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Hordeum chilense repetitive sequences. Genome characterization using biotinylated probes

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Summary. A library of random DNA fragment clones of wild barley *Hordeum chilense* was screened for clones of repeated nucleotide sequences. Five clones were isolated that gave a stronger hybridization signal in colony and dot blot hybridization with total *H. chilense* DNA in comparison to *Triticum aestivum* DNA. Clones labelled with biotinylated nucleotides were used as probes to investigate the repeated sequences organization in the *H. chilense* genome. Tandemly arranged and interspersed sequences have been found, together with homology differences with related sequences present in *T. aestivum*, which could allow the differentiation of *H. chilense* DNA when it is present in wheat. We show that biotin can replace the use of ^{32}P in preparing repeated sequence probes for Southern and DNA dot blot analyses.

Key words: *Hordeum chilense* – Wheat – Repeated sequences – Molecular probes – Biotin

Introduction

Intergeneric and interspecific hybridization have great potential for crop improvement as a first step towards introducing alien variation and transferring desirable traits from wild species into cultivated species. Hybridization between wheat and barley, both cultivated and wild, has been attempted a number of times with varying degrees of success (Kruse 1973; Martin and Chapman 1977; Islam et al. 1981; Shetti et al. 1986). However, the development of amphiploids to be used as a new crop is promising. The allohexaploid between *Hordeum chilense* and tetraploid wheat (called tritordeum) shows good cytological and agronomic characters (Martin and Sánchez-Monge Laguna 1982), and is under selection and trial testing to improve its favorable features. The amphiploid also offers the possibility of chromosome manipulation. Crosses with *Triticum aestivum ph1b* mutant would create a *ph1b/ph1b* background, which would permit homoeologous pairing between wheat and *H. chilense* chromosomes and should also allow the selection of spontaneous translocations between univalents belonging to the D and H^{ch} genomes.

The identification of recombinant plants could be undertaken by using cytological, biochemical, or molecular chromosome markers. The C-banding pattern of *H. chilense* chromosomes is different from the pattern of wheat chromosomes (Fernández and Jouve 1984; Armstrong et al. 1987). However, not all the chromosome arms or every chromosome region can be identified. The characterization of isozymes (Fernández and Jouve 1987; Miller and Reader 1987) and endosperm proteins (Payne et al. 1987) only allows screening for a few loci.

Another approach, the use of molecular markers, promises to be a powerful tool in the characterization of hybrid plants and recombinant chromosomes. The cromosomes of higher organisms contain repeated nucleotide sequences (Britten and Khone 1968). This kind of sequence can account for a considerable proportion of the total nuclear DNA in cereals (Flavell et al. 1977). A common feature of these sequences is that the repeated units of each family can diverge considerably during evolution, and, therefore, there are often large differences among the repeated sequences of related species (Flavell 1982; Dover et al. 1982). Isolation of species-specific repeated DNA has been carried out in a number of crop species and their wild relatives within the Triticeae (Bredbrook et al. 1980; Gerlach and Peacock 1980; Appels and Moran 1984; Prosnyak et al. 1985; Rayburn and Gill 1986; Dvorak et al. 1988). Both tandem arrays and dispersed sequences of the middle and highly repeated frac-

tion of DNA have been used as hybridization probes in dot and Southern blots to detect the presence of the genome under investigation in the DNA of hybrids, amphiploids, or derived plants (Hutchinson et al. 1985; Junghams and Metzlaff 1988; Gill et al. 1988).

Furthermore, chromosome mapping following in situ hybridization has allowed the location of some species-specific repeated sequences to chromosomes of different species (Jones and Flavell 1982; Hutchinson and Londsdale 1982; Rayburn and Gill 1986; Appels et al. 1986). Lapitan et al. (1986) were able to identify the breakpoints of certain wheat-rye translocations by analyzing the distribution of a dispersed, highly repeated sequence of rye.

Recently, it has been shown that biotin-labelled probes give a resolution that is comparable to that obtained with radioactive probes (McInnes et al. 1987; Craigh et al. 1988). Single-copy genes and repetitive sequences have been detected in digests of total human genomic DNA by Southern blot analysis using biotinylated probes. The progressive improvement obtained by the reduction of the background, together with the simplified procedures and the conservation of resources (as probes do not have to be continually made) are outstanding advantages.

In this study, we report the molecular cloning and characterization of members of five different repeated sequence families of *H. chilense*, as well as their relationship to related families in *T. aestivum*, by means of dot and Southern blot analysis and biotin-labelled probes. Our aim is to isolate *H. chilense*-specific repeated sequences that will further allow us to clearly identify its chromosomes in hybrids and derivatives.

Materials and methods

Plant material

Seed of *Triticum aestivum* cv 'Chinese Spring' *ph1b* mutant were obtained from E. R. Sears (University of Missouri, Columbia/MO). *Triticum turgidum* ssp. *durum* cv MA and *Hordeum chilense* line H-1 were obtained from A. Martín (CSIC, Córdoba, Spain).

Plant DNA cloning

High-molecular-weight DNA was extracted from isolated nuclei of 3-week-old seedlings of *H. chilense* following the procedures described by Evans et al. (1983) and Sharp et al. (1988). The DNA was further purified by CsCl centrifugation.

The isolated nuclear DNA was digested with MboI under conditions that generated a size distribution of fragments peaking in the range of 2 kb to 4.5 kb. The random fragments were cloned into the BamHI site of pUC19. Plasmids were used to transform *E. coli* JM83.

Clones containing repeated DNA sequences were identified by colony hybridization (Grundstein and Hogness 1975) and dot blot analysis of recombinant plasmids (Birnboim and Doly 1979), using biotinylated total nuclear DNA of *H. chilense*. Only repetitive DNA gave a strong hybridization signal in this system. These clones were also screened for *T. aestivum* sequences by hybridizing them with biotinylated total DNA of *T. aestivum*. Clones giving a negative hybridization signal, similar to the salmon testes DNA control, were selected.

Plasmids were further characterized by determining the length of their inserts and the sites of a number of restriction enzymes. One microgram of each plasmid preparation was digested with EcoRI, HindIII, BamHI, and HaeIII according to the manufacturer's instructions. Digested DNAs were fractionated by electrophoresis either in 0.7% agarose gels or 5% acrylamide vertical gels (Maniatis et al. 1982), and the fragment lengths were estimated by comparing them with DNA molecular-weight markers.

Southern blot hybridization

Five-microgram aliquots of *H. chilense* and *T. aestivum* DNAs were digested with various restriction endonucleases for 16 h, electrophoretically fractionated in 1% agarose/TAE gels, and blotted onto Zeta-probe nylon membranes by alkaline blotting (Reed and Mann 1985). Membranes were prehybridized in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 25 m*M* sodium phosphate pH 6.5, 5% dextran sulphate, 0.5% skimmed milk, and 0.5 mg/ml sheared, denatured salmon sperm DNA at 43 °C for 24 h.

Hybridization was carried out in a shaking bath at 43 °C for 21 h in a buffer containing 45% (v/v) formamide, 5 × SSC, 1 × Denhardt's solution, 20 m*M* sodium phosphate, pH 6.5, 0.5% skimmed milk, 5% dextran sulphate, 0.5% SDS, 0.20 mg/ml salmon sperm DNA, and 300 ng/ml of denatured biotinylated probe. Of the above solution, 75 µl was used per 1 cm^2 of membrane. The probes were labelled with biotin 11-dUTP (BRL) by nick translation (Rigby et al. 1977). The hydridization solution can be stored at −20 °C and reused up to six times without loss of sensitivity.

Filters were washed by shaking twice in 2 × SSC, 0,1% SDS for 3 min each time at room temperature, twice in 0.2 × SSC, 0.1% SDS for three min at room temperature, once in 0.16 × SSC, 0.1% SDS for 15 min at 50 °C, once in 0.16 × SSC, 0.1% SDS for 15 min at 60 °C, and then twice in 2 × SSC for 3 min at room temperature.

Colorimetric detection of biotinylated probes

Detection by a streptavidine-alkaline phosphatase conjugate was according to the manufacturer's protocol (BRL Blue Gene kit). Filters were incubated with substrate solution for 30 min–3 h in the dark. The reaction was stopped, and the membranes were washed in 20 m*M* *Tris*, pH 7.5, 0.5 m*M* EDTA. Membranes were photographed, air dried, and stored.

Results

From the colony hybridization experiments, 62 clones containing repeated nucleotide sequences (out of a library of 550) were initially selected, as they gave a strong signal when they were hybridized with *H. chilense* DNA. Twenty clones hybridized more intensely with *H. chilense* genomic DNA than with that from *T. aestivum*. Dot blot analysis of the 20 clones selected was carried out to accurately test the hybridization signals, since it is known that debris of bacterial cell walls can interfere with the DNA binding to the membrane, thereby affecting hybridization levels. Furthermore, when biotinylated probes are

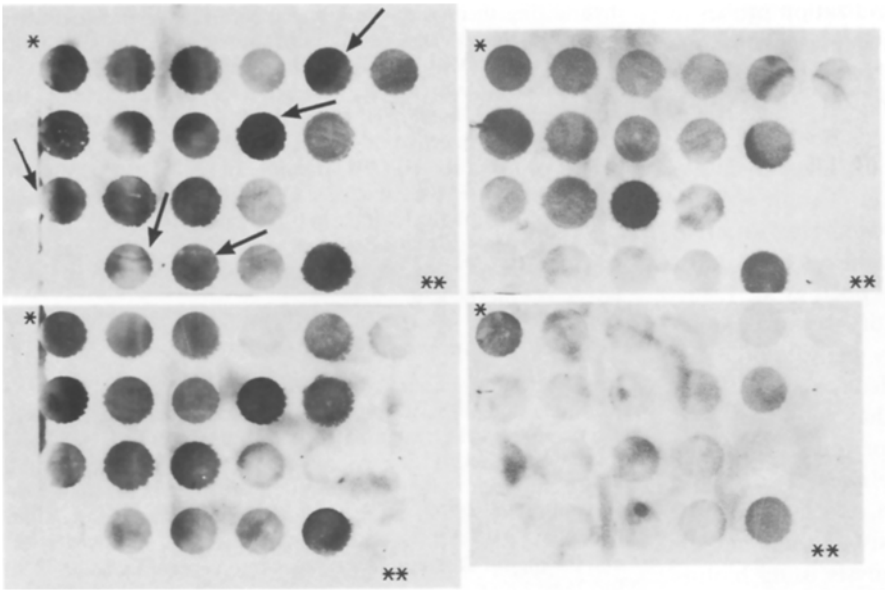

Fig. 1. Dot blot analysis of recombinant plasmids after hybridization with: *left* – biotin-labelled *H. chilense* total DNA; *right* – biotin-labelled *T. aestivum* total DNA. *Top line:* the last posthybridization wash was performed at 60 °C. *Bottom line:* the last posthybridization wash was performed at 65 °C. *Arrows* indicate the five selected plasmids. * stands for *H. chilense* DNA; ** stands for salmon testes DNA

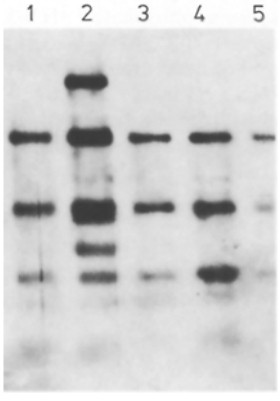

Fig. 2. Southern blot of Sau3A-digested plasmids (0.25 µg per lane) probed with biotinylated pHch1. pHch4 (*lane 1*); pHch1 (*lane 2*); pHch2 (*lane 3*); pHch5 (*lane* 4); pHch 3 (*lane 5*)

used, the antibody-enzyme conjugate could react unspecifically with bacterial cell wall components, disturbing the hybridization signal.

Five clones, designated pHch1 through pHch5 were selected in accordance with the high level versus low level of hybridization observed when probed with *H. chilense* and *T. aestivum* DNA, respectively, at two stringency washes (Fig. 1). These clones were expected to contain repetitive sequences from the H^{ch} genome, virtually absent from the A, B, and D genomes, and these could provide a means of indentifying alien chromatin introduced into wheat.

To estimate the total length of each insert in the recombinant plasmids, double digestions with EcoRI and HindIII were carried out. Clones pHch1, pHch2, pHch3, pHch4, and pHch5 contained 2.6-, 2.1-, 0.5-, 2.6-, and 2.0-kb inserts, respectively.

The degree of homology among the cloned sequences was investigated by means of Southern hybridizations of recombinant plasmids digested with Sau3A, probed with each of the clones in turn. This enzyme was employed instead of MboI, because Sau3A can recognize the same target as MboI, even when it has been modified by the *dam* methylase present in the *E. coli* strain used in this experiment. The results, obtained after probing with pHch1 (Fig. 2), only show that the bands corresponding to vector fragments shared by all the plasmids appeared. Similar patterns were obtained from the other four probes. It was therefore concluded that the five inserts originated from different repeated nucleotide sequence families.

Genomic organization of the cloned sequences

Repetitive sequences are usually organized either as tandem repeats, arranged in a similar manner to some satellite sequences, or as dispersed repeats located in various loci on the genome (Flavell 1982). Repetitive sequences representing both types of sequence organization have been reported (Bedbrook et al. 1980; Appels et al. 1986). In order to address the question of how the families to which our cloned fragment belonged are organized within the *H. chilense* genome, and to assess genome affinities to *T. aestivum* genomes, we performed Southern hybridization studies. Four restriction enzymes – EcoRI, HindIII, BamHI, and HaeIII – were used for digestion of genomic DNAs.

pHch1

The products of complete digestion of *H. chilense* DNA by HindIII, BamHI, and EcoRI, when separated on an

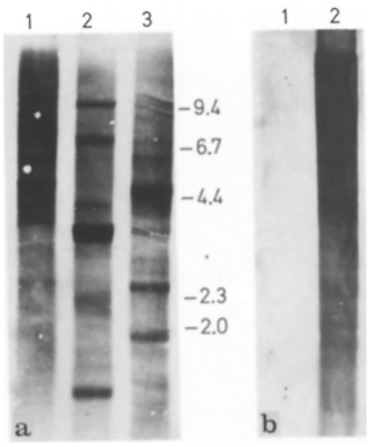

Fig. 3a and b. Southern blot hybridization of *H. chilense* and *T. aestivum* DNAs probed with biotinylated pHch1. **a** Five micrograms of total DNA of *H. chilense* was digested with EcoRI (*lane 1*), BamHI (*lane 2*), and HindIII (*lane 3*). **b** Five micrograms of total DNA of *T. aestivum* (lane 1) and 5 μg of total DNA of *H. chilense* (lane 2) were digested with EcoRI. Numbers refer to the molecular weights, in kb, of HindIII-digested lambda DNA

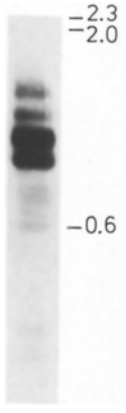

Fig. 4. Southern blot of HaeIII-digested *H. chilense* DNA (5 μg) probed with biotinylated pHch1. Numbers refer to molecular weights, in kb, of HindIII-digested lambda DNA

agarose gel, blotted, and probed with pHch1, resulted in a complicated pattern of distinct bands of various intensities, ranging from 1 kb to 10 kb in the BamHI digest and from 1.8 kb to 8 kb in the HindIII digest; they also resulted in a mixture of high-molecular-weight fragments inthe EcoRI digest (Fig. 3a). Hybridization also occurs with a smear of fragments over a wide range of sizes, but the fact that this is not due to nonspecific binding is shown by the complete absence of hybridization to nonhomologous DNA, such as that from salmon testes (data not shown).

Because none of the enzymes were able to cut the cloned sequence, the multiple bands that hybridized to pHch1 represent fragments of different lengths, which

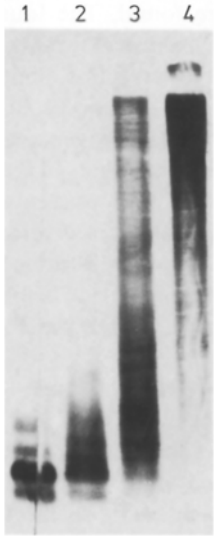

Fig. 5. Southern blot hybridization analysis of a digestion of *H. chilense* DNA probed with pHch1. An aliquot of 5 μg of DNA was digested for 1 h at 37 °C with 3.2 units of HaeIII/μg of DNA (*lane 1*) 0.8 units (*lane 2*), 0.4 units (*lane 3*), and 0.2 units (*lane 4*)

share homology with the cloned sequence and whose sizes depend on where the recognition sites of the enzymes are located on adjacent sequences. Therefore, the large size distributions of the BamHI, HindIII, and EcoRI restriction fragments with respect to the pHch1 sequence and their different equimolar amounts indicate that the family members of the cloned sequence are dispersed in the genome and are present in varying copy number at each site.

The internal heterogeneous nature of the cloned sequences family was revealed by digestion with HaeIII. The nucleotide sequence of pHch1 proved to be very rich in HaeIII sites, since HaeIII digests of pHch1 gave a fragment of 900 bp along with 11 fragments ranging from 190 to 60 bp. Hybridization of pHch1 to *H. chilense* DNA digested by HaeIII showed two main bands of 0.9 and 1 kb and other minor bands of 0.2, 0.3, 0.6, 1.2, and 1.4 kb (Fig. 4). The lack of any smear, in contrast to the results obtained with the other enzymes, indicates that every sequence homologous to pHch1 contains at least one internal fragment liberated by the HaeIII digestion. The results in Fig. 4 show that pHch1 hybridizes to specific restriction fragments that are not in equimolar amounts. The genomic fragments of 1, 1.2, and 1.4 kb could be generated by partial digestion of larger repeating structures that include the 0.9-kb fragment.

To test this hypothesis, *H. chilense* DNA samples were subjected to increasing amounts of HaeIII (Fig. 5). As the digestion progressed, a depletion of discrete large bands in concert with the appearance of smaller ones was evident. However, lengths of the larger bands were clearly different from those obtained after complete digestion

and no multimeric relationships were found. The four bands produced by complete digestion increased simultaneously in intensity during the period tested. Thus, none of the bands is the result of incomplete digestion of another. This indicates that pHch1 probes a heterogeneous collection of DNAs, presumably because members of the pHch1 family occur in a number of different sequence environments, being part of a larger and more diverse repeating unit. A precise restriction map of the cloned sequence and subcloning of different fragments are being carried out to ascertain the organization of the pHch1 repeated sequence family.

When *T. aestivum* and *T. turgidum* DNA were digested with EcoRI and BamHI and hybridized to pHch1, neither discrete bands nor smear was visible (Fig. 3 b). Therefore, pHch1 represents a good marker for *H. chilense* DNA, and it will be particularly useful to check the presence of specific *H. chilense* DNA in descendants of hybrids between *H. chilense* and *T. aestivum* or *T. turgidum*.

pHch2

This clone contains a 2.16-kb insert, which was cut by HaeIII generating two fragments of 0.36 and 1.8 kb, respectively. No EcoRI, BamHI, or HindIII sites were found. Probing *H. chilense* DNA digested with restriction enzymes that recognize a hexanucleotide DNA sequence, with pHch2 yielded bands varying from 0.8 kb to 6 kb, suggesting that sequences complementary to the probe are present in several discrete configurations (Fig. 6).

A common band of 1.85 kb was present in all digestions, indicating that some of the sequences could be organized in a short tandem of 1.85 kb units each, carrying for one site EcoRI, HindIII, and BamHI. However, no evidence of multimers remained after partial digestions with those enzymes.

The hybridization of pHch2 with *H. chilense* DNA digested with HaeIII showed a prominent band of 500 bp and some less clear bands ranging from 0.7 to 2.8 kb, and some smear was also observed (Fig. 7). This result indicated that most of the sequences homologous to the clone contained a fragment of 500 bp that is liberated by the HaeIII digestion. As this differs from the 360 bp HaeIII fragments from pHch2, it appears that either only a part of the homologous sequence to the 500 bp fragment has been cloned in pHch2, or that the clone belongs to a less representative subfamily lacking one HaeIII site. In any case, the high number of bands on the Southern hybridization of HaeIII digests would indicate a high level of sequence heterogeneity among members of the pHch2 family.

Clone pHch2 cross-hybridized with *T. aestivum* DNA. Common but weaker bands to those of *H. chilense*

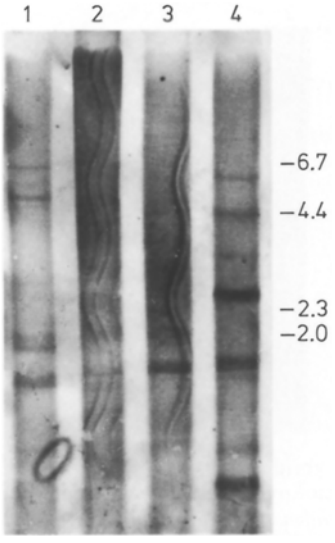

Fig. 6. Southern blot of *T. aestivum* DNA (5 µg per lane) digested with EcoRI (*lane 1*), *H. chilense* DNA (5 µg per lane) digested with EcoRI (*lane 2*), HindIII (*lane 3*), and BamHI (*lane 4*), and probed with biotinylated pHch2. Numbers refer to molecular weight, in kb, of HindIII-digested lambda DNA

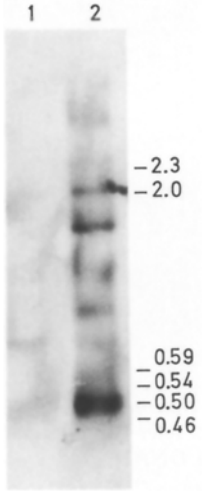

Fig. 7. Southern blot of HaeIII-digested genomic DNA (5 µg per lane) probed with pHch2. *T. aestivum* (*lane 1*) *H. chilense* (*lane 2*). Numbers refer to molecular weight, in kb, of HindIII-digested DNA and HaeIII-digested pBR322

were found after digestion of *T. aestivum* with HaeIII (Fig. 7). Either a lesser abundance of the sequence in the *T. aestivum* genome or a reduction in homology could account for the reduction of hybridization observed.

The hybridization pattern of *T. aestivum* digested by EcoRI showed a group of four discrete bands of different length to those of *H. chilense* (Fig. 6, lane 1). This indicates a more homogeneous dispersion of the sequence in the *T. aestivum* genome. In spite of the cross-hybridization, this clone need not be discarded when hybrids or

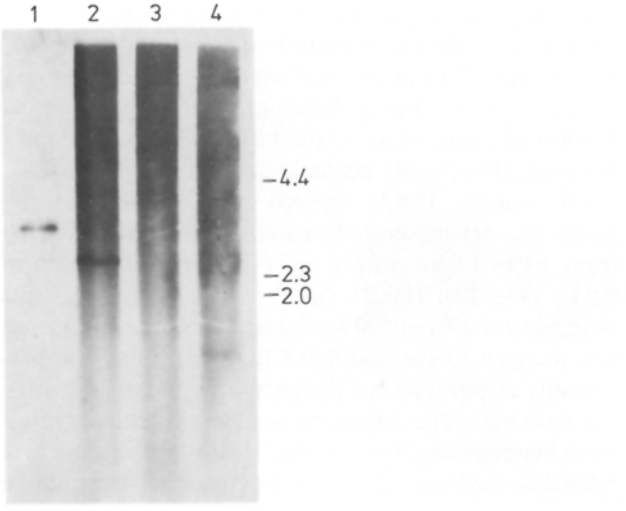

Fig. 8. Southern blot of *T. aestivum* DNA (*lane 1*) and *H. chilense* DNA (5 µg per lane) digested with EcoRI (*lanes 1 and 4*), BamHI (*lane 3*), HindIII (*lane 2*), and probed with biotinylated pHch3. Numbers refer to molecular weight, in kb, of HindIII-digested lambda DNA

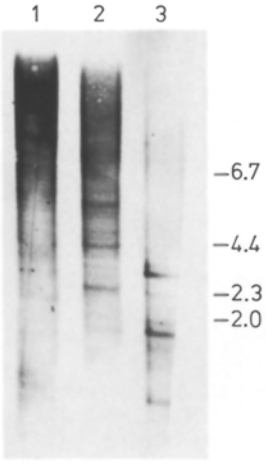

Fig. 10. Southern blot of *H. chilense* DNA (5 µg per lane) digested with EcoRI (*lane 1*), BamHI (*lane 2*), and HindIII (*lane 3*), and probed with biotinylated pHch4. Numbers refer to molecular weight, in kb, of HindIII-digested lambda DNA

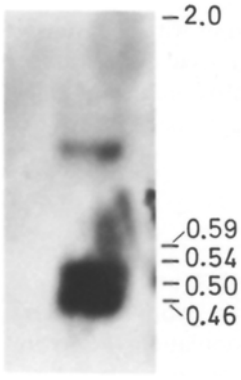

Fig. 9. Southern blot of HaeIII-digested *H. chilense* genomic DNA (5 µg) probed with biotinylated pHch3. Numbers refer to molecular weight, in kb, of HaeIII-digested pBR322

amphiploids are checked, since they might show higher levels of signal for the 500 bp band than that of *T. aestivum*.

pHch3

The insert of this clone had a length of 500 bp. On the enzymes tested only HaeIII was able to cut the insert, producing two fragments of about the same length.

H. chilense DNA digested with EcoRI or BamHI and hybridized with pHch3 did not produce distinct hybridizing fragments. Complete digestion by HindIII produced an intense hybridizing smear and a weaker band of 2.8 kb (Fig. 8). The hybridization patterns produced by enzymes whose sites were not present in the cloned sequences were taken as an indication of sequence conservation within the repeated family. On the other hand, the products of complete digestion by HaeIII produced three distinct hybridizing bands; two major ones in nearly equimolar amounts (450 and 500 bp), and a weaker one of 900 bp (Fig. 9). In some membranes a very faint band of 1,000 bp was observed. Southern hybridizations of MboI digests of *H. chilense* DNA showed a similar pattern of bands (data not shown). These data could suggest a tandem array organization of the homologous sequences to pHch3, which would constitute two related families differing by an insertion/deletion of about 50 bp. The occasional loss of a HaeIII or MboI site accounts for the 900 and 1,000 bp dimers.

A weak hybridization signal was apparent after probing *T. aestivum* DNA digested with EcoRI with pHch3 (Fig. 8), indicating a small degree of homology between the pHch3 insert and related families of *T. aestivum*.

pHch4

The hybridization pattern after digestion of *H. chilense* DNA with hexanucleotide target restriction enzymes of this probe was similar to that of pHch1: band size ranged from 1 kb to 5.5 kb without any obvious multimeric relationships, superimposed over a hybridization smear (Fig. 10). A dispersed nature of the sequences complementary to the probe was inferred from these patterns. The probe failed to give any prominent band on the blots of *H. chilense* DNA digested by HaeIII (Fig. 11). Since the insert of the clone assesses three HaeIII sites, and its digestion yields fragments of size 1,560, 480, 242, and

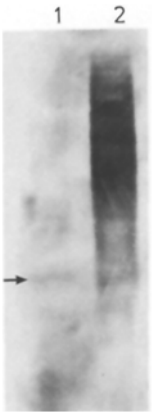

Fig. 11. Southern blot of *T. aestivum* DNA (*lane 1*) and *H. chilense* DNA (*lane 2*) digested with HaeIII and probed with biotinylated pHch4. *Arrow* indicates the 0.9 kb band

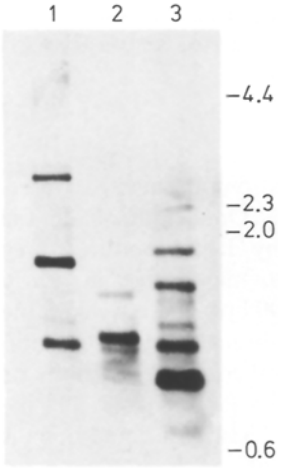

Fig. 12. Southern blot of *H. chilense* DNA (5 µg per lane) digested with EcoRI (*lane 1*), AluI (*lane 2*), and HaeIII (*lane 3*), and probed with biotinylated pHch5

122 bp, this family appears to be heterogeneous and is not very abundant in the *H. chilense* genome.

T. aestivum DNA hybridized very poorly with pHch4 after digestion with HaeIII (Fig. 11). A faint band of 0.9 kb was observed.

pHch5

This clone has a 2-kb insert, and hybridized strongly to three fragments of 1.09, 1.81, and 2.9 kb length in *H. chilense* DNA digested by EcoRI (Fig. 12). Most of genomic DNA homologous to this clone hybridized to these three fragments, as the hybridization smear along the track is greatly reduced when compared to the other clones. Digestion with BamHI or HindIII did not generate any prominent band. Since both enzymes were able to cut the cloned sequence at one site, it can be deduced that this family of repeats is dispersed. Considering that the insert sequence in plasmid pHch5 lacks EcoRI sites, the hybridization pattern obtained with this enzyme would indicate that the repeated unit was dispersed among very few different sequences, heterogeneous with respect to EcoRI sites, and would account for the fragment lengths obtained after EcoRI genomic digestion.

H. chilense DNA digested with HaeIII and hybridized to pHch5 showed a pattern of fragments ranging from 0.3 to 1.9 kb, with a most prominent fragment of 0.9 kb (Fig. 12). HaeIII cut the insert into fragments ranging in size from 900 to 190 bp. The largest fragment was mapped to the middle of the insert, and was presumably responsible for the prominent fragment of the genomic blot. The remaining genomic fragments result from heterogeneity for the HaeIII sites in the sequences related to the clone. However, a detailed restriction map of the cloned sequence must be done to ascertain such a hypothesis.

Digestion of *H. chilense* DNA with AluI (Fig. 12) produces a prominent band of 1.15 kb and some other weaker bands of 1.5, 1, and 0.9 kb. Because AluI was also able to cut into the cloned sequence, the deduced heterogeneity of the sequence after digestion with HaeIII is corroborated by these results.

pHch5 showed cross-hybridization with *T. aestivum*. The major bands of hybridization seen in *H. chilense* after digestion with HaeIII were also present in wheat DNA, digested with HaeIII, although the hybridization signal was considerably reduced.

Discussion

Clones of plant-specific repeated DNA and their use as probes both to detect variability at the evolutionary level and to characterize segregating populations of hybrid origin have gained considerable importance over the past few years (Bedbrook et al. 1980; Appels et al. 1986; Junghams and Metzlaff 1988; Gill et al. 1988; Dvorak et al. 1988). Simple and efficient cloning strategies have contributed to this growth. However, detection systems based on radio-labelled probes still present a severe limitation both in cost and handling, if the technology is to be used in high-volume, routine laboratory experiments. Recent developmet of nonisotopic detection methods has demonstrated the possibility that they may substitute for radio-labelling in many research fields (review by Wilcheck and Eduard 1988).

In this paper we report the cloning of several repeated sequences of *Hordeum chilense* by using biotinylated nucleotides for labelling DNA. The reported low sensitivity of biotin-coupled detection systems has been pointed out as the main disadvantage of the method (McInnes et al. 1987; Craigh et al 1988). However, if the amount of sampled DNA is not a limiting factor, the hybridization signal can be raised by increasing the quantity of DNA

bound to the filter. The good sensitivity of the hybridization reactions suggest that the biotin labelling procedure and subsequent denaturation for hybridization does not impair the resulting probe's ability to recognize complementary base sequences. An additional advantage of the method is that no detectable loss of sensitivity in hybridization reactions occurred, either with biotinylated DNA probes stored for 2 or 3 months at −20 °C or with probes reused five or six times.

The results presented in this paper show that part of the genome of *H. chilense* is constituted by repeated sequences, as with other cereal species (reviews by Appels and Moran 1984; Flavell 1985). Four of our clones, namely, pHch1, 2, 4, and 5, represent repeated families whose members are interspersed with non-repeated DNA segments and/or unrelated repeated sequences. Such an arrangement of the probe sequences is strongly suggested by the bands produced by hybridization to Southern blots of restricted genomic DNA. Only the family represented by pHch3 seems to be organized in the *H. chilense* genome in tandem arrays. This sequence is the shortest analyzed and it would confirm other observations suggesting that short repeated sequences are more probably organized in this way (Bedbrook et al. 1980).

The interspersion patterns shown by the repeated families studied are different with respect to heterogeneity and copy number of the flanking sequences. The pHch5 sequence is found within a larger conserved repetitive element, as is evident from a very simple hybridization pattern after digestion with EcoRI (Fig. 12). On the other hand, sequences homologous to pHch1, 2, and 4 produce a large range of restriction fragments of different molar amounts, which would indicate that members of the repeated families are in different genomic locations with different neighboring repeated sequences in each location. This kind of sequence organization has been postulated in a wide variety of organisms, including the most relevant cereals (Rimpau et al. 1978, 1980; Flavell et al. 1981; Appels et al. 1986).

Variation has been found among members of a repeated family. Fragments produced by restriction enzymes recognizing sites inside the hybridizing sequence are not always of a length predicted from the restriction digest of the plasmid. This is particulary evident for the sequences related to pHch4, which present a high heterogeneity for HaeIII sites in spite of the fact that the plasmid possesses three HaeIII sites (Fig. 11). Furthermore, the postulated two structural subfamilies homologous to pHch3 differing by about 50 bp indicate that an insertion or deletion distinguishes the two subsets of the hypridization family. Point mutations creating variation in HaeIII sites in the *H. chilense* genome could account for all the hybridization patterns observed. However, given the presumably compound nature of the cloned sequences (in the sense that flanking sequences repeated in variable degrees have been introduced into the plasmids), it cannot be ruled out that distinct bands correspond to genomic fragments homologous to different fragments of the cloned inserts.

The model proposed by Flavell (1982) and Dover (1982) is based upon variability for the nucleotide sequences of the family members, transposition to different genomic locations, reamplification of one sequence and their flanking elements to give a chimeric repeated sequence together with occasional deletions. This model would explain the patterns of interspersion observed.

It has been postulated that the amplification of complex DNA segments that have arisen by sequence rearrangement explain the different organization of related repeats in the cereal genomes that have envolved from a common ancestor (Flavell et al. 1977, 1981; Rimpau et al. 1978, 1980). These authors propose that species-specific sequences have been amplified at different evolutionary times. From the comparison between the hybridization patterns of *H. chilense* and *T. aestivum* DNAs with the different probes analyzed, it can be concluded that the family homologous to pHch1 has been amplified since wheat and wild barley diverged. The other analyzed sequences cross-hybridized with *T. aestivum* DNA, which indicates their presence in the common ancestor. However, the weaker hybridization bands obtained in the Southern blot of *T. aestivum* reflect different degrees of reamplification of the sequences since the divergence of these species.

An important consequence of the evolutionary divergence of the repeated sequences is that isolated species-specific DNAs can be used as probes to mark chromosomes or chromosome segments of one species when they are introduced into another. Most of the sequences described in this paper seem to serve this purpose: pHch1 and pHch3 on the hybridization conditions used in this experiment; pHch2 and pHch4 increasing the temperature of the posthybridization washes to reduce the hybridization signal due to sequences less closely related to the probe, as has been suggested by Appels et al. (1986). *Triticum aestivum-Hordeum chilense* addition lines are being analyzed to find out whether the repeated sequences of *H. chilense* show particular chromosome locations.

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