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Repeated DNA sequences isolated by microdissection. II. Comparative analysis in *Hordeum vulgare* and *Triticum aestivum*

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Abstract The genomic organization of two satellite DNA sequences, pHvMWG2314 and pHvMWG2315, of barley (*Hordeum vulgare*, 2n=14, HH) was studied by comparative *in situ* hybridization (ISH) and PCR analysis. Both sequences are members of different *Rsa*I families. The sequence pHvMWG2314 is a new satellite element with a monomer unit of 73 bp which is moderately amplified in different grasses and occurs in interstitial clusters on D-genome chromosomes of hexaploid wheat (*Triticum aestivum*, 2n=42, AABBDD). The 331-bp monomer pHvMWG2315 belongs to a tandemly amplified repetitive sequence family that is present in the Poaceae and preferentially amplified in *Aegilops squarrosa* (2n=14, DD), *H. vulgare* and *Agropyron elongatum*. (2n=14, EE). The first described representative of this family was pAs1 from *Ae. squarrosa*. Different sequences of one satellite DNA family were amplified from *Ae. squarrosa*, *A. elongatum* and *H. vulgare* using PCR. Characteristic differences between members of the D and H genome occurred in a variable region which is flanked by two conserved segments. The heterogeneity within this element was exploited for the cytogenetic analysis of Triticeae genomes and chromosomes. Comparative ISH with pHvMWG2315 identified individual wheat and barley chromosomes under low (75%) and high (85%) hybridization stringency in homologous and heterologous systems. We propose the designation Tas330 for the *T*riticeae *a*mplified *s*equence (Tas) satellite family with a 330 bp average monomer length.

Key words Satellite DNA · Fluorescence *in situ* hybridization (FISH) · Polymerase chain reaction (PCR) · 'Species specificity'

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Introduction

Triticeae genomes consist of more than 70% of moderate to highly amplified repetitive DNA sequences (Rimpau et al. 1978, 1980; Flavell et al. 1980). These generally non-coding sequences often undergo rapid evolutionary changes and hence are often modified and/or differentially amplified in members of closely related species (McNeil et al. 1994). In contrast, low-copy sequences are frequently conserved (Jelenik and Schmid 1982). Differentially amplified repetitive DNA sequences have been widely used for evolutionary studies in *Secale* (Jones and Flavell 1982; Appels et al. 1989), *Agropyron* (McIntyre et al. 1988; Zhang and Dvorak 1990), *Triticum* (Dvorak and Zhang 1992; McNeil et al. 1994) and *Hordeum* (Svitashev et al. 1994; Vershinin et al. 1994). Several of these sequences are valuable for assaying the introgression of alien genetic material into crop plants (Appels et al. 1978; Zhang and Dvorak 1990; Guidet et al. 1991; The et al. 1992).

In grasses, subterminal satellite sequences occur in rye (Bedbrook et al. 1980), *Ae. squarrosa* (Rayburn and Gill 1987), rice (Wu et al. 1991; Wu and Tanksley 1993), *Ae. speltoides* and *Ae. sharonensis* (Anamthawat-Jònsson and Heslop-Harrison 1993), and barley (Röder et al. 1993). Although, some of these amplified sequences were labelled species-specific, they show considerable variation within closely related species. However, the Dgas44-3 sequence, isolated from *Ae. squarrosa*, is limited to different D genomes of diploid, tetraploid and hexaploid Triticeae species (McNeil et al. 1994).

Coupled with *in situ* hybridization (ISH) various repetitive sequences are useful in karyotypic analysis. The repeated sequence pAs1 from *Ae. squarrosa* was used to identify the seven D-genome chromosomes in wheat (Rayburn and Gill 1986), six A- or B-genome chromosomes (Mukai et al. 1993), as well as the *Hordeum vulgare* and *H. chilense* chromosomes (Brandes et al. 1995; Cabrera et al. 1995). The repetitive rye sequence pSc119.2 hybridized to all chromosomes of rye and to 12 chromosomes of *T. aestivum* (McIntyre et al. 1990; Mukai et al. 1993). Dou-

ble hybridization with pSc119.2 and pAs1 identified 17 of the 21 wheat chromosomes (Mukai et al. 1993). Recently, we have reported the karyotyping of *H. vulgare* using the tandemly repeated sequence pHvMWG2315 (Busch et al. 1995). This element shares approximately 85% sequence homology with the pAs1 monomers, 75% homology with the interspersed 'genome-specific' DNA sequence pHcKB6 from *H. chilense* (Anamthawat-Jónsson and Heslop-Harrison 1993), and up to 85% with sub-regions of the dpTa1 sequence from *T. aestivum* (Vershinin et al. 1994).

Here, we report on the sequence composition and the comparative ISH on two barley derived sequences, pHvMWG2315 and pHvMWG2314. These sequences can be used for chromosome differentiation in homologous and heterologous hybridization experiments under various stringency conditions. In diploid barley, the pHvMWG2314 sequence is moderately amplified, dispersed, and generates no ISH signals. In wheat, however, pHvMWG2314 shows characteristics of a new satellite DNA family.

Materials and methods

DNA probes

Two clones, designated pHvMWG2314 (EMBL No. X85383) and pHvMWG2315 (EMBL No. X85384), were originally isolated from a library of microdissected long arms of chromosome 3 (3HL) from a wheat-barley telosome addition line (Busch et al. 1995). The plasmid pTa71 contains a single wheat 18*S*·5.8*S*·25*S* rRNA gene repeat unit of 8.9 kb (Gerlach and Bedbrook 1979) and the plasmid pTa794 the 5*S* rDNA sequence of 410 bp (Gerlach and Dyer 1980). The plasmid pAs1 carries a 1-kb fragment from *Ae. squarrosa* (Rayburn and Gill 1986).

DNA sequencing

Nucleotide sequences were determined by the chain-termination procedure (Sanger et al. 1977) using ^{35}S-labelled dATP (500 mCi/mM, Amersham/USB, Braunschweig, Germany) and the sequenase sequencing kit from USB accordinge manufacturer's instructions. Samples were subjected to electrophoresis in 6% polyacrylamide gels in 1× TBE, 50% urea, at 43.5 V/cm for 3 h. The gels were dried for 2 h in a vacuum gel dryer and exposed to BIOMAX™MR film (Kodak, Stuttgart, Germany) overnight.

Fluorescence in situ hybridization (FISH)

Barley (cv Marinka), wheat (cv Chinese Spring) and wheat/barley addition line 3HL (Islam 1983) were germinated and chromosome preparations made according to Busch et al. (1994). Probes were labelled by nick translation with either biotin-16-dUTP or digoxigenin-11-dUTP following the instructions of the supplier (Boehringer, Mannheim, Germany). The *in situ* hybridization, signal detection and amplification procedures were according to Busch et al. (1995). The stringency conditions of post-hybridization washes were of approximately 70 or 80% (for pHvMWG2314) and 75 or 85% (for pHvMWG2315) and were calculated according to Meinkoth and Wahl (1984). The specimens were mounted in VECTASHIELD™ (Vector, Burlingame, USA) antifading medium.

Microscopy

Metaphases were examined using a Zeiss Axioplan epifluorescence microscope equipped with the filter sets 487901 (DAPI), 487917 (FITC), and a Cy3-specific filter set supplied by AHF analysentechnik (Tübingen, Germany). Pictures of counterstained chromosomes and probe signals were taken separately with a CCD camera (Photometrics, Tucson, Arizona, USA) and processed using the IPLab Spectrum and MultiProbe (Signal Analytics Corp., Vienna, Virginia, USA) and Adobe Photoshop (Adobe Systems Inc. Mountain View, California, USA) software.

PCR (polymerase chain reaction)-derived sequences

Two sets of oligonucleotide primers flanking the 5′ and 3′ ends of the repeated DNA sequences pAs1 and pHvMWG2315 were synthesized (MWG-Biotech, Ebersberg, Germany). Primers No. 1 (5′-ACAAAACGGACAATCTC-3′) and No. 2 (5′-ATCCAGTTTTTGCCGTA-3′) were used to amplify the internal sequences; No. 3 (5′-GAGATTGTCCGTTTTGT-3′) and No. 4 (5′-TACGGCAAAAACTGGAT-3′) were used for the inverse PCR reaction to amplify the flanking region or linker DNA sequences of two neighbouring 330-bp elements.

The PCR reaction was performed in a volume of 25 µl containing 5 ng of template DNA (from *H. vulgare*, *Ae. squarrosa* or *A. elongatum*), 1 mM of primer, 80 µM of dNTPs, 1 U of *Taq* DNA polymerase (EUROGENTEC, Seraing, Belgium) and buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% Tween 20). The reactions were incubated in a Programmable Thermal Controller (MJ Research, Watertown, USA) for 30 cycles of 1 min at 94°C, 30 s at 40°C and 30 s at 72°C with an initial de-naturation step of 5 min at 94°C and a final extension step of 5 min at 72°C. Five microliters of the PCR products were separated in a 1% agarose gel. Two microliters of the remaining volume were used for cloning the different PCR fragments into the TA Cloning Kit vector pCRIII following the instructions of the supplier (Invitrogen, San Diego, California, USA). Single recombinant clones were identified by blue-white colony selection. Three to six cloned PCR products from *Ae. squarrosa*, *H. vulgare* and *A. elongatum* were randomly selected and sequenced. The primer sequences were excluded from the calculation of sequence similarities.

Results

In situ hybridization

The sequence of pHvMWG2314 (thereafter MWG2314) is 146 bp long and represents a dimer of two monomeric units of 73 bp with 96% homology and an internal *Rsa*I restriction site at the 73-bp position. ISH experiments with MWG2314 at 80% homology uncovered single interstitial major hybridization sites on the long arms of wheat chromosomes 1D, 3D and 6D with the most prominent signal on chromosome 1D (Fig. 1a). The signal-carrying chromosomes were identified in double and triple hybridization experiments as outlined below. Chromosome 5D carried three distinct hybridization signals. Several unidentified chromosomes also displayed signals. In addition, hybridization was observed near the centromeric or telomeric end of the telosome 3HL (Fig. 1a, 2n=45). At a 70% wash stringency, an additional dispersed hybridization signal became apparent (data not shown).

The sequence MWG2315 also hybridized to chromosomes of hexaploid wheat. The distribution of the hybridization sites was compared with the published idiogram of wheat based on simultaneous hybridization with pAs1 and pSc119.2 (Mukai et al. 1993). At an 85% wash stringency,

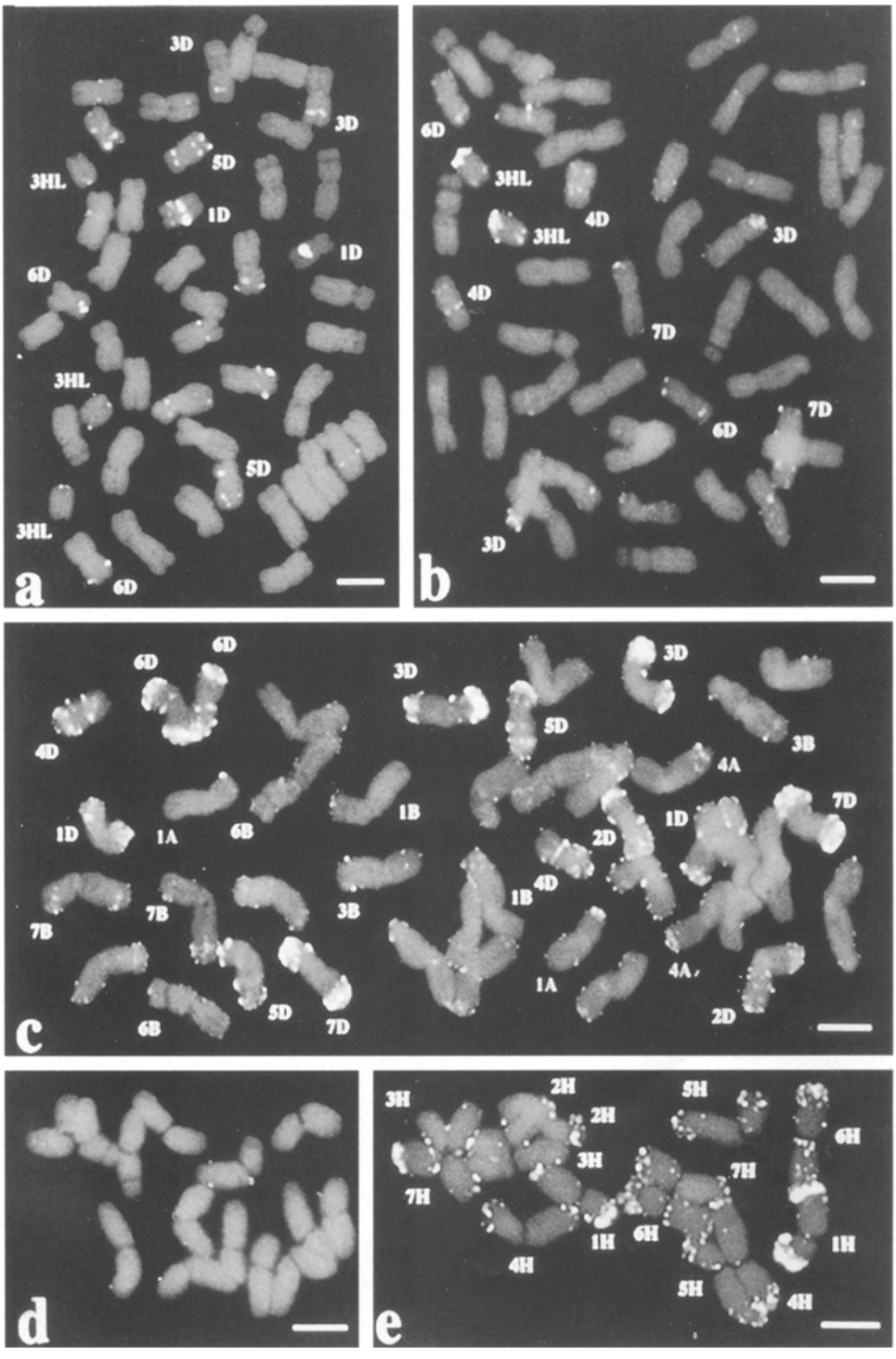

Fig. 1 Metaphase spreads of ditelosomic 3HL addition lines of Chinese Spring wheat (**a** and **b**), Chinese Spring wheat (**c**), and barley (**d** and **e**) after hybridization with the sequences pHvMWG2314 (**a**), pHvMWG2315 (**b** and **c**) and pAs1 (**d** and **e**). The wash stringency differed: 70% (**a**), 75% (**c** and **e**) and 85% (**b** and **d**). The biotinylated probe was detected with avidin-FITC (**a**, **b** and **c**) or avidin-Cy3 (**d** and **e**). Note: One of the telosomes in **a** originates from a neighboring metaphase cell. Bar: 5 μm

major binding of the sequence was found on chromosomes 3D, 4D, 6D and 7D (Fig. 1b). Chromosome 3D was the most prominently labelled wheat chromosome and the barley telosome 3HL carried the strongest signal. At 75% stringency, the hybridization sites generated by MWG2315 were useful to identify all D-genome chromosomes, similar to pAs1 (Fig. 1c). In contrast, pAs1 gave no specific hybridization on barley chromosomes at 85% stringency (Fig. 1d). However, the banding pattern and hybridization intensities at 75% (Fig. 1e) were comparable to barley chromosomes with MWG2315 (Busch et al. 1995).

The hybridization sites detected in single hybridizations using MWG2314 (Fig. 1a) or MWG2315 (Fig. 1b,c) were compared with those obtained after triple hybridization (Fig. 2) to allocate sites of MWG2314 or MWG2315 and pAs1. Co-hybridization of signals from pAs1 and

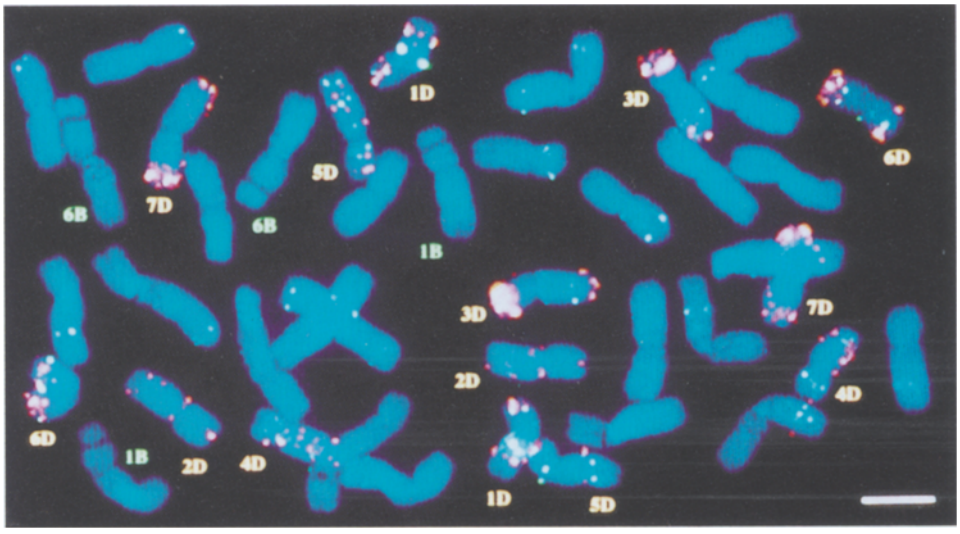

Fig. 2 Simultaneous *in situ* hybridization with pHvMWG2314, pHvMWG2315 and pAs1 in wheat. The pHvMWG2314 and pHvMWG2315 sequences were labelled with biotin-16-dUTP, and pAs1 with digoxigenin-11-dUTP. The biotin-labelled probes were detected *via* avidin-FITC (*green*), and pAs1 *via* monoclonal mouse-anti-digoxigenin and a Cy3-coupled rabbit-anti-mouse antibody (*red*). Bar: 5 µm

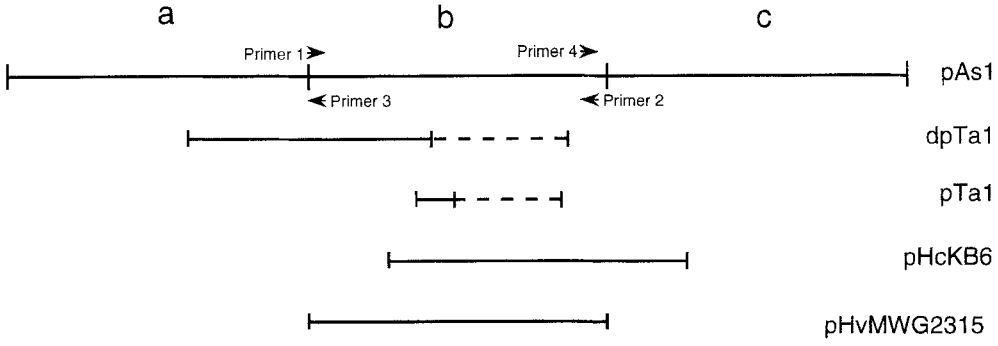

Fig. 3 Overall structure of pAs1 (Nagaki et al. 1995) and the related sequences dpTa1 (Vershinin et al. 1994), pTa1 (Metzlaff et al. 1986), pHcKB6 (Anamthawat-Jònsson and Heslop-Harrison 1993) and pHvMWG2315. The *arrows* identify primers Nos. 1, 2, 3, and 4 that were used for PCR experiments. The *dashed lines* indicate low sequence homology (<50%)

MWG2315 on all D-genome chromosomes was observed (Fig. 2). Simultaneous ISH of MWG2314 with pTa794 (5*S* rDNA) and/or pTa71 (18*S*·5.8*S*·25*S* rDNA) confirmed the allocation of the signals on chromosomes 1D and 5D (data not shown).

PCR and DNA sequencing of related MWG2315 elements

A sequence comparison of the three monomeric units, pAs1a, pAs1b and pAs1c, of the *Ae. squarrosa* pAs1 element with the tandem repeat MWG2315 resulted in the identification of conserved boundary regions of the monomers in different members of the Triticeae (Fig. 3). PCR primer pairs from the conserved region of these units were exploited to examine the organization and sequence divergence of the almost entire 336-bp elements in different Triticeae species. Primers 1 and 2 amplified internal fragments of approximately 320 bp. Primers 3 and 4, in turn, revealed the external linking fragments that included the *Rsa*I restriction sites. No PCR products were obtained using only one primer, which proved the simple tandem arrangement of the monomers and excludes the existence of inverted repeats. Preferentially, the PCR products of those species were analysed that had the highest amplification of the monomers in the genome, i.e. *H. vulgare*, *Ae. squarrosa* and *A. elongatum*. However, sequences of similar length were amplified from the DNA of *Ae. speltoides* (2n=14, SS), *Ae. sharonensis* (2n=14, S^lS^l) *T. turgidum* (2n=28, AABB) and *Secale cereale* (2n=14, RR) (data not shown). The amplification of DNA from *T. boeoticum* (2n=14, AA) has so far failed. Detailed sequence comparison and alignment of internal regions of the monomeric unit displayed intraspecific (Fig. 4) and interspecific (Fig. 5) point mutations, insertions and deletions within and among the diploid species. The entire 331 bp monomer shows distinctive intraspecific sequence homology in *Ae. squarrosa* (90.6–97.6%), *H. vulgare* (90.3–94.8%) and *A. elongatum* (92.6–94.3%). Interspecific sequence comparisons uncovered overall sequence similarities of between 82.7 and 90.6% (Table 1). The monomer can be subdivided into three subregions (I–III), approximately 136 bp (I), 70 bp (II) and 120 bp (III) in length. Subregions I and III are more conserved (both intra- and inter-specifically) than the median subregion II. The most striking interspecific diversity (68.7–70.2%) between *Ae. squarrosa* and barley was detected in subregion II. The intraspecific similarity for that subregion (bp 137–207) varied from 60.3% (*H. vulgare*) to 97.3% (*Ae. squarrosa*).

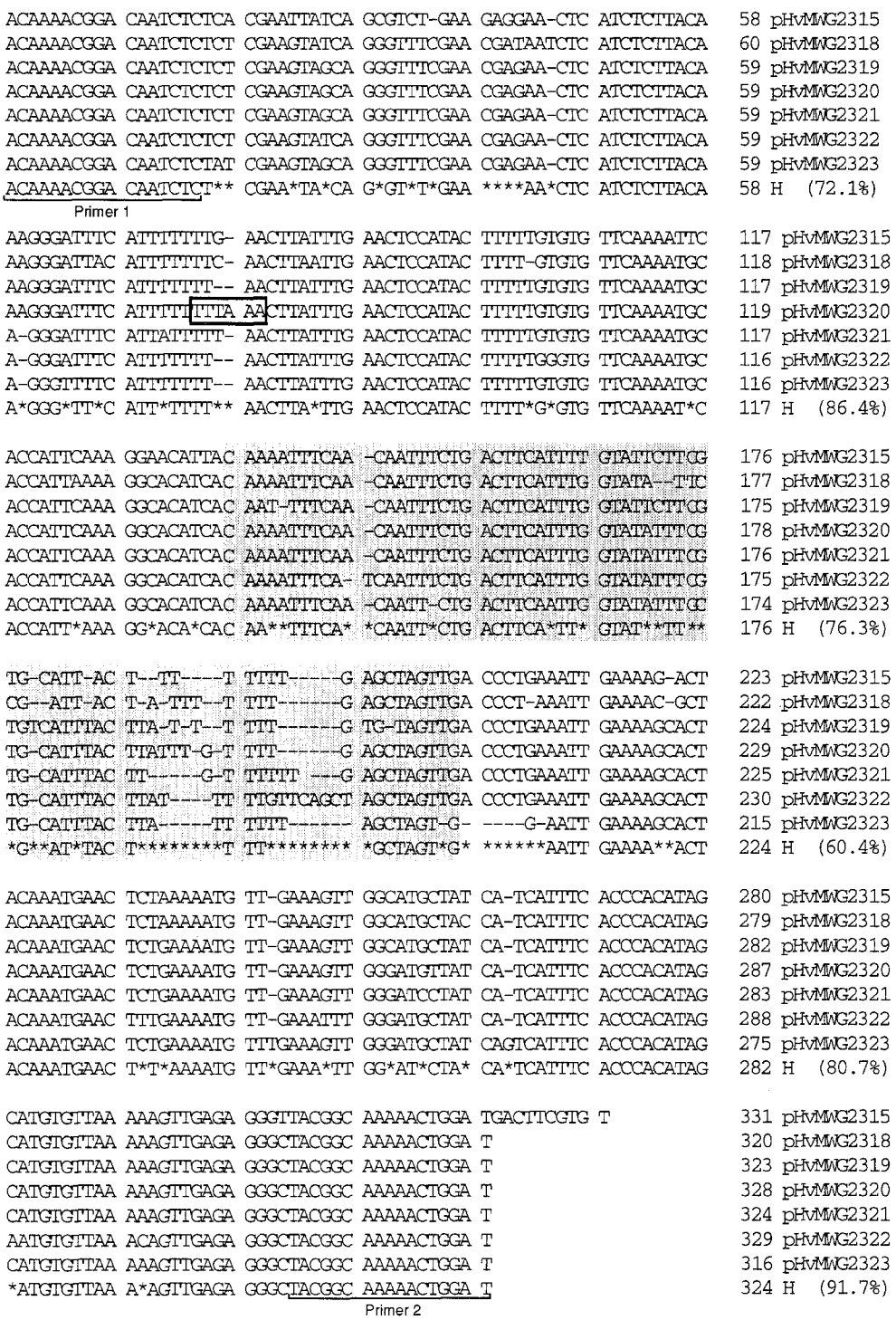

Fig. 4 Aligned sequences of DNA segments recovered from PCR products. The PCR used total genomic DNA from *H. vulgare* as a template *plus* primers Nos. 1 and 2 (defined in Fig. 3). A consensus sequence is shown in the lower line. The frequency of common nucleotides in the consensus sequence (per line) is given in *brackets*. An *asterisk* represents a mismatching base and a *dash* a gap in the sequence alignment. The location of a *Dra*I restriction site (at position 75) that was detected in one sequence is shown by a *box*. The region of high intraspecific sequence variation is *shaded*. The nucleotide sequence data are stored in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession numbers X85384 (pHvMWG2315), Z54371 (pHvMWG2318), Z54372 (pHvMWG2319), Z54373 (pHvMWG2320), Z54374 (pHvMWG2321), Z54375 (pHvMWG2322) and Z54376 (pHvMWG2323)

A comparison of the consensus sequences of the PCR products from *H. vulgare*, *Ae. squarrosa* and *A. elongatum* indicated an organization of the monomers into conserved and variable subregions (Fig. 5). The intraspecific variability in the conserved regions varied from 68.3–94.7%. For bp 172–217 in subregion II of the D-, H- and E-genomes the sequence similarities ranged from 60 to 95.6%. Only 26.1 or 26.8% of the consensus sequences of all three genomes are identical in regions bp 18–58 and bp 172–217, respectively (Fig. 5). No preferential transitions or transversions were detected. In tandemly organized monomers the conserved subregions I and III are bordered and connected by the linking fragment that contains the *Rsa*I endonuclease restriction site. Note, that the *Rsa*I restriction enzyme was originally used in the microdissection experiment. Another internal restriction site was found in one

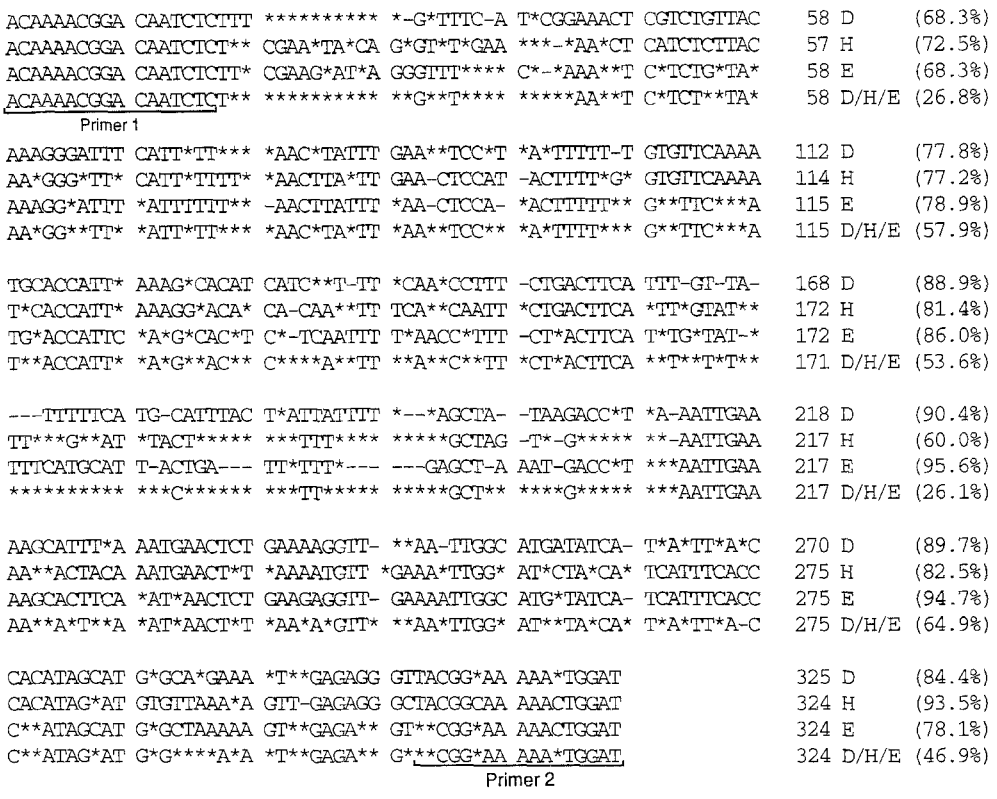

Fig. 5 Aligned consensus sequences of DNA elements recovered from PCR products and from sub-sequences of pAs1. Total genomic DNA from *Ae. squarrosa* (2n=14, DD), *H. vulgare* (2n=14, HH), and *A. elongatum* (2n=14, EE) as template *plus* the primers Nos. 1 and 2 (defined in Fig. 3), were used for the PCR assays. *Asterisks* represent mismatching bases and *dashes* gaps in the alignment of sequences

Table 1 Interspecific DNA sequence homology between the internal (pAs1b) and external (pAs1a and pAs1c) 336-bp elements of pAs1 from *Ae. squarrosa* and of pHvMWG2315 from *H. vulgare*. Homologies of sequence representatives amplified by PCR from *Ae. squarrosa* (pAsMWG2317), *H. vulgare* (pHvMWG2318) and *A. elongatum* (pAeMWG2325), and of the subregions of the respective sequences, are shown below

Sequence designation	Origin	Overall homology (in %)	Homology in sub-regions (%)		
			I (bp 1–136)	II (bp 137–207)	III (bp 207–336)
pAs1a	*Ae. squarrosa*	97.1	95.2	100	98.1
pAs1b	*Ae. squarrosa*	100	100	100	100
pAs1c	*Ae. squarrosa*	97.6	98.3	100	96.1
pHvMWG2315	*H. vulgare*	85.4	94.7	68.7	88.7
pAsMWG2317	*Ae. squarrosa*	90.6	92.1	91.7	90.9
pHvMWG2318	*H. vulgare*	82.7	85.9	70.2	86.4
pAeMWG2325	*A. elongatum*	89.0	93.8	92.3	91.8

member of the family (Fig. 4) for the enzyme *Dra*I (at position 75). This observation is consistent with the Southern experiments since in barley most elements are organized as *Rsa*I monomers or *Dra*I monomers and dimers of approximately 330 bp and 660 bp in length, respectively.

Discussion

The MWG2314 sequence represents a non-species-specific sequence that has been inserted and amplified at certain interstitial chromosome regions of wheat after the separation of the genera *Triticum* and *Hordeum*. It is dispersed in wheat and barley but exists as a satellite DNA in multiple copies at distinct sites of wheat chromosome arms 1DL, 3DL, 6DL, 5DS and 5DL. This is supported by the apparent dispersed signals under 70% stringency in wheat. An analogous phenomenon has been observed for the *Agropyron* amplified sequence 1-E66HcII-1. It hybridizes to all *Agropyron* chromosomes in a dispersed fashion. In wheat, only a single hybridization site on one chromosome pair was detected by Bournival et al. (1994) in their experiments.

ISH experiments with differentially labelled and simultaneously hybridized probes are useful in classifying sets of chromosomes (Leitch et al. 1991; Mukai et al. 1993) and may also be useful to physically order sequences along the chromosome. Using pAs1 (Mukai et al. 1993), pTa794 (Mukai et al. 1990) and pTa71 (Appels et al. 1980; Hutchinson and Miller 1982; Jiang and Gill 1994; Mukai et al. 1991) all wheat chromosomes except 2A, 2B, 3A, 4B, 6A,

7A can be identified. In a triple hybridization experiment with the sequences MWG2314, MWG2315 and pAs1 (Fig. 2), a few chromosome pairs with distinct hybridization sites were noted in addition to those that were labelled in single ISH experiments. At the moment it is not clear if those signals result from mutual enhancement of interspersed, most likely clustered, sequences of MWG2314 and MWG2315 that become visible only in simultaneous hybridization and detection experiments. Thus, MWG2314 may be useful for the identification of single wheat chromosomes in single and multiple ISH experiments. This could be further verified by simultaneous ISH with new sequences or by sequential hybridization techniques.

The MWG2315 (Busch et al. 1995) and the related pAs1 (Brandes et al. 1995; Cabrera et al. 1995; Mukai et al. 1993; Rayburn and Gill 1987) elements have shown the importance of this sequence family for chromosome diagnosis within the Triticeae. The 5′ (128 bp) and 3′ ends (132 bp) of MWG2315 share 85 and 82% homology to the tandemly repeated sequence dpTa1 from *T. aestivum* (Metzlaff et al. 1986; Vershinin et al. 1994). Southern analysis indicates that dpTa1 is present in all the Triticeae species analysed so far (Vershinin et al. 1994). Brandes et al. (1995) observed a background smear on pulse-field gels and a partially dispersed distribution of the tandemly repeated pAs1 unit in *H. vulgare*. ISH revealed only a few hybridization signals on barley chromosomes under stringency conditions of approximately 80%. Here, the signal pattern in *H. vulgare* consists of distinct signals under 75% stringency. The signal distribution with pAs1 is identical to the distribution of signals with MWG2315 which detects 50 hybridization sites and allows the karyotyping of barley (Busch et al. 1995).

The tandemly organized MWG2315 elements share approximately 70–95% sequence homology between representatives in *H. vulgare*, *Ae. squarrosa* and *A. elongatum*. Within the 330-bp repeat unit a subregion of approximately 70 bp in length shows higher intra- and inter-specific variability. The two flanking subregions of about 130 bp are highly conserved and occur as neighbours in tandem arrays of monomers. Consequently, this conserved subregion of 260 bp can be amplified by PCR (Nagaki et al. 1995). The overall sequence difference of the 330-bp unit between various species was used here to study the hybridization patterns of these elements in homologous and heterologous systems under different stringency conditions. For example, at low stringency the pAs1 sequence from *Ae. squarrosa* hybridized to barley chromosomes (Isono et al. 1991) and is useful for the identification of all barley chromosomes (Busch et al. 1995), while at 80% stringency only few specific signals are detectable (Brandes et al. 1995; Busch, unpublished).

Some limitations of Southern analysis in the establishment of 'species-specificity' have been noted. For instance, stable hybridization between representatives of the 350–480 bp family of rye and a telomeric sequence of *Criteson bogdanii*, which share 75% DNA sequence homology, does not occur (Appels et al. 1989). Thus, repetitive DNA sequences that previously were described as 'species-specific' may have significant sequence homology in related species. Repetitive DNA sequences often show low intraspecific variation so that only one or a few representatives per taxon or cytotype have to be analysed in comparative studies (Dvorak and Zhang 1992). The amplification of species-characteristic sequences that are related to the pAs1 family from different diploid Triticeae offers the opportunity of studying the organization and amplification of repetitive elements in the tribe. We propose that these elements will be of even greater interest for the cytogenetic identification of chromosomes that carry clusters of tandemly repeated 'genome-specific' sequences and for the study of the evolution of genomes, chromosomes and even chromosome regions.

Southern analysis showed that MWG2314 is amplified in wheat relative to barley (Busch et al. 1995). No signal could be detected in chromosomes of diploid barley using ISH. However, the barley telosome 3HL when added to wheat showed a clear subterminal hybridization. Thus, the hybridization and detection sensitivity with MWG2314 on the telosome is higher than on the long arm of chromosome 3H in diploid barley. The same phenomenon has been observed for MWG2315. The reduced signal detection in diploid barley cv Marinka may be due to the fact that different barley varieties, cv Marinka and cv Betzes, have been analysed in the diploid and the addition line, respectively. Further experiments with different barley varieties can clarify this observation. In addition, a possible modification of the added barley telosome in a wheat background could be analysed with probes like the barley specific subtelomeric sequences (Röder et al. 1993).

It is still a challenge to detect single or low-copy sequences by ISH with high efficiency and reproducibility. Most likely, the organization of single or low-copy sequences, as well as of moderately and highly amplified sequences, influences the signal detection in plant genomes. Here, we have shown that two amplified sequences that have a clustered and dispersed organization pattern produce additional binding sites if the sequences are simultaneously labelled and detected. The pAs1 family with a monomer length of 330 bp is not species specific but it is a Triticeae amplified sequence (Tas) which we propose to designate as the Tas330 family and to employ for further comparative analysis of the Triticeae species.

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