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IRAP and REMAP: two new retrotransposon-based DNA fingerprinting techniques

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Abstract The *BARE*-1 retrotransposon is an active, dispersed, and highly abundant component of the genome of barley (*Hordeum vulgare*) and other species in its genus. Like all retrotransposons of its kind, *BARE*-1 is bounded by long terminal repeats (LTRs). We have developed two amplification-based marker methods based on the position of given LTRs within the genome. The IRAP (*I*nter-*R*etrotransposon *A*mplified *P*olymorphism) markers are generated by the proximity of two LTRs using outward-facing primers annealing to LTR target sequences. In REMAP (*RE*trotransposon-*M*icrosatellite *A*mplified *P*olymorphism), amplification between LTRs proximal to simple sequence repeats such as constitute microsatellites produces markers. The methods can distinguish between barley varieties and produce fingerprint patterns for species across the genus. The patterns indicate that although the *BARE*-1 family of retrotransposons is disperse, these elements are locally clustered or nested and often found near tandem arrays of a simple sequence repeat.

Key words *Hordeum vulgare* · Retrotransposon *BARE*-1 · REMAP · IRAP · Molecular marker

Introduction

In recent years, the generation and display of DNA "fingerprints," distinctive fragment patterns in samples, have proven extremely valuable for forensic, mapping, and biodiversity applications. Sufficiently high polymorphism allows the component bands of the fingerprints to serve as genetic markers, with recombination and segregation of the markers being used to construct genetic maps. These have proven superior to both biochemical (protein) and phenotypic markers, which suffer from a fairly low degree of polymorphism, lack of sufficient loci to generate dense maps, and environmentally variable expression. The DNA-based techniques rely either on the detection of genomic sequences polymorphic for internal restriction sites by hybridization of cloned probes, such as in restriction fragment length polymorphism (RFLP) (Botstein et al. 1980), on polymerase chain reaction (PCR) amplification between random primers, such as in random amplified polymorphic DNA (RAPD) (Williams et al. 1990), or on amplification between simple sequence repeats or microsatellites (Zietkiewicz et al. 1989). The amplified fragment length polymorphism (AFLP) method (Vos et al. 1995) is conceptually a hybrid, relying on polymorphism in the recognition sites for restriction enzymes but using adapters and adapter primers to obviate the need for sequence knowledge to detect the polymorphisms.

The dispersion (Katsiotis et al. 1996; Suoniemi et al. 1996a), ubiquity (Flavell et al. 1992; Voytas et al. 1992), and prevalence (Pearce et al. 1996, 1997; Suoniemi et al. 1996a) of retrotransposons in plant genomes provide an excellent basis for the development of marker systems. Retrotransposons replicate by successive transcription, reverse transcription, and insertion of the new cDNA copies back into the genome, very much like retroviruses (Adams et al. 1987; Grandbastien 1992). The structure and replication strategy of retrotransposons give them several advantages as markers. They contain long, defined, conserved sequences which can be used for cloning of specific markers and flanking sequences. Secondly, replicationally active members of a retrotransposon family will produce new insertions in

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the genome, leading to polymorphism. The new insertions may then be detected and used to temporally order insertion events in a lineage, thereby helping to establish phylogenies (Shimamura et al. 1997).

In barley, the endogenous copies of the *BARE*-1 retrotransposon family are highly conserved (Manninen and Schulman 1993; Suoniemi et al. 1997, 1998), dispersed (Suoniemi et al. 1996a), and transcriptionally active (Suoniemi et al. 1996b). In addition, *BARE*-1 is present throughout *Hordeum* and related genera and appears to have played a major role in genome evolution in the genus. The *BARE*-1 family would thus appear to be well-suited for marker development. Recently, a modified AFLP method based on *BARE*-1, Sequence-Specific Amplified Polymorphism (S-SAP), was introduced in which a PCR primer facing outward from the *BARE*-1 long terminal repeat (LTR) is used in combination with an AFLP adapter primer (Waugh et al. 1997). The method gives several advantages over AFLP: more polymorphism, a more even chromosomal distribution, and more codominance.

Nevertheless, S-SAP relies on restriction digestion to provide sites for adapter ligation. The sensitivity of commonly used enzymes such as *Pst*I and *Eco*RI to DNA methylation, combined with the high and potential variable degree of CG and CXG methylation in plant DNA, means that some apparent polymorphism may neither be sequence-based nor heritable. We present here two retrotransposon-based methods which require no DNA digestion: IRAP (*I*nter-*R*etrotransposon *A*mplified *P*olymorphism) and REMAP (*RE*trotransposon-*M*icrosatellite *A*mplified *P*olymorphism). The application of these methods has been demonstrated on a set of *Hordeum* species representing the diversity of the genus and on a set of barley cultivars. The resulting data have interesting implications for genome organization within *Hordeum*.

Materials and methods

Plant material and DNA preparation

The barley (*Hordeum vulgare*) varieties examined were kind gifts from the following sources: 'Djau Kaputak', 'Pallidum 107', and 'Pallidum 107 × Ros', Dr. A.A. Linchevski, Institute of Plant Breeding and Genetics, Odessa, Ukraine; 'NK 1558', Northrup King Company, Minneapolis, Minn., USA; 'Bomi', Hans Doll, Risø National Laboratories, Roskilde, Denmark; 'Kusta', Risto Lampinen, Kesko Ltd, Hahkiala Breeding Station, Hauho, Finland; 'Forrest' and 'Schooner', R.J. Puckridge, Department of Agriculture, Northfield Research Laboratories, Adelaide, Australia; 'Pomo' and 'Tampar', Minna Nurminiemi, Department of Biotechnological Sciences, Norwegian University of Agriculture, Ås, Norway. Ten-day-old seedling leaves or 5-day-old etiolated leaves were collected for use in DNA preparation. DNA was prepared by the CTAB method (Ausubel et al. 1995) with RNase A treatment.

IRAP

Primers were designed to match the 5′ LTR of the *BARE*-1a sequence (accession Z17327, bases 309-2137). The reverse primer (6150, Table 1) is complementary to bases 418-439, and the forward primer (6149) matched bases 1993-2012. The location of the primers corresponded to conserved stretches of the *BARE*-1 LTR, particularly at the primers' 3′ ends, based on initial alignments (Suoniemi et al. 1997).

The IRAP PCR was performed in a 20-µl reaction mixture containing 20 ng DNA, 1 × PCR buffer (20 m*M* TRIS-HCl, pH 8.8, 50 m*M* KCl, 0.01% Tween-20, 5% glycerol), 2 × Enchancer (Epicentre), 2 m*M* $MgCl_2$, 5 pmol each primer, 200 n*M* dNTP, 1 U *Taq* polymerase (Epicentre). Amplification was performed in a PTC-225 DNA Engine Tetrad (MJ Research) in 0.2-ml tubes (Robbins Scientific). The PCR reaction program consisted of: 1 cycle at 94°C, 2 min; 1 cycle at 94°C, 30 s; 60°C, 30 s; ramp + 0.5°C s^{-1} to 72°C; 30 cycles of 72°C, 2 min + 3 s; 1 cycle at 72°C, 10 min; 4°C. Products were

Table 1 Primers for IRAP and REMAP

Primer	Sequence	Annealing temperature (T_a)
IRAP		
LTR reverse 6150	*CTGGTTCGGCCC*ATGTCTATG TATCCACACATGGTA[a]	60.0°C
LTR forward 6149	*CTCGCTCGCCCA*CTACATCAA CCGCGTTTATT[b]	60.0°C
REMAP		
LTR reverse 7286	*GGAATTC*ATAGCATGGATAA TAAACGATTATC	
8081	$(GA)_9C$	54°C
8082	$(CT)_9G$	54°C
8385	$(CAC)_7G$	58°C
8386	$(GTG)_7C$	58°C
8387	$(CA)_{10}G$	54°C
8564	$(CAC)_7T$	58°C
8565	$GT(CAC)_7$	58°C

[a] *Italics*, tail for non-ligational cloning in vector pDirect™ (Clontech) vector
[b] *Italics*, tail containing *Eco*RI

analyzed by electrophoresis on 2% NuSieve 3:1 agarose (FMC Bioproducts) and detected by ethidium bromide staining.

REMAP

The reverse primer (Table 1, 7286) corresponded to nt 369-393 of the *BARE*-1a (accession Z17327) LTR with the addition of a tail containing an *Eco*RI site at the 5′ end. A total of seven forward primers were designed (Table 1) for microsatellites based on two dinucleotide repeats, $(GA)_n$ and $(CT)_n$, and two trinucleotide repeats, $(CAC)_n$ and $(GTG)_n$. The primers are listed in Table 1. Most primers (8081, 8082, 8385, 8386, 8387, and 8564) are anchored at the 3′ ends of the microsatellite repeats, while 8565 is anchored at 5′ end. The PCR reactions and program and product resolution were as for IRAP, except that the annealing temperature (T_a) used was as specified.

ISSR

The inter-simple sequence repeat (ISSR) amplification was carried out by a method similar to those previously described (Godwin et al. 1997; Salimath et al. 1995; Sivolap Iu et al. 1994; Zietkiewicz et al. 1994). The reaction mixture was as for IRAP and REMAP. The PCR reaction program consisted of: 1 cycle at 94°C, 2 min; 30 cycles of 94°C, 30 s; T_a for primer, 30 s; 72°C, 2 min; 1 cycle at, 72°C, 10 min; 4°C. Products were analyzed as for IRAP and REMAP.

Phylogenetic analysis

Phylogenetic trees were constructed by a distance-based method. Evolutionary distances were calculated from tables of bands scored for presence or absence using equations of Nei and coworkers (Nei and Li 1979; Saitou and Nei 1987). Trees were constructed by neighbor-joining (Saitou and Nei 1987) implemented in a DOS-based program, Tree version 4.3, written by and available from one of the authors (R.K.)

Results

IRAP and REMAP strategy

Both IRAP and REMAP examine polymorphism in retrotransposon insertion sites, IRAP between retrotransposons and REMAP between retrotransposons and microsatellites (SSRs) (Fig. 1). Retrotransposons, unlike DNA transposons, spread daughter copies rather than excising and moving directly. Hence, stable insertions behave as Mendelian loci during crosses and segregate accordingly. Retrotransposon-based markers would be expected to be co-dominant. For both IRAP and REMAP, we have designed primers facing outward from the long terminal repeats (LTRs) of the *BARE*-1 retrotransposon. The LTRs of retrotransposons contain sequences which are essential for expression (promoter and processing signals) and integration. These sites tend to be highly conserved. From these conserved LTR regions, primers for PCR amplification can be generated which will anneal to the dispersed members of a retrotransposon family. Other regions of a retrotransposon, such as the highly conserved (−)- and (+)-strand reverse transcriptase priming sites, can be used instead. Due to the 1.8-kb length of the *BARE*-1 LTRs, the LTR primers in this case serve best. In an earlier work, analysis of a set of genomic *BARE*-1 LTRs enabled highly conserved regions to be defined for primer design (Suoniemi et al. 1997). The regions chosen are 109 bp in from the 5′ terminus and 144 bp in from the 3′ terminus of the LTR, and are conserved in the *Wis*-2 retrotransposon family of wheat as well. In later experiments, it was established that the terminal region of the LTR yields the same band pattern.

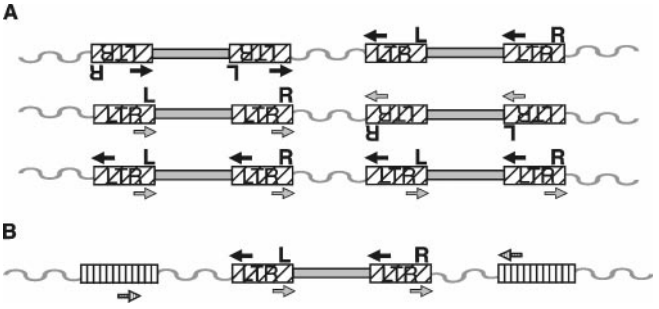

Fig. 1A, B Amplification strategy for IRAP (**A**) and REMAP (**B**). **A** *BARE*-1 retrotransposons are depicted as LTRs (long terminal repeats, labeled *L* and *R* for left and right, respectively) bounding the internal coding region (*dark box*) inserted within flanking DNA (*wavy line*). PCR primers facing outward from the 5′ (*dark arrows*) and 3′ (*light arrows*) ends of the LTRs will amplify intervening DNA from *BARE*-1 elements in any of the three possible orientations. **B** In REMAP, a primer similar to one of those for IRAP is used together with a primer consisting of simple sequence repeats (*segmented arrow*). The corresponding microsatellite is shown as a *segmented box*

Retrotransposons may integrate in principle in either orientation into the genome, and hence any two members of a retrotransposon family may be found head-to-head, tail-to-tail, or head-to-tail. For the first two orientations, a single primer suffices to generate PCR products from elements sufficiently close to one another (Fig. 1A). To amplify intervening genomic DNA for elements in head-to-tail orientation, we must use both 5′ and 3′ LTR primers, which in turn should produce some bands from the other orientations as well.

The REMAP method relies on one outward-facing LTR primer and a second primer from a microsatellite. Primers were designed to the $(GA)_n$, $(CT)_n$, $(CA)_n$ $(CAC)_n$, $(GTG)_n$, and $(CAC)_n$ microsatellites and were anchored (all but one) to the microsatellite 3′ terminus by the addition of a single selective base at the 3′ end. This was done to avoid polymorphism from variation in microsatellite repeat length, but at the same time to permit amplification from multiple microsatellite loci. The $(GA)_n$ microsatellites have been estimated to occur in 3.6×10^4 blocks in the wheat genome (Röder et al. 1995) and have been used in several barley mapping projects (Becker and Heun 1995; Russell et al. 1997).

Interspecies polymorphism with IRAP and REMAP

Sixteen species were compared by IRAP and REMAP (Fig. 2). The degree of polymorphism was very high, 100% by either method. The number of bands visible by IRAP reflects the number of *BARE*-1 elements situated close enough to one another to permit PCR amplification, which in turn is a function of both *BARE*-1 number and genome organization. The conflation of these factors can be seen by comparing the number of bands in the *H. murinum* subspecies (Fig. 2A), which have approximately 300×10^3 LTRs per haploid genome (our unpublished data) and display approximately 15 bands, to *H. roshevitzii* (360×10^3 LTRs) with approximately 30 bands and to *H. vulgare* or *H. spontaneum* (180×10^3 LTRs), likewise with approximately 30 bands. The amount of polymorphism revealed on the species level within *Hordeum* is too great to permit the use of IRAP in interspecies phylogenetic analyses. The **H**-genome species (*H. euclaston*, *H. pusillum*, *H. brachyanterum*, *H. erectifolium*, *H. bogdanii*, *H. stenostachys*, *H. patagonicum*, *H. roshevitzii*, *H. depressium*, *H. muticum*) share few IRAP bands. However, IRAP is useful for analyses below the species level, as can be seen in Fig. 2A by comparing the lanes for the *H. vulgare* and *H. spontaneum* and for the *H. murinum* subspecies. Improved resolution and the scoring of the IRAP bands at 50–800 bp can be obtained by using Cy-5® cyanine -labeled (Pharmacia) LTR primers followed by separation and detection on an automated sequencer.

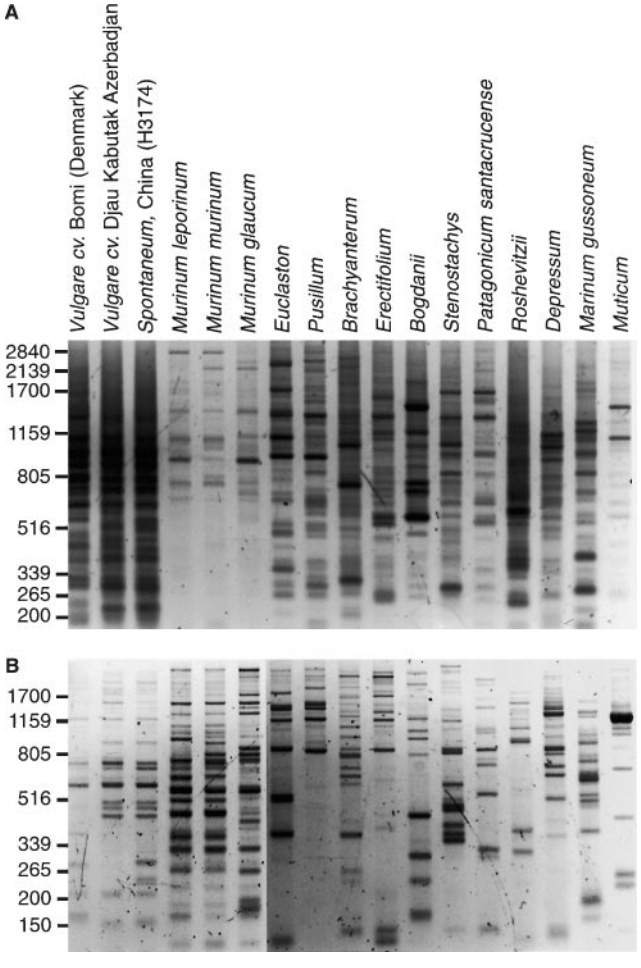

Fig. 2A, B Banding patterns from *Hordeum* species generated by IRAP **A** and REMAP **B**. LTR primers 7058 and 6149 were used for IRAP and LTR primer 7086 together with 8081 [$(GA)_9C$] for REMAP. Marker sizes in basepairs are shown on the *left axes*

The REMAP banding pattern (Fig. 2B) results from the proximity of microsatellites, in this case $(GA)_9$ (primer 8081, Table 1), to *BARE*-1 LTRs. No bands are shared with the ISSR pattern (data not shown), indicating that all bands span intervening domains between LTRs and microsatellites in the genome. By REMAP, *H. vulgare* and *spontaneum* accessions could be both grouped and distinguished one from another, as could the *H. murinum* subspecies. The pattern, as for IRAP, is so polymorphic within the genus that few bands are shared even between the **H**-genome species. However, the morphologically-distinct *H. erectifolium* and *H. euclaston*, both accessions from Buenos Aires province in Argentina, have similar REMAP patterns. Due to the polymorphism, similarities seen by protein and RAPD markers (Jacobsen and von Bothmer 1992; Jørgensen 1986; Marillia and Scoles 1996) between *H. brachyanterum* and *H. bogdanii*, *H. stenostachys* and *H. pusillum*, and between *H. vulgare* and *H. murinum* could not be detected by REMAP. The number of bands detected by REMAP better reflected the number of LTRs in the genomes examined than by IRAP: *H. patagonicum* ssp. *santacrucense*, with a comparatively few 87×10^3 LTRs, displayed 13 bands; *H. erectifolium* (128×10^3 LTRs), approximately 17 bands; *H. depressum* (153×10^3 LTRs), 19 bands; *H. brachyanterum* (162×10^3 LTRs), 23 bands. The *H. marinum* ssp. *gussoneanum* was an exception, with 407×10^3 LTRs but only 15 marker bands. The banding pattern seen was robust, and identical patterns were obtained with various brands of tubes and several makes of cyclers.

Use of IRAP and REMAP to distinguish barley varieties

We examined a set of barley varieties from various regions to see if either the IRAP or the REMAP markers were polymorphic enough to permit the techniques to be used in breeding applications. The REMAP bands generated were polymorphic with all of the microsatellite primers tried, including primers 8081 [$(GA)_9$] (Fig. 3A) and 8082 [$(CT)_9$] (Fig. 3B). The REMAP bands were not generated by amplification between the microsatellite repeats (ISSR), as can be seen by comparing the ISSR pattern for primer 8081

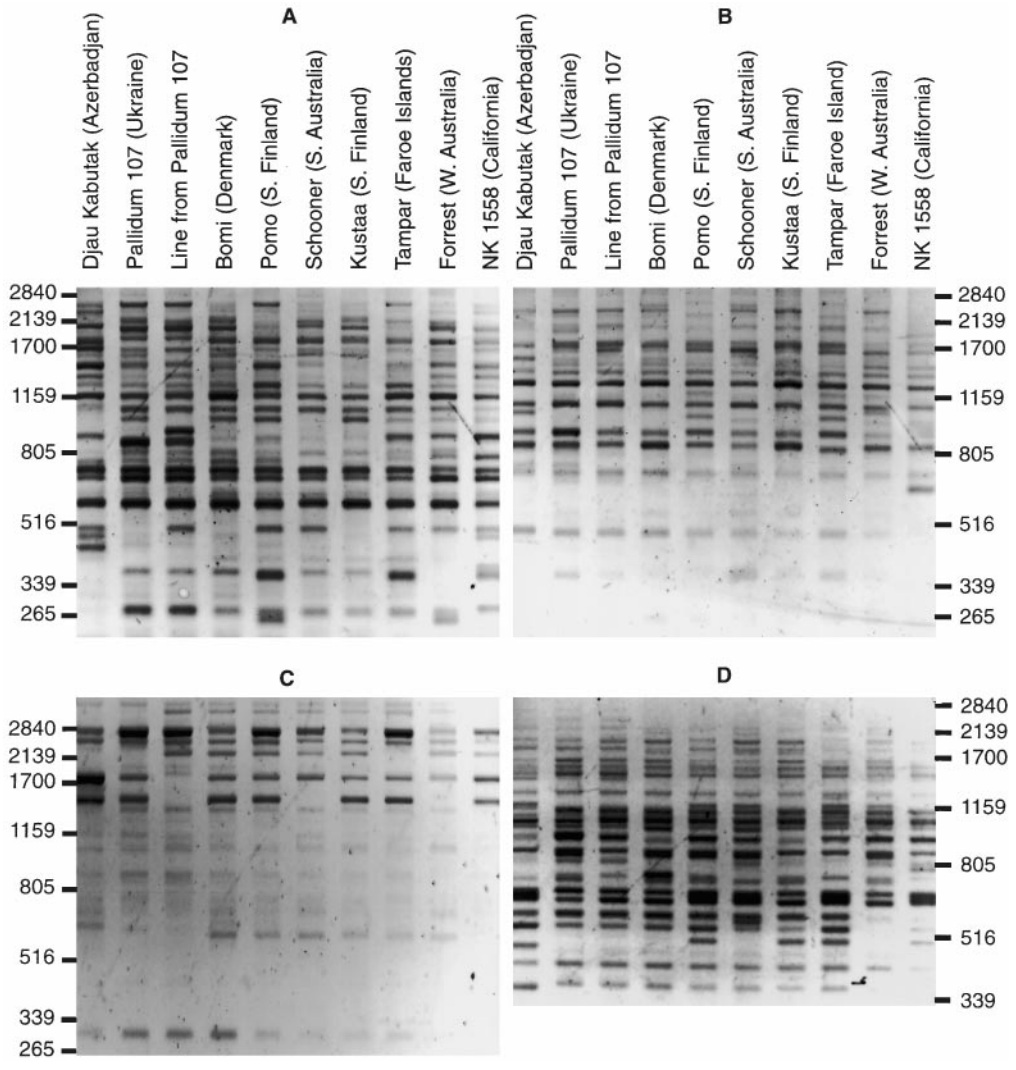

Fig. 3A–D Banding patterns from barley varieties generated by REMAP, IRAP, and ISSR. **A** REMAP with primer 8081 [$(GA)_9C$] and LTR primer 7086. **B** REMAP with primer 8082, [$(CT)_9G$] and LTR primer 7086. **C** ISSR with primer 8081 as in **A**. **D** IRAP with LTR primers 7058 and 6149 as in Fig. 2. Marker sizes in basepairs are shown adjacent to the gels

in Fig. 3C with Fig. 3A. The ISSR pattern shows considerably less variation between barley cultivars, implying that much of the variation seen is a result of the insertion or loss of *BARE*-1 elements rather than of the microsatellites. The IRAP pattern as well (Fig. 3D) is polymorphic between barley varieties. A summary of the results for the various primer pairs is found in Table 2. The levels of polymorphism for IRAP and REMAP are comparable. A similarity index was constructed to estimate the overall degree of polymorphism in the banding pattern, based on the presence or absence of bands scored. A value of 1 indicates that the patterns are totally identical between the accessions compared and a value of 0, total dissimilarity. By this standard, the IRAP and REMAP primer pairs tried gave an overall average of 0.61 ± 0.07 (S.E.M.), or 0.64 when pooling bands from all primer combinations.

Pooled REMAP data were used to generate a phylogenetic tree by the distance-based neighbor-joining method (Fig. 4). The reticulate relationships between cultivars in breeding programs as well as the uncertain identity of their ancestral landraces makes such analysis difficult. However, for this initial test of the methods, the material chosen was geographically diverse and was well resolved by REMAP. 'Djau Kabutak', a variety from Azerbadjan clustered with 'NK 1558', a California variety derived from Mediterranean stock. 'Forrest', a feed variety from Western Australia, branched from the same node as 'Schooner', a malting variety from South Australia. 'Tampar' is a six-rowed landrace from the Faroe Islands, presumably derived from stocks brought to the islands by the original Norse settlers a millennium ago. It clustered with 'Pomo', a six-rowed Finnish variety which derives exclusively from crosses of selections from Swedish

Table 2 Products displayed by IRAP and REMAP

Primer combination	Total bands	Polymorphic bands	Percent polymorphic	Total accessions	Average phenotypic frequency[a]	Similarity index[b]
IRAP						
6149 + 6150	27	18	67	10	0.1	0.563
6149 + 7286	27	18	67	10	0.1	0.563
REMAP						
7286 + 8081	23	19	83	10	0.1	0.5
6149 + 8081	35	22	63	10	0.1	0.72
6149 + 8082	26	16	62	10	0.1	0.333
7286 + 8385	29	15	52	10	0.1	0.667
7286 + 8564	36	19	53	10	0.1	0.9
Total	149	91	61	10	0.1	0.636
ISSR						
8081	15	4	27	10	0.2	1
8082	3	2	67	10	0.4	1

[a] Phenotypic frequency = number occurrences of phenotype ÷ total number of assays
[b] Similarity index = 1 − (unique bands + uniquely absent bands)/(total band positions for accessions compared)

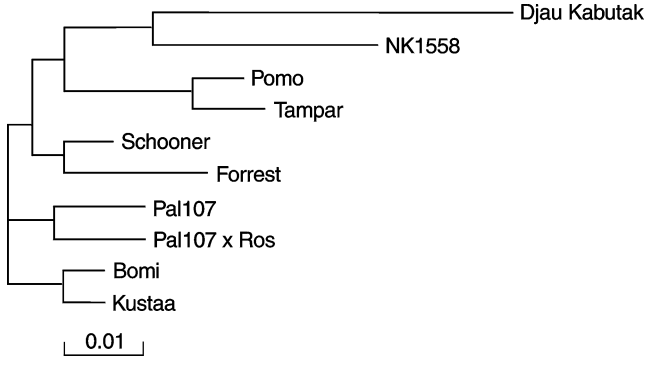

Fig. 4 Neighbor-joining phylogenetic model for barley varieties. Data pooled from REMAP markers generated with primers 8081, 8082, 8385, 8386, 8367, and 8564 with LTR primer 7086 were used for construction of the tree

landraces and so is likely to have common ancestry with 'Tampar' (personal communication, Reino Aikasalo, Boreal Plant Breeding, Jokionen, Finland). 'Bomi' and 'Kustaa' are two-rowed malting barleys from Denmark and southern Finland, respectively, and clustered together. 'Pallidum 107' is a two-rowed malting barley from the Ukraine. The neighbor-joining tree clustered 'Pallidum' with a line derived by crossing 'Pallidum 107' and 'Ros'.

Conclusions

We have presented two methods which exploit the repetitive, dispersed nature of many LTR-containing retrotransposon families for the visualization of genomic polymorphism. Several conceptually related methods have been reported. One (Purugganan and Wessler 1995) examines internal differences in the sequence or structure of retrotransposons themselves as diagnosed by restriction site polymorphism within the transposon. The S-SAP method (Waugh et al. 1997) is similar to AFLP but uses only one restriction site adapter-primer, the other being an outward-facing primer anchored in a *BARE* (or other) LTR. Variability in the band pattern is derived from variation in the distance between a restriction site and a *BARE*-1 element. The IRAP and REMAP methods, in contrast, require no restriction enzyme digestion but rely instead on the amplified polymorphism either between two LTRs nearby in the genome or between an LTR and a microsatellite.

The appearance of new bands would arise from the insertion of new *BARE*-1 copies into the genome within amplifiable distance of another *BARE*-1 LTR (IRAP) or a microsatellite (REMAP), or from an increase in the repeat number of a microsatellite to a point sufficient for amplification (REMAP). The fact that IRAP works at all, or produces so many polymorphic bands, may be somewhat surprising. Waugh and coworkers, in reporting the S-SAP method (Waugh et al. 1997), were unable to generate bands from a single, outward-facing *BARE*-1 LTR primer. Such products are indeed generated in IRAP from *BARE*-1 elements in head-to-head orientation. In situ hybridization studies (Suoniemi et al. 1996a) and the S-SAP method itself (Waugh et al. 1996) indicate that *BARE*-1 is dispersed throughout the genome, in some $3–7 \times 10^4$ copies per genome (Manninen and Schulman 1993; Suoniemi et al. 1996a). An equal dispersion of copies would yield an average of

48–124 kb between elements, making IRAP amplification impossible with current PCR methods.

The banding pattern which is obtained in IRAP, using outward-facing primers anchored either at the 5′ or 3′ end of the LTR (Fig. 1), lends strong support for the local clustering but large-scale dispersion of *BARE*-1 elements in the genome. Both the nested insertion of one *BARE*-1 element into another as well as a nearby insertion would generate an IRAP band. The shortest IRAP bands observed (approx. 200 bp) are close to or smaller than the minimum (approx. 300 bp) which would be generated from two complete, adjacent LTRs by the primers used. This is consistent with the recent demonstration of tightly clustered and nested retrotransposons in the maize genome (SanMiguel et al. 1996) and, conversely, of "islands" of genes in barley and other grasses (Barakat et al. 1997; Panstruga et al. 1998). However, no direct demonstration of clustered retrotransposons has heretofore been made for barley. The multitude of bands generated by REMAP may indicate either that *BARE*-1 tends to be inserted into regions of repetitive DNA, consistent with "gene islands," or that the genome contains many arrays of SSRs which are too short to generate microsatellites but which are available for amplification, or both.

Loss of REMAP or IRAP bands may result from dominant amplification of certain PCR products, point mutations at the 3′ end of the primer annealing sites, recombinational loss of the microsatellite or LTR, or decrease in the repeat number of the microsatellite below that sufficient for primer annealing. However, the microsatellite primers used in REMAP had a minimum repeat length of 7; earlier studies with barley (Becker and Heun 1995; Saghai Maroof et al. 1994) and humans (Weber and May 1989) showed that such short microsatellites show little variation in repeat number.

Although both IRAP and REMAP patterns proved to be too polymorphic to be applicable in phylogenetic studies between *Hordeum* species, these methods visualize sufficient polymorphism within cultivated barley to be useful for the generation of markers. The lack of similarity in either the IRAP or REMAP banding patterns between *Hordeum* species appears therefore more likely to reflect differential amplification and differing insertion sites for *BARE*-1 subsequent to the last common ancestor for these species. The wide variation in LTR and *BARE*-1 copy number within the same accessions (our unpublished data) supports this assertion. Within cultivated barley, the presence of a unique IRAP band (Fig. 3D, approx. 500 bp) in 'Nordic Pomo', 'Kustaa', and 'Tampar' may indicate the transposition of a new *BARE*-1 copy in a shared ancestor prior to the Norse settlement of the Faroe Islands 800–1050 A.D. The cv 'Bomi', although 'Nordic' as well, shows an IRAP pattern differing from these others.

The single greatest advantage of the retrotransposon-based marker systems IRAP and REMAP may be this ability to track an insertion event and its subsequent vertical radiation through a pedigree or phylogeny (Shimamura et al. 1997). Since the methods are PCR-based, they may be multiplexed through the use of fluorescent dyes and subjected to analysis on an automated sequencer. Hence, multiplexed REMAP may allow the detection and isolation of active, newly inserted retrotransposon copies by analysis of subsequent generations within an otherwise genetically homogeneous material. The methods are applicable to any plant with dispersed families of LTR-retrotransposons where either the LTR or nearby internal sequences are known. These, in turn, may be fairly easily established by PCR based on highly conserved domains such as the reverse transcriptase priming sites (Suoniemi et al. 1997).

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