC, 1% SDS for 15 min each and exposed to Kodak XAR-5 film at −70 °C between two intensifying screens.

DNA dot blot method

Two hundred and fifty nanograms of total genomic DNA was digested with 1 unit of HindIII for 1 h and transferred onto Hybond N$^+$ membrane using a dot-blot apparatus (BioRad). The membrane was processed as above.

Results

Chromosomal location

Two of the four low-copy clones hybridised to sequences on each chromosome in homoeologous group 5 or homoeologous group 1, and one clone hybridised to sequences in both groups 3 and 5. The remaining clone, PSR454 (600 bp), hybridised to fragments of only a single chromosome, namely *3B*. The lack of detectable hybridising sequences in N3BT3A and DT3BS indicates that this probe is specific for sequences on the long arm of chromosome *3B* (Fig. 1). This result was so unexpected that the experiment was repeated, this time washing at a lower stringency of 2 × SSC, without a second 0.2 × SSC wash, and extending the exposure time from 3 days to 1 month (results not shown). Even after this length of time, the N3BT3A and DT3BS lanes were still completely blank with no significant differences in the other lanes, demonstrating that PSR454 contained a sequence specific to *3B* alone.

Copy number estimation

The multiplicity of restriction fragments hybridising to PSR454 shows that the copy number of related sequences in Chinese Spring is greater than one. Depending on the restriction enzyme used to digest the genomic DNA, 8–12 bands were observed, with some showing a much stronger hybridisation signal than others. To estimate the relative copy number of this sequence per haploid genome, PSR454 insert DNA was loaded on an agarose gel in amounts equivalent to a single sequence (0.4 pg), a sequence present in 10, 20, 30, 40, 50 and 100 copies, and 10 µg of EcoRI-restricted Chinese Spring DNA. After Southern transfer, the membrane was probed with PSR454 and the intensities of hybridisation were compared. The results (not shown) indicated that the sequence represented by PSR454 is present in Chinese Spring in at least 30 copies per haploid genome.

RFLP analysis

PSR454 was hybridised to genomic DNAs of 13 wheat varieties, 13 barley varieties and two rye varieties digested with HindIII, EcoRI, EcoRV or DraI. Of the 13 wheat varieties tested, 9 carried hybridising fragments, while four (Timgalen, RL4137, Condor and Synthetic) showed no detectable hybridisation at all, i.e. had a null phenotype for the PSR454 sequence (Fig. 2a). Among the 13 varieties analysed, three patterns were observed

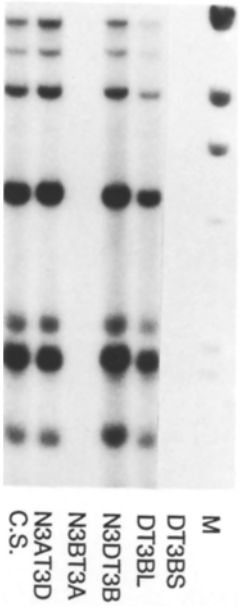

Fig. 1. Autoradiograph after hybridisation of EcoRI-digested genomic DNAs of the Chinese Spring (CS) homoeologous group 3 nullisomic-tetrasomic and ditelosomic lines with PSR454. M = HindIII-digested lambda DNA size marker

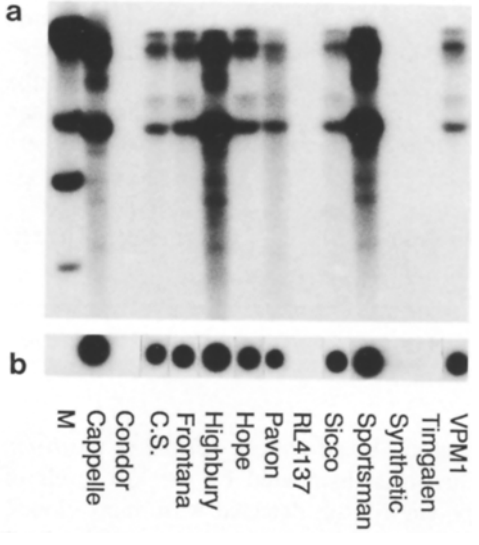

Fig. 2a and b. Autoradiograph after hybridization of HindIII-digested genomic DNAs of 13 wheat varieties with PSR454. **a** Overnight electrophoretic separation of 10 µg of digested DNA followed by Southern blotting. Blank lanes show the null phenotype. M = HindIII-digested lambda DNA size marker. **b** Direct transfer of 250 ng of HindIII-digested DNA using a dot blot apparatus

with EcoRI and four with DraI, EcoRV and HindIII, including the null phenotype. Overall, the level of RFLP, as measured by the proportion of differences in pattern detected in all possible pairwise comparisons between varieties, was 73% (69% for EcoRI, 72% for DraI and 76% for EcoRV and HindIII). None of the barley varieties and only one of the ryes showed any detectable hybridisation to PSR454 (results not shown).

Detection of RFLPs using dot blots

Dot blot analysis demonstrated that the null phenotype varieties could be detected without electrophoretic separation followed by Southern blotting (Fig. 2b). Reexposure for a further month still detected no hybridisation on the null dots (results not shown).

Genetic analysis

The NT and DT analyses show that the sequence is restricted to the long arm of *3B*. In order to determine whether the several copies are all closely linked or spatially dispersed on chromosome *3BL*, two further experiments were carried out. Firstly, pulse-field gel electrophoresis (PFGE) separations of genomic DNA restricted with rare-cutting enzymes were performed to determine whether the several copies can be shown to be carried on only one large DNA fragment. Secondly, an analysis of a F_2 segregating population of a Chinese Spring × Synthetic cross was used to determine whether the copies recombine.

Chinese Spring genomic DNA digested with NotI and NruI was separated using a contour-clamped homogeneous electric field (CHEF) apparatus, with a pulse phase designed to provide a resolution of up to 3.3 Mbp and transferred to nylon membranes as described in Cheung and Gale (1990). Probing with PSR454 detected three NotI fragments with approximately equal hybridisation signals totalling 6.4 Mbp and a major NruI band of 2.8 Mbp, with less intense hybridisation elsewhere (results not shown). This indicates that the minimum length of DNA containing sequences related to PSR454 is at least 2.8 Mbp, but it does not define the maximum size. When this minimum size is compared to the sum of the sizes of the seven fragments observed on the autoradiograph in Fig. 1, approximately 224 kb (56 kb multiplied by 30/7 to account for copy number), it is clear that these related sequences cannot be arranged in tandem.

Segregation analysis was performed on the EcoRI-restricted DNA of 96 F_2 progeny of a cross between the wheat varieties Chinese Spring and Synthetic. Segregation was consistent with single-locus inheritance (1:2:1), where presumptive heterozygotes were identified by a reduced hybridisation signal representing a halving of the number of copies (Fig. 3). However, as dominant homozygotes were difficult to distinguish from heterozygotes and some misclassification could have occurred, the segregation was also treated as a 3:1 ratio, with which it was also consistent. Thus, all sequences represented by PSR454 are inherited as a single linkage block. From the PFGE data this "locus" is at least 2.8 Mbp in size.

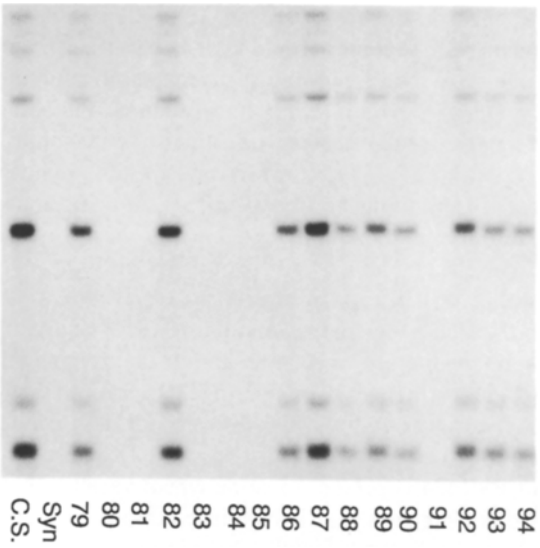

Fig. 3. Autoradiograph from the hybridisation of EcoRI-digested genomic DNAs of 16 of the 96 F_2 progeny of a Chinese Spring (CS) × Synthetic (Syn) cross with PSR454. Blank lanes show the Synthetic phenotype. Progenies 86, 88, 89, 90, 93 are probably heterozygotes

Discussion

In this paper we have described the isolation of a member of a family of related chromosome-specific, moderately repeated DNA sequences in wheat. Of 73 random wheat cDNA clones described by Sharp et al. (1989), none were found to be chromosome specific. Random genomic libraries are expected to be a source of noncoding, and thus perhaps chromosome-specific, sequences. In addition, as non-coding regions may be less conserved than coding regions (Helentjaris and Gesteland 1983), genomic libraries may contain sequences that are more likely to demonstrate RFLP than cDNA libraries. The isolation of PSR454 from one such library would seem to support this. Indeed, the level of RFLP (73%) observed using PSR454 is the highest recorded in our laboratory with a RFLP probe. For comparison, Chao et al. (1989) recorded a mean of 8.7% for RFLP at 54 loci identified with 18 cDNA clones.

Null alleles resulting from the lack of detection of sequences hybridising to single-copy clones have been reported in lettuce (Landry et al. 1987), tomato (Zamir and Tanksley 1988) and rice (McCouch et al. 1988). Since

in wheat, most low-copy clones detect sequences in all three constituent genomes (Chao et al. 1989) then, even if null alleles in one genome are found, conventional Southern analysis is required to detect them in the presence of related sequences in the other two genomes. However, since PSR454 is chromosome specific, related sequences are absent in the other genomes, allowing analysis by the dot blot method, avoiding the necessity of agarose gel electrophoresis and Southern blotting. We estimate that this method is at least as twice as fast as, and less than half the cost of, standard RFLP analysis.

It is interesting to speculate about the origins of the PSR454 sequence. Considering its chromosome specificity and variability, its discovery in a genomic rather than in a cDNA library is expected. The observation that PSR454 is present in some wheat varieties but not in others suggests that it has little adaptive significance. Its location on a single chromosome and in a single linkage block implies a recent origin, as one might have expected an "older" repeat sequence to be present on more than one chromosome due to transposition and other events. This suggests that the source could be a single ancestral variety. However, an exhaustive pedigree study did not reveal such a result. Indeed, analysis of one accession of each of 31 alien relatives of wheat, including *Triticum*, *Hordeum*, *Aegilops*, *Agropyron* and *Dasypyrum* species, showed this sequence to be present or absent in a way which yielded no clue to its origin; for example, it is present in one accession of each of *Hordeum bulbosum*, *Aegilops uniaristata* and *Aegilops sharonensis*, but not in 13 different *Hordeum vulgare* cultivars, nor in an accession of *Aegilops triaristata* or *Aegilops longissima*. We are screening a wider distribution of species to include other cereals such as maize and rice, in order to determine whether these species also have sequences related to PSR454.

Acknowledgements. We would like to thank P. J. Sharp and S. Chao for helpful advice, and R. M. D. Koebner and G. Moore for critical comments on the manuscript. We are grateful to W. Y. Cheung for providing the PFGE membranes and to C. M. Bowman for the wheat chloroplast DNA. We thank J. B. Smith, C. L. Gibbs and C. N. Chinoy for technical assistance. RLH acknowledges the receipt of a John Innes Trust Fund scholarship and an Overseas Research Student award.

References

Chao S, Sharp PJ, Worland AJ, Warham EJ, Koebner RMD, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group 7 chromosomes. Theor Appl. Genet 78: 495–504

Cheung WY, Gale MD (1990) The isolation of high molecular weight DNA from wheat, barley and rye for analysis by pulse-field gel electrophoresis. Plant Mol Biol 14: 881–888

Gale MD, Chao S, Sharp PJ (1990) RFLP mapping in wheat – progress and problems. In: Gustafson JP (ed) Gene manipulation in plant improvement II. Plenum Press, New York, pp 353–364

Hanahan D (1983) Studies on transformation of *Escherichia coli* with plasmids. J Mol Biol 166: 557–580

Helentjaris T, Gesteland R (1983) Evaluation of random cDNA clones as probes for human restriction fragment polymorphisms. J Mol Appl Genet 2: 237–247

King PV, Blakesley RW (1986) Optimizing DNA ligations for transformation. Focus 8: 1–3

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: a laboratory manual. Cold Spring Harbour Laboratory Press, Cold Spring Harbour/New York

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

Sears ER (1954) The aneuploids of common wheat. Mo Agric Exp Stn Res Bull 572: 1–58

Sears ER (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulation and plant genetics. Oliver and Boyd, London, pp 29–45

Sharp PJ, Kreis M, Shewry PR, Gale MD (1988) Location of β-amylase sequences in wheat and its relatives. Theor Appl Genet 75: 286–290

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78: 342–348

Zamir D, Tanksley SD (1988) Tomato genome is comprised largely of fast-evolving, low-copy-number sequences. Mol Gen Genet 213: 254–261