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Genetic distribution of Bare–1-like retrotransposable elements in the barley genome revealed by sequence-specific amplification polymorphisms (S-SAP)

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Abstract Retrotransposons are present in high copy number in many plant genomes. They show a considerable degree of sequence heterogeneity and insertional polymorphism, both within and between species. We describe here a polymerase chain reaction (PCR)-based method which exploits this polymorphism for the generation of molecular markers in barley. The method produces amplified fragments containing a Bare–1-like retrotransposon long terminal repeat (LTR) sequence at one end and a flanking host restriction site at the other. The level of polymorphism is higher than that revealed by amplified fragment length polymorphism (AFLP) in barley. Segregation data for 55 fragments, which were polymorphic in a doubled haploid barley population, were analysed alongside an existing framework of some 400 other markers. The markers showed a widespread distribution over the seven linkage groups, which is consistent with the distribution of the Bare–1 class of retrotransposons in the barley genome based on in situ hybridisation data. The potential applicability of this method to the mapping of other multicopy sequences in plants is discussed.

Key words Bare-1 · Retrotransposons · Barely · Linkage analysis · Sequence-Specific Amplification Polymorphisms (S-SAPs)

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Introduction

Retrotransposons are the commonest class of eukaryotic transposable element. They are separated from other transposons by their ability to transpose via an RNA intermediate, which they convert to DNA by reverse transcription prior to reinsertion. The retrotransposons include long terminal repeat (LTR) and non-LTR classes (Boeke and Corces 1989; Grandbastien 1992; Smyth 1993; Kumar 1996). The most studied group of LTR retrotransposons is the *Ty1-copia* group, named after the best studied elements in *Saccharomyces cerevisiae* and *Drosophila melanogaster*. *Ty1-copia* retrotransposons are also present throughout the plant kingdom (Flavell et al. 1992a, b; Voytas et al. 1992; Hirochika and Hirochika 1993; Lindauer et al. 1993). Several of the plant elements have been fully sequenced including Ta1 of *Arabidopsis thaliana* (Voytas and Ausubel 1988), Tnt1 of tobacco (Grandbastien et al. 1989), Tst1 of potato (Camirand et al. 1990) and Bare–1 of barley (Manninen and Schulman 1993). They are generally present in high copy number and occupy a considerable proportion of some plant genomes Flavell et al. 1992b; Hirochika et al. 1992; Leeton et al. 1993). In addition, retrotransposons are frequently found in the regions flanking known plant genes (White et al. 1994).

Although a growing body of knowledge is available on the phylogenetic relationships among the populations of plant *Ty1-copia* retrotransposons, less is known of the distribution of these elements within plant genomes. In situ hybridisation of *Ty1-copia* retrotransposon sequences to metaphase chromosomes in sugarbeet (Schmidt et al. 1996) and faba bean (Pearce et al. 1996) has revealed that the *Ty1-copia* elements of these species are located throughout the euchromatin and appeared to be mostly absent from heterochromatic regions.

Since the development of polymerase chain reaction (PCR) technology (Saiki et al. 1988), a large number of approaches have been developed which allow the detection of polymorphism at the DNA level. These in-

clude sequence-specific methods such as cleaved amplified polymorphisms (CAPs; Konieczny and Ausubel 1993) and simple sequence repeats (SSRs; Tautz 1988) and a range of generic approaches such as randomly amplified polymorphic DNA (RAPDs; Williams et al. 1990), arbitrary primed-polymerase chain reaction (AP-PCR; Welsh et al. 1991), DNA amplification fingerprinting (DAF; Caetano-Annoles 1993) and random amplified microsatellite polymorphism (RAMPs; Zietkiewicz et al. 1994). The most recent, and arguably one of the most powerful techniques, is amplified fragment length polymorphism (AFLP; Zabeau and Vos 1993). AFLP relies on PCR amplification of a subset of small restriction fragments generated by digesting genomic DNA with a frequent and a rare cutting restriction endonuclease and ligation of known sequence adaptors to the fragment ends (Vos et al. 1995). The main attraction of AFLP is its high multiplex ratio (Powell et al. 1996) which allows the generation of a large body of information from a relatively small number of individual assays. A few recent publications demonstrate the utility of AFLP for both saturation mapping (Ballvora et al. 1995; Meksem et al. 1995; C.M. Thomas et al. 1995) and genome-wide linkage mapping (van Eck et al. 1995). In barley, studies by ourselves (Powell et al. 1996; Waugh et al. 1996) and others (Becker et al. 1995) have shown that, while AFLP will be a very powerful approach, scope does exist for improvement on a number of fronts. For example, the level of detectable polymorphism is relatively low and few markers are codominant. In addition, preliminary observations suggest that AFLPs generated after digestion of the DNA with the recommended enzyme pair of *Eco*RI and *Mse*I are frequently clustered in centromeric regions and at the edges of genomic segments which are heavily mapped with RFLPs (Becker et al. 1995; Waugh et al. 1996).

The ubiquitous distribution, high copy number and widespread chromosomal dispersion of the *Ty1-copia* group of retrotransposons in plants provides excellent potential for developing a multiplex DNA-based marker system (Kumar 1996). Here we describe the use of Bare–1-like retroelement LTR sequences in barley for detecting DNA polymorphisms based on the location of the retroelements relative to adjacent restriction endonuclease sites. The usefulness of this system has been demonstrated by following the inheritance of a number of these DNA polymorphisms relative to a range of other markers in a doubled haploid (DH) barley population. This has allowed us to show that the genetical distribution of Bare–1 elements in barley correlates well with the physical distribution, as revealed by in situ hybridisation.

Materials and methods

Materials

A DH barley population (B × E) derived from the F_1 of a cross between the cv Blenheim and breeding line E224/3, which has previously been extensively mapped with RFLPs, RAPDs and AFLPs (W.T.B. Thomas et al. 1995), was used for all studies. DNA was extracted from 3-week-old plants according to Saghai-Maroof et al. (1984).

In situ hybridisation

Seeds of *Hordeum vulgare* cv Blenheim were germinated by placing on moist filter paper. Root tips 1 cm in length were cut off and placed on 0.5% colchicine for 4 h, then fixed in 3:1 methanol:acetic acid. Fixed root tips were washed in enzyme buffer (6 mM sodium citrate, 4 mM citric acid, pH 4.6) followed by digestion with cellulase and pectinase (1.6% cellulase, Calbiochem; 0.4% cellulase, Onasaka; 20% pectinase, Sigma) at 37° C for 80 min. Squashes were made in 45% acetic acid and coverslips removed after freezing on dry ice. In situ hybridisation followed the technique of Leitch et al. (1994). Briefly, 1 μg Bare–1 DNA was labelled with biotinylated dUTP (Boehringer Mannheim) by the random primed method. Chromosome preparations were pretreated with RNase (100 mg/ml for 1 h at 37° C). The hybridisation mixture consisted of 50% formamide, 10% dextran sulphate, 0.1–0.5% SDS, autoclaved salmon sperm DNA (25–100 ng/l), 2 × SSC with 50 ng/slide of labelled probe. The probe mix was denatured at 70° C for 10 min and held on ice for 5 min before being added to the slides. Probe and root tip preparations were then denatured at 80° C for 5 min, before hybridisation overnight at 37° C. Post-hybridisation washes were carried out, the most stringent being 20% formamide in 0.1 × SSC at 37° C. Sites of hybridisation were detected using fluorescein isothiocyanate extravidin-(FITC) conjugate (Sigma). The chromosomes were counterstained with 2 g/ml 4′, 6-diamidino-2-phenylindole (DAPI) in McIlvaines citric buffer pH 7, before examination by epifluorescent light microscopy.

Sequence-specific amplification polymorphism (S-SAP) marker analysis

Total DNA (1.0 μg) was restricted with *Pst*I and *Mse*I (5 U of each) for 1 h in 1 × RL buffer (10 mM TRIS-acetate pH 7.5, 10 mM magnesium acetate, 50 mM potassium acetate, 5 mM DTT, 5 ng/μl BSA) (Vos et al. 1995) in a total volume of 20 μl. Template DNA was prepared by adding 4 μl of a ligation mix [4 pmol *Mse*I (5′-GACGATGAGTCCTGAG-3′ plus 5′-TACTCAGGACTCAT-3′), 2 pmol *Pst*I adaptor (5′-CTCGTAGACTGCGTACATGCA-3′ plus 5′-TGTACGCAGTCTAC-3′), 1.2 mM ATP, 1 × RL buffer and 0.5 U T4 DNA ligase] and the samples incubated for 3 h at 37° C, then stored at 4° C. The template DNA was then preamplified to select and bulk restriction fragments of the correct size and configuration using primers homologous to the adaptor sequences (P: 5′-GACTGCGTACATGCAG-3′; M: 5′-GATGAGTCCTGAGTA-3′) in 25 μl reactions containing 0.75 μl template DNA, 75 ng P and 75 ng M oligonucleotides, 0.2 mM dNTPs, 1 × PCR buffer (Perkin Elmer) and 1 U *Taq* DNA polymerase (Perkin Elmer). PCR on a Perkin Elmer 9600 instrument comprised 30 cycles of 94 °C (30 s), 60 °C (30 s), 70 °C (1 min). Then, 55 μl TO.1E (10 mM TRIS-HCl, pH 8.0, 0.1 mM EDTA) was then added and the preamplified DNA stored at 4° C. The Bare–1-like LTR oligonucleotide (Bare-1-LTR: 5′-CTAGGGCATAATTCCAACA-3′) was end-labelled by combining 0.1 μl γ-[^{33}P]ATP (3000 Ci/mmol), 0.1 μl 10 × T4 buffer (0.25 M TRIS-HCl pH 7.5, 0.1 M $MgCl_2$, 50 mM DTT, 5 mM spermidine), 0.134 μl Bare–1-LTR (50 ng/μl stock), 0.25 U T4 kinase (0.025 μl) and 0.641 μl sterile distilled H_2O per subsequent reaction. Each selective amplification reaction contained 1 μl ^{33}P-labelled Bare–1-LTR, 0.5 μl unlabelled Bare–1-LTR (50 ng/μl), 0.6 μl $P_{(\ )}$ or $M_{(\ )}$ selective amplification primer (50 ng/μl stocks), 2 μl dNTP mix (2 mM stocks), 2 μl dNTP mix (2 mM stocks), 2 μl 10 × PCR buffer, 0.5 U *Taq* DNA polymerase (Perkin Elmer), 11.8 μl distilled H_2O and 2 μl preamplified DNA. $M_{(\ )}$ and $P_{(\ )}$ primers had the same sequence as the P and M primers given above but included one to three additional selective nucleotides at the 3′ end. The primers used were M, $M_{(C)}$, $M_{(AC)}$, $M_{(ACA)}$ P, $P_{(C)}$, $P_{(CC)}$, $P_{(CG)}$, $P_{(GT)}$, $P_{(TC)}$, $P_{(AAT)}$,

$P_{(AAA)}$, $P_{(ACG)}$, $P_{(AGC)}$, $P_{(ATT)}$, $P_{(CGA)}$, $P_{(GGA)}$, $P_{(GTA)}$, $P_{(TAC)}$ and $P_{(TCG)}$. The touchdown PCR protocol of Vos et al. (1995) was followed exactly.

Gel electrophoresis/autoradiography

After PCR, 20 µl gel loading buffer (94% formamide, 10 mM EDTA, 0.5 mg/ml xylene cyanol FF, 0.5 mg/ml bromophenol blue) was added to each tube and the samples denatured by incubation at 90 °C for 5 min and then placed directly on ice. A 3.8-µl aliquot of each sample was loaded onto 6% denaturing polyacrylamide gels ($40 \times 30 \times 0.04$ cm) (Sambrook et al. 1989) which had been prerun at 80 W for 30 min. Samples were electrophoresed for 1 h 45 min at 80 W constant power alongside an M13 sequencing marker. Gels were dried directly onto the glass plates and exposed to Kodak X-Omat film for 1–5 days at room temperature.

Linkage analysis

Segregating Bare–1-LTR-derived marker data were entered alongside existing marker data for the B × E population and analysed using Joinmap V 2.0 (Stam and van Ooijen 1995). Parameters were set for F_1 DH-derived progeny using *a* for markers derived from Blenheim and *b* for markers from E224/3. Mapping was carried out as described by Powell et al. (1996) except that the results from the third cycle of JOINMAP were used to examine the distribution of the S-SAPs.

Results

Physical distribution of Bare–1-like retrotransposable elements in the barley genome

An important feature of a molecular assay system which will affect its general application is the overall distribution of markers in the genome of the species under study. To determine the distribution of Bare–1 elements in the barley genome, a Bare–1 probe was hybridised to metaphase chromosome spreads from barley root tip preparations. Figure 1 shows the sites of Bare–1 hybridisation. Bare–1 elements are present at high copy number and are distributed throughout the barley genome. A less intense staining is apparent at both centromeric and nucleolar organiser regions. A control experiment using a portion of the reverse transcriptase domain of a different barley retrotransposon gave much reduced staining throughout the genome (not shown). These results are consistent with those recently reported by Suoniemi et al. (1996).

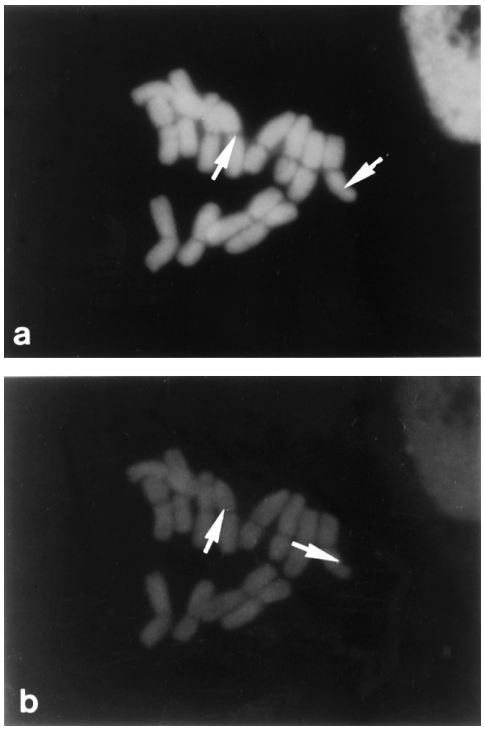

Fig. 1 a Metaphase spread of barley chromosomes counterstained with 4′,6-diamidino-2-phenylindole (DAP). **b** Metaphase chromosomes showing distributions of hybridised biotinylated Bare–1 elements visualised by binding to streptavidin fluorescein isothiocyanate-(FITC) conjugates. The Bare–1 elements appear to be present on all chromosomes with the exception of the centromeric and nucleolar organiser regions (*arrow*)

Bare–1-LTR-derived sequence-specific amplification polymorphisms (S-SAPs)

The observed physical distribution of Bare–1 elements suggested that the relatively highly conserved sequences of these elements could be exploited to develop a genome-wide multiplex assay for revealing molecular polymorphisms between related individuals. There are a number of possible approaches to developing a PCR-based marker from retrotransposable elements, exploiting the inherent variation either within the element itself or the variation in the DNA flanking its site of insertion. We chose the latter approach by modifying the recently described AFLP technique (Vos et al. 1995) to detect Bare–1-like elements. The procedure is outlined in Table 1. The initial steps were identical to the AFLP protocol. However, the selective amplification employed a single adaptor-homologous primer, along with a Bare–1-derived radiolabelled primer originating from the highly conserved terminus of the LTR (Manninen and Schulman 1993). The $P_{(\ )}$ or $M_{(\ )}$ adaptor-homologous primers carried 0, 1, 2 or 3 selective nucleotides at their 3′ ends. Figure 2a shows the effect of adding selective nucleotides to an $M_{(\ )}$ primer when used in combination with labelled Bare–1-LTR on two barley lines, Blenheim

Table 1 Steps involved in Bare–1-LTR-driven sequence-specific amplification polymorphisms (S-SAP)

1.	Digest genomic DNA with rare and frequent cutting restriction enzyme
2.	Ligate on compatible adaptors
3.	Preamplify prepared template DNA with adaptor-homologous primers
4.	Selectively amplify with [^{33}P]-labelled sequence-specific oligonucleotide (Bare–1-LTR) and either rare or frequent site adaptor homologous oligonucleotide
5.	Denature and separate in high solution gel

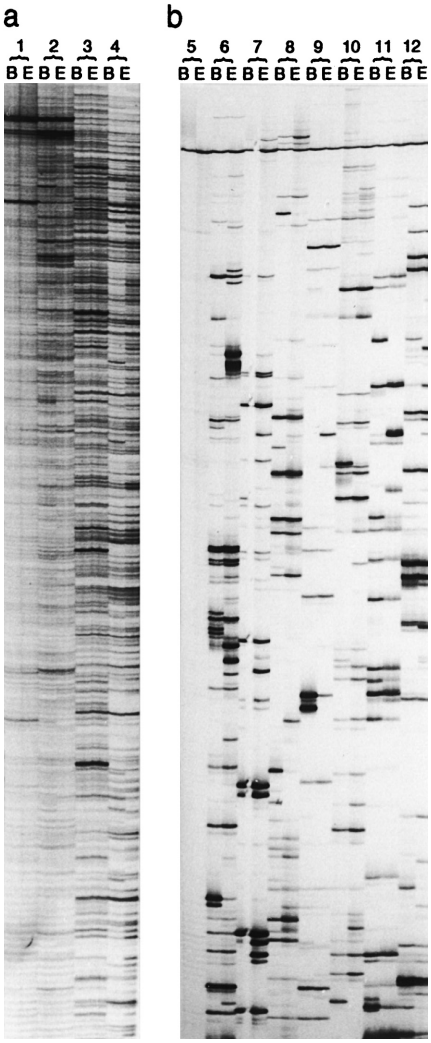

Fig. 2 a Effect of one to three additional selective nucleotides on an M-homologous adaptor oligonucleotide used in polymerase chain reaction (PCR) with ^{33}P-labelled Bare–1-LTR. Addition of selective nucleotides effectively reduces the number of amplification products to a useful number at +3. *Lanes1* Bare–1-LTR + M-homologous oligonucleotide, *2* Bare–1-LTR + $M_{(C)}$, *3* Bare–1-LTR + $M_{(AC)}$, *4* Bare–1-LTR + $M_{(ACA)}$. **b** Combination of $P_{(+3)}$ primers with labelled Bare–1-LTR oligocucleotide. *Lanes 5* Bare–1-LTR, *6* Bare–1-LTR + $P_{(AAA)}$, *7* Bare–1-LTR + $P_{(AAT)}$, *8* Bare–1-LTR + $P_{(ACG)}$, *9* Bare–1-LTR + $P_{(AGC)}$, *10* Bare–1-LTR + $P_{(ATT)}$, *11* Bare–1-LTR + $P_{(CGA)}$, *12* Bare–1-LTR + $P_{(GGA)}$. (*B* Blenheim, *E* E224/3)

and E224/3. With no selective nucleotides, a major product is amplified on a less intense background of other fragments. This could be expected because the Bare–1-LTR primer can prime amplification both into the flanking DNA sequence and into the core of the element itself, as Bare–1 LTRs are direct repeats. The largest subclass of Bare–1 elements with a conserved *Mse*I site within an easily amplifiable distance from the LTR would therefore be expected to generate a major product. The sequenced Bare–1 has an *Mse*I site 843 bp inside the 3′ LTR. In our case, the major product is ca 450 bp, suggesting that it originates from a class of Bare–1 elements which is different from that characterised previously. A survey of the published Bare–1 sequence revealed 36 sequence motifs within 500 bp of the internal Bare–1 LTR sequence which could potentially form an *Mse*I site by mutation at a single position. At approximately 450 bp 5′ of the internal LTR sequence, there is a cluster of three potential *Mse*I sites within a 10-bp window.

The addition of up to three selective nucleotides effectively reduces the contribution of this individual class to the overall profile obtained and reduces the number of products to a level which is easily interpreted on a band presence/absence basis. Using the $P_{(\ )}$ series of primers produced broadly similar results, with resolution of individual fragments requiring fewer selective nucleotides due to the lower relative frequency of *Pst*I restriction endonuclease sites in the barley genome. Only two potential (1 bp mismatch) *Pst*I sites were found within the 500 bp immediately 5′ to the internal LTR sequence, suggesting that the contribution of the Bare–1 element itself to the overall profile obtained with the $P_{(\ )}$ homologous primers in Bare–1-LTR-driven S-SAP is probably minor (Fig. 2b). No products were generated when Bare–1-LTR was used on its own. This suggests that no (intact) Bare–1 elements exist in the barley genome in a head-to-head orientation within a distance which is readily amplifiable by standard PCR (Fig. 2b). The level of polymorphism detected by Bare–1-LTR-driven S-SAPs is compared to that derived from AFLP using either *Eco*RI (+3)/*Mse*I or *Pst*I (+3)/*Mse*I on the same parental lines (Table 2). With the primers tested, Bare–1-LTR-driven S-SAP produces fewer products. This is probably a reflection of the selective nucleotides added. However, a higher proportion of the S-SAPs are polymorphic.

Table 2 Comparison of Bare–1-LTR sequence-specific amplification polymorphisms (*S-SAPs*) and amplified fragment length polymorphisms (*AFLPs*) between Blenheim and E224/3

		Number of primer combinations	Total number of amplified products	Number of polymorphic products	Proportion of polymorphic products
AFLP	*Eco*RI (+3)/*Mse*I (+ 3)[a]	39	92.3 ± 3.9	8.5 ± 1.03	0.092 ± 0.0049
	*Pst*I (+3)/*Mse*I (+3)	18	42.67 ± 4.23	8.0 ± 0.68	0.193 ± 0.015
S-SAP	*Pst*I (+3)/Bare–1-LTR	10	36.27 ± 2.71	10.62 ± 1.79	0.26 ± 0.039

[a] From Powell et al. (1996)

The two barley genotypes used to test the S-SAP approach (Blenheim and E224/3) have been used previously to generate a DH population (B × E) which has in turn been used to construct a genetic linkage map (W. T. B. Thomas et al. 1995; Powell et al. 1996). To establish the utility of S-SAPs in genetic linkage studies, we examined the segregation of Bare–1-LTR-derived amplicons in this population. Six different $P_{(\ )}$ and $M_{(\ )}$ primers used in combination with Bare–1-LTR generated 54 segregating markers (an average of 10.62 per primer pair). An example of the segregation patterns observed is given in Fig. 3. At low frequency, products were observed which segregated in the progeny within expected ratios which were not present in either of the parental lines (highlighted in Fig. 3.) This has been observed previously in this population with other molecular assays (RFLP, RAPD, AFLP) and has been attributed to residual heterozygosity in one of the parental lines used to construct the DH population. These were excluded from the linkage analysis. At very low frequency, amplicons which were monomorphic in both of the parental lines and the progeny were missing from a single individual progeny line (the highlighted case was the only clear cut observation). There are several possible explanations for this. Either a genomic rearrangement (insertion, deletion or inversion) has occurred which has removed the Bare–1-LTR sequence, a point mutation has occurred in the adjacent *Pst*I site or in the 5′ end of the LTR which abolishes effective primer binding or the *Pst*I site has become methylated in this line. The exact cause was not determined.

Of the 54 S-SAPs segregating in the B × E population, 48 were mapped to individual linkage groups using the existing marker information (Fig. 4). All behaved as dominant markers. The Bare–1-LTR-derived S-SAPs are located on all seven barley linkage groups. Their distribution largely reflects that observed for other types of markers in this population (Powell et al. 1996) and correlates well with expectations based on the physical distribution of Bare–1 elements revealed by in situ hybridisation (Fig. 1). Their inclusion did not result in the merging of any segments from the same group and, with one exception, did not extend beyond the preexisting terminal markers of groups.

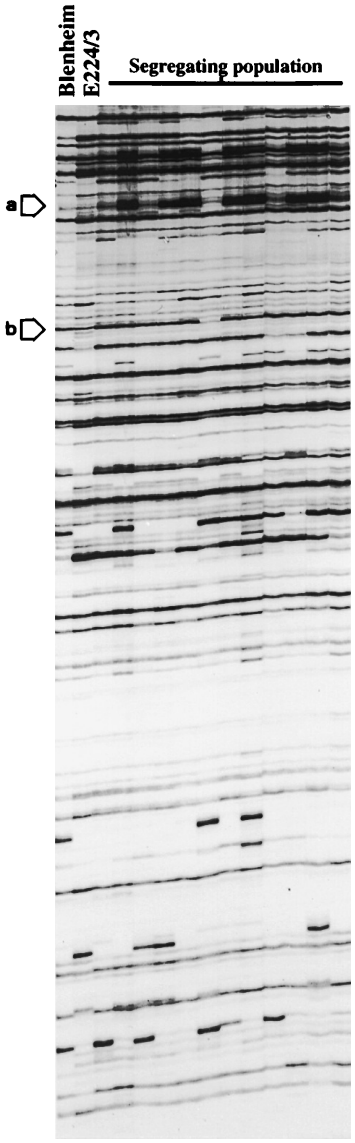

Fig. 3 Segregation of Bare–1-LTR + $P_{(GT)}$-derived sequence-specific amplification polymorphisms (S-SAPs) in the first 12 progeny of a doubled haploid barley population. A number of bands which are polymorphic between the parental lines can be seen to segregate in the progeny. At position **a**, a band segregates in the progeny which is not present in either of the parental lines. At position **b** in the ninth track from the left, a product is absent which is present in both parents and all other progenies

Discussion

The barley genome is estimated to contain upwards of 70 000 Bare–1 elements (A. Schulman, personal communication), giving enormous scope for the development of new classes of markers based on Bare–1 sequences. Here, using the sequence of Bare–1, we have developed an assay for revealing polymorphism (S-SAP) which uses a combination of AFLP and sequence-specific PCR. The Bare–1 sequence was from the terminal 5′ end of the LTR. By using a transposable element sequence, we hoped to increase the level of polymorphism detectable in barley to above that revealed by AFLP. While the number of polymorphisms detected per Bare–1-LTR-driven S-SAP assay was not significantly different from AFLP in the same population (Powell et al. 1996), the proportion of polymorphic products apparently was. This will, however, need testing across a wider range of genotypes before we can confirm this observation.

Assuming that all products are derived from Bare–1-like LTR sequences (which was not definitively determined), a fraction of the polymorphism revealed will

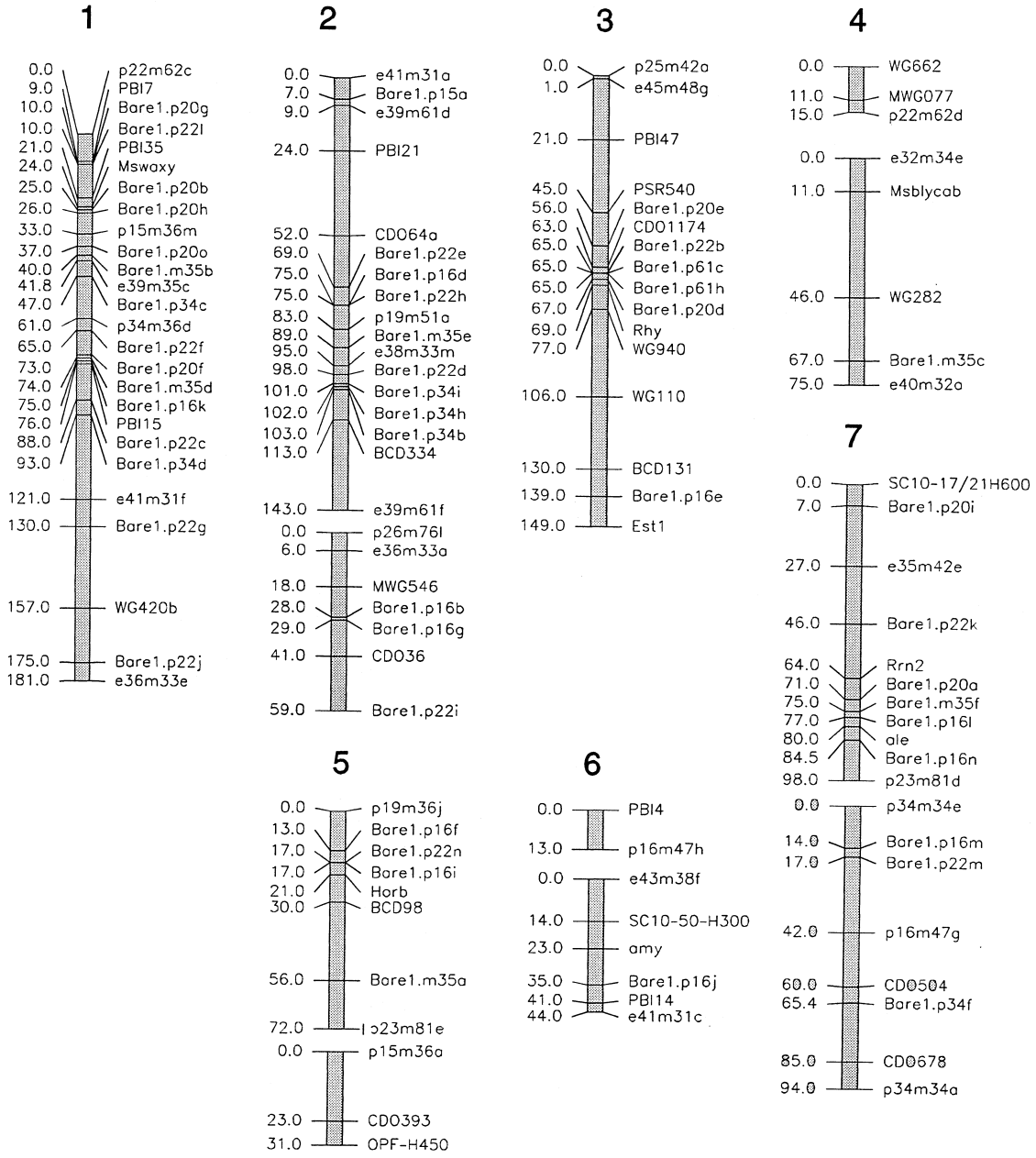

Fig. 4 Genetic distribution of 48 segregating Bare–1 elements in the Blenheim × E224/3 doubled haploid population (Powell et al. 1996). The Bare–1-LTR-derived S-SAPs map to all seven barley chromosomes. For clarity, most other markers have been omitted

be derived from sequence variation (or DNA methylation in the case of *Pst*I) at flanking or internal restriction sites, another portion with variation at the 5′ terminal end of the Bare–1-like LTR and the remainder due to insertional polymorphism of the Bare–1-like retrotransposable element. The polymorphism associated with the first parameter is directly comparable to the variation detected by AFLP. The latter two are retrotransposon specific. Assuming that single nucleotide changes are the primary cause of AFLP (with a mutation rate of ca 10^{-7}–10^{-9}/nucleotide per generation), the observed increase in the frequency of polymorphism with Bare–1-LTR-driven S-SAP indicates that Bare–1 transposition in barley occurs at approximately 25% of the frequency of point mutations. This conclusion is reinforced by the segregation analysis where recent insertional polymorphisms would be detected as novel bands present in one (or more) of the progeny lines but not in the parents. No clear products of this type were observed out of the ca 24 500 elements visualised (ca 350 elements per sample × 70 samples). The transposition rate of Bare–1-like elements in these lines is therefore very low.

Given that the majority of Bare–1-like elements are transpositionally inactive (although they may still contribute to the pool of Bare–1-derived RNA in the cell),

that there are upwards of 70 000 elements in the genome and that transposition frequency is low, the identification of functional elements – whether via a cDNA-based approach or phenotypic selection – is a difficult task. A thorough analysis of a range of samples of the same genotype with Bare–1-LTR-driven S-SAPs is one possible route to the isolation of active elements which, as mentioned above, would appear as novel, unique bands in isogenic samples. As plant cell culture is known to increase the transposition frequency of retrotransposons (Hirochika 1995), a comparison of isogenic lines taken through a tissue culture phase would increase the possibility of identifying mobile elements. In this respect, while we have used the rare cutting enzyme *Pst*I, which is methylation-sensitive, such an experiment would be better conducted using a methylation-insensitive enzyme, as tissue culture is also known to affect methylation status (Brown 1989). Nevertheless, the identification of active elements is an important objective which will facilitate further studies into the mechanism, induction and consequences of transposition on genome structure, evolution and biodiversity (Kumar 1996). Bare–1-LTR-driven S-SAP offers an approach towards achieving this objective.

The approach outlined could be used in other species from which retrotransposable elements have been characterised. The 5′ terminal LTR sequence from a number of these are given in Table 3. The Bare–1 LTR sequence used here is identical to the analogous sequence of the Wis–2 retrotransposon in wheat. Precisely the same approach could therefore be used directly in wheat, where it may also offer the potential for revealing higher levels of polymorphism than the limited levels detected by other molecular polymorphism assays. Also, the genetic and physical distributions of Bare–1 elements are consistent and show good genome coverage. This is an important attribute, as uneven distribution would restrict general applicability. Initial studies with *Eco*RI/*Mse*I- and *Pst*I/*Mse*I-derived AFLPs in barley (Powell et al. 1996) and wheat (M. Gale, personal communication) indicate significant differences between their distribution along certain linkage groups or clustering around the borders of genomic segments heavily mapped with RFLPs. Many of the RFLP probes used are either genomic *Pst*I or cDNA clones which identify low copy sequences. Homology searches in plant DNA sequence databases frequently reveal the proximity of retrotransposable elements to coding sequences. While this may be simply a function of the number of randomly distributed elements in the genome, it may also indicate preferential integration of Bare–1 into low copy or transcriptionally active regions. While the consequences of this on gene expression, genome structure and evolution are unclear, identification of these types of sequence is a valuable characteristic of a marker system, allowing relatively easy development of diagnostic single locus markers from a complex molecular fingerprint.

Table 3 5′ Terminal-long terminal repeat (LTR) sequences of plant *Ty-copia*-like retrotransposons

Bare–1 (barley)	TGTTGGAATTATGCCCTAG
Wis–2 (wheat)	TGTTGGAATTATGCCCTAG
Tos–1 (rice)	TGTTGGGAATAGTCCCACA
Tos–2 (rice)	TGTTGAATAGTTCCACATT
Tos–3 (rice)	TGTTAGAAGTATAATATGT
Bs–1 (maize)	TGTTAGCAACCCAATACCA
Cin–1 (maize)	TGTTGGGGACCTTTCTCTT
Tnt–1 (tobacco)	TGATGATGTCCATCTCATT
Ta–1 (*Arabidopsis*)	TGTTGGAGTTATGATCC

While we have described here the potential for generating genome-wide markers based on the conserved sequences of the transposable element Bare–1, we are currently using S-SAP as a general approach to obtain linkage information on a range of other conserved sequences in the barley genome. The same approach could also be applied to any other species. The basic requirement is the proximity of a given sequence (conserved or not) to a relatively rate cutting restriction enzyme site to which adaptors can be ligated. By adjusting both the enzyme and/or number of selective nucleotides on the adaptor-homologous oligonucleotide and/or by incorportating an initial asymmetric PCR with a chosen primer, it should be possible to amplify from almost any oligonucleotide sequence. While this will be most efficient for multicopy consensus sequences because of the higher information content per assay, motifs such as the nucleotide binding domains (which are feature of a class of cloned plant resistance genes) and even low copy sequences should also be appropriate.

In conclusion, we have combined the general principle of AFLP with sequence-specific PCR to develop an approach which reveals the genetic distribution of Bare–1-like retrotransposable elements in the barley genome. The high copy number of Bare–1 elements makes them virtually impossible to map genetically by hybridisation or conserved-internal sequence PCR. In S-SAP. high copy number and dispersion throughout the genome are advantages, providing more information per assay. The level of polymorphism detected appears to be higher than that revealed by AFLP, which will be of benefit in species such as wheat where polymorphism is low. In addition, S-SAP is potentially applicable to any known sequence, given appropriate primer design.

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References

Ballvora A, Hesselbach J, Niewohner J, Leister D, Salamini F, Gebhardt C (1995) Marker enrichment and high resolution map of the sequence of potato chromosome VII harbouring the nematode resistance gene *Gro*I. Mol Gen Genet 249:82–90ff

Becker J, Vos P, Kuiper M, Salamini F, Heun M (1995) Combined mapping of AFLP and RFLP markers in barley. Mol Gen Genet 249:65–73

Boeke JD, Corces VG (1989) Transcription and reverse-transcription of retrotransposons. Annu Rev Microbiol 43:403–434

Brown PTH (1989) DNA methylation in plants and its role in tissue culture. Genome 31:717–729

Caetano-Anolles G (1993) Amplifying DNA with arbitrary oligonucleotide primers. PCR Methods 3:85–94

Camirand A, St Pierre B, Martineau C, Brisson N (1990) Occurrence of a *copia*-like transposable element in one of the introns of the potato starch phosphorylase gene. Mol Gen Genet 224:33–39

Eck H van, Voort JR van der, Draaistra J, Zandvoort PV, Enckevort E van, Segers B, Peleman J, Jacobsen E, Helder J, Bakkar J (1995) The inheritance and chromosomal localisation of AFLP markers in a non-inbred potato offspring. Mol Breeding 1:397–410

Flavell AF, Dumbar E, Anderson R, Pearce SR, Hartley R, Kumar A (1992a) *Ty1-copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. Nucleic Acids Res 20:3639–3644

Flavell AF, Smith D, Kumar A (1992b) *Copia-Ty* family retrotransposon heterogeneity in plants. Mol Gen Genet 231:233–242

Grandbastien M-A (1992) Retroelements in higher plants. Trends Genet 8:103–108

Grandbastien M-A, Spielmann A, Caboche M (1989) Tnt1 a mobile retroviral-like transposable element of tobacco isolated by plant cell genetics. Nature 337:376–380

Hirochika H (1995) Activation of plant retrotransposons by stress In: Oono K, Takaiwa F (eds) Modification of gene expression and non-Mendelian inheritance. NIAR, Japan, pp 15–22

Hirochika H, Hirochika R (1993) *Ty1-copia* retrotransposons as ubiquitous components of plant genomes. Jpn J Genet 68:35–46

Hirochika H, Fukuchi A, Kikuchi F (1992) Retrotransposon families in rice. Mol Gen Genet 233:209–216

Konieczny A, Ausubel F (1993) A procedure for mapping *Arabidopsis* mutations using codominant, ecotype-specific PCR-based markers. Plant J 4:403–410

Kumar A (1996) The adventures of *Ty1-copia* group of retrotransposons in plants. Trends Genet 12:41–43

Leeton PJ, Smyth DR (1993) An abundant LINE-like element amplified in the genome of *Lilium speciosum*. Mol Gen Genet 237:97–104

Leitch AR, Schwarzacher T, Leitch IJ (1994) The use of fluorochromes in the cytogenetics of the small grained cereals. Histochem 26:471–479

Lindauer A, Frazer D, Bruderlein M, Schmitt R (1993) Reverse transcriptase families and a *copia*-like retrotransposon in the green alga *Volvox carteri*. FEBS Letts 319:261–266

Manninen I, Schulman AH (1993) BARE–1 a *copia*-like retroelement in barley (*Hordeum vulgare*). Plant Mol Biol 22:829–864

Meksem K, Leister D, Peleman J, Zabeau M, Salamini F, Gebhardt C (1995) A high resolution map of the vicinity of the R1 locus on chromosome V of potato based on RFLP and AFLP markers. Mol Gen Genet 249:74–81

Pearce SR, Harrison G, Li D, Heslop-Harrison JS, Kumar A, Flavell AJ (1996) The *Ty1-copia* group retrotransposons in *Vicia* species: copy number, sequence heterogeneity and chromosomal localisation. Mol Gen Genet 250:305–315

Powell W, Thomas WTB, Baird E, Lawrence P, Booth A, Harrower B, McNicol JW, Waugh R (1996) Analysis of quantitative traits in barley by the use of amplified fragment length polymorphism. Heredity (in press)

Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horu GT, Mullis KB, Erlich HA (1988) Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Schmidt T, Kubis S, Heslop-Harrison JS (1996) Analysis and chromosomal localisation of retrotransposons in sugarbeet (*Beta vulgaris* L.): LINEs and *Ty1-copia*-like elements as major components of the genome. Chromosome Res 3:335–345

Smyth DR (1993) Plant retrotransposons. In: Verma DPS (ed) Control of gene expression. CRC Press, London, pp 1–15

Stam P, Ooijen JW van (1995) Joinmap (tm) version 2.0: software for the calculation of genetic linkage maps. CPRO-DLO, Wageningen

Suoniemi A, Kesara A-J, Arna T, Schulman AH (1996) Retrotransposon BARE–1 is a major, dispersed component of the barley (*Hordeum vulgare* L.) genome. Plant Mol Biol 30:1321–1329

Tautz D (1988) Hypervariability of simple sequence repeats as a general source for polymorphic DNA markers. Nucleic Acids Res 17:6463–6471

Thomas CM, Vos P, Zabeau M, Jones PA, Norcott KA, Chadwick BP, Jones JDG (1995) Identification of amplified restriction fragment polymorphism (AFLP) markers tightly linked to the tomato CF-9 gene for resistance to *Cladosporium fulvum*. Plant J 8:785–794

Thomas WTB, Powell W, Waugh R, Chalmers KJ, Barua UM, Jack P, Lea V, Forster BP, Swanston JS, Ellis RP, Hanson PR, Lance RCM (1995) Detection of QTL for agronomic, yield, grain and disease characters in spring barley. Theor Appl Genet 91:1037–1047

Vos P, Hogers R, Bleeker M, Reijans M, Lee T van de, Hornes M, Fritjers A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new concept for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Voytas DF, Ausubel FM (1988) A *copia*-like transposable element family in *Arabidopsis thaliana*. Nature 336:342–344

Voytas DF, Cummings MP, Konieczny A, Ausubel FM, Rodermel SR (1992) *Copia*-like retrotransposons are ubiquitous among plants. Proc Natl Acad Sci USA 89:7124–7128

Waugh R, Bonar N, Baird E, Lawrence P, Harrower B, Booth A, Khalil W, Hayes P, Graner A, Thomas WTB, Ellis RP, Powell W (1996) Conservation of AFLP marker order in different barley populations. Abstracts of International Barley Genetics Symposium, Saskatoon, Canada (in press)

Welsh J, Petersen C, McClelland M (1991) Polymorphisms generated by AP-PCR in the mouse: applications to strain identification and genetic mapping. Nucleic Acids Res 19:303–306

White SE, Habers L, Wesseler SR (1994) Retrotransposons in the flanking regions of normal plant genes. A role for *copia*-like elements in the evolution of gene structure and expression. Proc Natl Acad Sci USA 91:11792–11796

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Zabeau M, Vos P (1993) Selective restriction fragment amplification: a general method for DNA fingerprinting. European Patent Application 92402629.7 (Publication number:0 534 858 A1)

Zietkiewicz E, Rafalski A, Labuda D (1994) Genome fingerprinting by simple sequence repeats (SSR)-anchored PCR amplification. Genomics 20:176–183