Molecular Breeding (2006) 17: 173–184
DOI 10.1007/s11032-005-4885-4

Integration of retrotransposons-based markers in a linkage map of barley

Monica Rodriguez[1,*], Donal O'Sullivan[2], Paolo Donini[2], Roberto Papa[3], Elena Chiapparino[2], Fiona Leigh[2] and Giovanna Attene[1]

[1]*Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria, Università degli Studi di Sassrai, Via E. De Nicola, 07100 Sassari, Italy;* [2]*Molecular Research Group, NIAB, Huntingdon Road, Cambridge, CB3 0LE, UK;* [3]*Dipartimento di Scienze degli Alimenti, Università Politecnica delle Marche, Via Brecce Bianche, 60131 Ancona, Italy; *Author for correspondence (e-mail: mrodrig@uniss.it; phone +39 079-22-92-25, fax: +39 079-22-92-22)*

Received 28 January 2005; accepted in revised form 3 November 2005

Key words: Barley, Molecular mapping, Retrotransposons, S-SAP (Sequence-Specific Amplified Polymorphism) markers

Abstract

A deeper understanding of random markers is important if they are to be employed for a range of objectives. The sequence specific amplified polymorphism (S-SAP) technique is a powerful genetic analysis tool which exploits the high copy number of retrotransposon long terminal repeats (LTRs) in the plant genome. The distribution and inheritance of S-SAP bands in the barley genome was studied using the Steptoe × Morex (S × M) double haploid (DH) population. Six S-SAP primer combinations generated 98 polymorphic bands, and map positions were assigned to all but one band. Eight putative co-dominant loci were detected, representing 16 of the mapped markers. Thus at least 81 of the mapped S-SAP loci were dominant. The markers were distributed along all of the seven chromosomes and a tendency to cluster was observed. The distribution of S-SAP markers over the barley genome concurred with the knowledge of the high copy number of retrotransposons in plants. This experiment has demonstrated the potential for the S-SAP technique to be applied in a range of analyses such as genetic fingerprinting, marker assisted breeding, biodiversity assessment and phylogenetic analyses.

Introduction

Barley (*Hordeum vulgare* L.) is a cereal crop that has been extensively studied all over the world. Its self-compatability, chromosome number ($2n = 2x = 14$) and the development of double haploid lines have facilitated the construction of genetic maps (Graner et al. 1991; Kleinhofs et al. 1993; Liu et al. 1996; Qi et al. 1996; Waugh et al. 1997; Ramsay et al. 2000; Li et al. 2003).

The first conventional maps of barley, obtained with isozyme and morphological markers, were enriched with molecular markers such as RFLPs (Graner et al. 1991; Heun et al. 1991; Kleinhofs et al. 1993; Sherman et al. 1995). Those maps were efficiently improved with PCR-based markers such as SSRs (Ramsay et al. 2000; Li et al. 2003) and AFLPs (Becker et al. 1995; Powell et al. 1997) which have been shown to be efficient tools for creating high-density maps, and being less time consuming than hybridization-based methods (Mohan et al. 1997; Powell et al. 1997).

Beside these markers a new AFLP-like technique, that produces Sequence-Specific Amplification Polymorphisms (S-SAPs) anchored to specific sequence motifs, has been introduced (Waugh

et al. 1997). This method, which exploits the widespread distribution of retrotransposons in the genome, has been demonstrated to be highly useful for molecular mapping (Waugh et al. 1997), for marker assisted selection (Mohan et al. 1997), and for a better understanding of gene function and gene regulation in plants (Kumar and Hirochika 2001). The advantage in using the S-SAP method relies on the high levels of polymorphism detected with these markers (Waugh et al. 1997; Ellis et al. 1998; Yu and Wise 2000; Leigh et al. 2003; Porceddu et al. 2002). This relates to several features of retrotransposons that play a major role in plant gene and genome evolution (Gribbon et al. 1999; Kumar and Hirochika 2001).

Retrotransposons are mobile genetic elements that are able to insert at different positions in the genome through the reverse transcription of an RNA intermediate. They can be divided in to LTR- containing and non-LTR-containing classes (Boeke and Corces 1989; Hull and Will 1989). Furthermore, LTR retrotransposons can be subclassified into Ty1-*copia* and Ty3-*gypsy* groups (Kumar and Bennetzen 1999).

In plants retrotransposons 'display a number of specific features that single them out from retroelements of other kingdoms' (Grandbastien 1992). Their copy number is quite variable and often very high. In addition, their replication and integration mechanism leads these elements to be ubiquitously present all over the chromosomes (commonly in the euchromatic regions) and in many cases, to account for more than 50% of the nuclear genome (Grandbastien 1992; San Miguel et al. 1996; Kumar and Bennetzen 1999).

In barley the most studied family is the *BARE*-1, a *copia*-like retrotransposon (Manninen and Schulman 1993; Suoniemi et al. 1997; Jääskeläinen et al. 1999). S-SAP markers derived from LTRs of this family have been already mapped in barley (Waugh et al. 1997). Shirasu et al. (2000) have also demonstrated the presence in the barley genome of *gypsy*-like retrotransposons (e.g. BAGY-1, BAGY-2 and Sabrina families), hybrid retroelements families called Sukkula, Nikita and Stowaway elements. Sukkula has since been shown to be a Large Retrotransposon Derivatives (LARD) (Kalendar et al. 2004). Leigh et al. (2003) have shown that the properties of all these families can be easily exploited via S-SAP technique.

Here we report the use of the S-SAP method in barley, and the integration of the markers derived from 4 retroelement families (*BARE*-1, Sukkula, Nikita and BAGY-2) into a previously published map. The aim of this study was to develop a set of genetically characterized markers that may be used for basic and applied studies as genetic diversity analyses and marker assisted selection.

Materials and methods

Plant materials

A population of 150 doubled haploid (DH) lines, derived from the 'Steptoe'×'Morex' (S×M) cross (Kleinhofs et al. 1993) through the *Hordeum bulbosum* method (Chen and Hayes 1989), was chosen to construct the map. The seed of the parents and of the DH lines used by the North American Barley Genome Mapping Project (NABGMP) was kindly provided by P. Hayes (Oregon State University, Corvallis, Ore). The DH lines and the parents were grown in a greenhouse and the DNA was extracted from fresh leaf tissue using the DNeasy 96 Plant Kit (Qiagen, Crawley, UK).

S-SAP analyses

The S-SAP method as described by Waugh et al. (1997), and modified by Leigh et al. (2003), was used. Nine primer combinations were used to screen the parents. The sequences of the LTR-primers (Table 1) were derived from the barley Sukkula, Nikita, BAGY-2 retroelements described by Shirasu et al. (2000) and the *BARE*-1 retroelement (Manninen and Schulman 1993). Of those, six primer combinations were selected which provided the best amplification patterns (band intensity vs. background intensity, distribution and abundance of bands on the gel). Primer combinations employing primers from different retrotransposons were chosen, rather than changing the selective bases of *Mse*I primer, in order to gain information about all of the retroelements available.

The polymorphic S-SAP fragments were coded for mapping according to the retrotransposon and *Mse*I primer name, and to their molecular weight (Table 2).

Table 1. Primers used in S-SAP analysis.

Retrotransposon-based primers	Position in LTR[a]	Orientation[b]	Sequence[c]
BARE-1-5980	5′ terminus + A	Reverse	CTAGGGCATAATTCCAACAA
Sukkula-E0228	5′ 6	Reverse	GGAACGTCGGCATCGGGCTG
Sukkula-9900	3′ 1	Forward	GATAGGGTCGCATCTTGGGCGTGAC
BAGY-2-C0589	5′ 35	Forward	TTCGACACTCTTACTTATCGAAAGG
Nikita-E2647	5′ 80	Forward	ACCCCTCTAGGCGACATCC
Mse I primers			
Basic *Mse* I primer	n.a.	–	GATGAGTCCTGAGTAA-
Selective 3′ bases	n.a.	–	-CAG, -CTA, -CTG, -CTT

[a]Position is given relative to the 5′ or 3′ end of the LTR as the number of nucleotides internal to the LTR terminus.
[b]Forward primers match the sense or 'upper' strand with respect to transcription; reverse primers match the antisense strand.
[c]All sequences are given in the 5′ to 3′ direction.

Table 2. Primer combinations used in S-SAP analyses and their respective map codes.

Retrotransposon-derived primer (code)	*Mse*I primer (code)	Molecular weight (nucleotides) (code)	Map code
Nikita-E2647 (Nik)	CTT (M62)	130(a)–500(k)[a]	NikM62a (k)[a]
Sukkula-E0228 (SukE)	CAG (M49)	220(a)–420(k)	SukEM49a (k)
BAGY-2-C0589 (BAGY2C)	CTG (M61)	112(a)–561(w)	BAGY2CM61a (w)
Sukkula-9900 (Suk9)	CAG (M49)	200(a)–634(m)	Suk9M49a (m)
Sukkula-E0228 (SukE)	CTA (M59)	110(a)–435(o)	SukEM59a (o)
BARE-1-5980 (BARE1–5)	CTA (M59)	74(a)–344(q)	BARE1-5M59a (q)

[a]The small letter identifies polymorphic bands starting at the smallest (a).

Linkage map analysis

To locate the S-SAP markers on the linkage map, 223 RFLP markers already mapped on S × M (Kleinhofs et al. 1993; GrainGenes database, http://wheat.pw.usda.gov/) were used as anchors. Furthermore, the RFLP map and the S × M bin map downloaded from the NABGP website (http://barleygenomics.wsu.edu/arnis/linkage_maps/maps-svg.html) were used to infer chromosome designation, polarity and centromeric position.

First the χ^2 test was performed to detect the deviation from the 1:1 ratio as expected under the null-hypothesis of no segregation distortion. The combined RFLP and S-SAP segregation data were then analysed using Joinmap v. 2.0 (Stam and van Ooijen 1995).

Linkage groups were established as suggested by Stam (1993), to prevent incorrect assignment of markers. A LOD score of 5.0 was used for chromosomes 1(7H), 4(4H), 5(7H), and 7(5H). For chromosome 6(6H) the LOD score was lowered to 3.0 in order to retain the telomeric RFLP marker MWG798A on the map; no other differences were observed compared to the linkage group obtained at LOD 5.0. A LOD score of 9.0 was eventually chosen for chromosomes 2(2H) and 3(3H) since at lower values, a grouping of the RFLP markers from the two chromosomes was observed. Map distances were calculated using the Kosambi function (Kosambi 1944) and only maps produced by the third cycle of mapping were chosen. A final mapping process was done using the 'fixed order' option to avoid minor changes in RFLP order, such as the inversion of two marker pairs. The maps produced by this last analysis were chosen, and drawn using the MapChart computer package (Voorrips 2002).

Mapped markers were then used to identify possible linkages with QTLs for agronomic and malting quality traits, already mapped in the S × M population (Hayes et al. 1993).

Distances between S-SAP markers and QTLs were calculated relatively to the QTL peak positions, identified according to sCIM (simplified Composite Interval Mapping) values available on GrainGenes website (http://wheat.pw.usda.gov/ggpages/S×M/, verified 26 October 2005). Distances from QTLs located in the interval between two markers were excluded for simplicity.

Associations between S-SAP markers and phenotypic trait means were calculated using one-way ANOVA (Table 3). Only markers ≤3.4 cM

Table 3. Association between QTLs (yield, height, diastatic power, malt extract, lodging, grain protein, α-amylase) mapped on S×M population and flanking S-SAP markers (distance ≤ 3.4 cM).

Chr.	Trait	QTL peak	Closest S-SAPs	S-SAP-QTL distance (cM)	One-way ANOVA	
					p	R^2
1	Yield	ABC167A	BARE1-5M59i	3.1	n.s.	
	Height	MWG911	BAGY2CM61n	2.6	n.s.	
			Suk9M49b	1.8	0.013	0.045
	Diastatic power	KFP194	SukEM59g	1.0	0.012	0.047
			BAGY2CM61o	3.0	< 0.001	0.118
	Malt extract	ABG476	BAGY2CM61q	3.4	n.s.	
			NikM62h	2.4	n.s.	
			BAGY2CM61b	0.9	< 0.001	0.133
2	Lodging	MWG557	NikM62a	3.4	0.007	0.053
			BARE1-5M59c	2.7	0.001	0.077
			SukEM49h	0.0	n.s.	
			BAGY2CM61l	2.6	< 0.001	0.280
	Grain protein	MWG557	NikM62a	3.4	< 0.001	0.166
			BARE1-5M59c	2.7	< 0.001	0.142
			SukEM49h	0.0	n.s.	
			BAGY2CM61l	2.6	n.s.	
3	Yield	ABG399	BAGY2CM61j	2.0	n.s.	
			BARE1-5M59f	0.7	< 0.001	0.304
			BAGY2CM61k	1.2	< 0.001	0.259
	Lodging	ABG399	BAGY2CM61j	2.0	n.s.	
			BARE1-5M59f	0.7	< 0.001	0.295
			BAGY2CM61k	1.2	< 0.001	0.230
	Height	BCD828	BARE1-5M59f	2.5	< 0.001	0.183
			BAGY2CM61k	0.6	< 0.001	0.157
	Grain protein	ABG399	BAGY2CM61j	2.0	0.009	0.048
			BARE1-5M59f	0.7	n.s.	
			BAGY2CM61k	1.2	n.s.	
	α-amylase	Glb4	BAGY2CM61f	1.8	n.s.	
6	Diastatic power	MWG820	Suk9M49a	1.5	n.s.	
7	Grain protein	Ubi2	BAGY2CM61s	0.8	n.s.	
			BAGY2CM61v	2.7	n.s.	
	Diastatic power	Ubi2	BAGY2CM61s	0.8	n.s.	
			BAGY2CM61v	2.7	n.s.	

One-way ANOVA was performed to test associations between phenotypic traits and S-SAP markers. The level of significance of the association (p) and the proportion of the total variance explained (R^2) are also indicated. n.s. = not significant.

distant from QTL peaks were used to test the association. Phenotypic trait means used for the analysis were obtained by the average of each available environmental mean of the 150 S×M lines, reported on the GrainGenes website.

Results

S-SAP polymorphism

The polymorphic S-SAP bands were, in general, clear and easy to score as shown on Figure 1.

In total, six primers combinations were screened against the 150 DH lines. As shown in Table 4, the number of visible bands screened for each primer combination ranged from 33 (SukEM49) to 78 (BAGY2CM61) with a mean of 53.3, while the number of polymorphic bands ranged from 12 (NikM62, SukEM49) to 27 (BAGY2CM61), with a mean of 16.3. No significant correlation (Pearson: $r = 0.69$, $n = 6$, $p = 0.13$) between total number of bands and the number of polymorphic bands detected by each primer combination, was found.

The resulting level of polymorphism calculated for the primer combinations was variable, ranging

from 19.0% (NikM62) to 37.0% (*BARE1*-5M59). When six primer combinations were considered, 98 out of 320 bands were polymorphic (30.6%, Table 4), 53 of which originated from the parent Steptoe and 45 from Morex.

Marker segregation and mapping

Sequence specific amplified polymorphism markers are recorded as dominant markers (Waugh et al. 1997). However, among our segregating products, 8 band pairs (16 polymorphic bands) were found to co-segregate as alleles of co-dominant loci. These pairs of co-segregating bands were always observed within the profile generated by a single primer combination and their molecular size differed by a few base pairs (1–3 bases). Each of the 8 band pairs were considered to result from the amplification of two alleles of a single locus (indicated with a 'C' in the Figure 2). Thus out of 98 polymorphic markers 82 were dominant loci and 16 were considered to represent 8 putative co-dominant loci (Table 4). Only 90 polymorphic markers, out of the 98 originally scored, were therefore retained for mapping.

Segregation analysis was performed on the S-SAP markers (Table 5). Twenty-three (23.5%) out of 98 showed a significant deviation ($p \leq 0.05$) from the 1:1 expected ratio under the null-hypothesis of no segregation distortion. Only 9 (9.2%) were significantly distorted with a p value ≤ 0.01. In total, 16 alleles skewed towards the Steptoe parent and 7 towards Morex. The overall frequency over the DH population of the Steptoe and Morex alleles was 48.1 and 44.2%, respectively; the remaining 7.7% were missing data (Table 5).

Combined segregation data for the 90 polymorphic S-SAP loci were added to the 223 RFLP markers to construct the linkage map (Figure 2). No co-mapping S-SAP markers were found, indicating that no putative co-dominant loci were present between primer combinations. As a result, 89 loci (81 dominant and 8 putative co-dominant) were unambiguously mapped on the seven barley chromosomes. Of those 16 were derived from *BARE*-1, a retrotransposon family whose properties were already exploited for mapping in barley (Waugh et al. 1997). The remaining 73 were derived from three retrotransposons families never used before for linkage mapping analysis (Nikita, BAGY2 and Sukkula).

Marker distribution

A total of 312 loci (223 RFLP + 89 S-SAP) were placed on the seven chromosomes with a resulting map length of 1138 cM and an average distance between markers of 3.6 cM.

The number of S-SAP loci mapped on each chromosome varied from 7 (chromosome 4(4H)) to 18 (chromosome 2(2H)) with a mean of 12.7

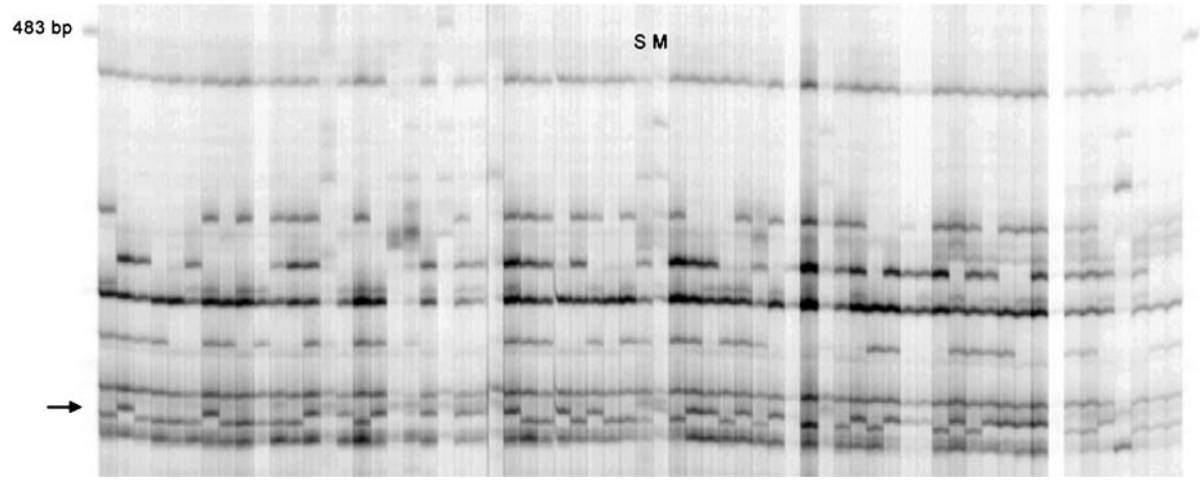

Figure 1. Example of S-SAP bands produced by the SukEM59 primer combination in the S×M population and the parental lines. The arrow indicates one of the hypothetic co-dominant bands.

Table 4. Descriptive statistics of S-SAP markers resulting from analysis of the S×M population.

Primer combination	Scored markers	Polymorphic markers (%)	Dominant loci	Putative co-dominant loci[a]	Putative polymorphic loci (%)	Un-mapped loci
NikM62	63	12 (19.0)	10	1	11 (17.5)	0
SukEM49	33	12 (36.4)	10	1	11 (33.3)	0
BAGY2CM61	78	27 (34.6)	19	4	23 (29.5)	0
Suk9M49	52	13 (25.0)	13	0	13 (25.0)	0
SukEM59	48	17 (35.4)	13	2	15 (31.3)	0
BARE1-5M59	46	17 (37.0)	17	0	17 (37.0)	1
Mean	53.3	16.3 (31.2)	13.7	1.3	15.0 (28.9)	0.2
Total	320	98 (30.6)	82	8	90 (28.1)	1

[a]Only one of the co-segregating bands was considered to calculate descriptive statistics for mapping.

Table 5. Marker distortion and allele frequency in the S×M mapping population.

Primer combination	Distorted markers (%)		Steptoe/Morex alleles (%) and (missing data %)	
	($p \leq 0.05$)	($p \leq 0.01$)		
NikM62	4 (36.4)	2 (18.1)	50.4/42.1	(7.4)
SukEM49	1 (9.1)	1 (9.1)	45.8/47.1	(7.2)
BAGY2CM61	7 (30.4)	1 (4.3)	49.4/44.0	(6.6)
Suk9M49	3 (23.1)	1 (7.7)	46.4/45.5	(8.2)
SukEM59	3 (20.0)	0 (0.0)	47.1/43.4	(9.6)
BARE1-5M59	5 (29.4)	4 (23.5)	48.4/44.1	(7.5)
Total	23 (23.5)	9 (9.2)	48.1/44.2	(7.7)

markers per chromosome (Table 6). The number of chromosomes covered by each primer combination varied from 6 (NikM62, BAGY2CM61 and BARE1-5M59) to 7 (SukEM49, Suk9M49 and SukEM59) (Table 6). No significant correlation was found between the chromosome length (cM) and the total number of loci (Pearson: $r = 0.45$, $n = 7$, $p = 0.31$). The S-SAP markers were interspersed among the RFLPs markers, as for example on chromosomes 2(2H), 5(1H) and 7(5H) (Figure 2). Notwithstanding S-SAP marker clusters were present on chromosomes 1(7H), 2(2H), 3(3H), 4(4H) and 7(5H). It is however noteworthy, that some of these clusters cover some of the gaps presents on the RFLP map as in chromosome 1(7H) between ABC156D and MWG911, on chromosome 3(3H) between ABG471 and ABG399 and on chromosome 4(4H) between bBE54A and BCD453B. Moreover, the big gap between WG364 and MWG514B on chromosome 7(5H), was reduced by the marker SukEM49f. As a result the S-SAP markers covered all the seven barley chromosomes (Figure 2).

Among the 89 S-SAPs mapped, the skewed markers showed a non-random distribution since they were mainly positioned near other skewed markers as shown by Kleinhofs et al. (1993). This is particularly clear on chromosome 2(2H), 5(1H) and 7(5H) (Figure 2).

Linkage between QTLs and S-SAP markers

Markers closely linked to QTLs on a high-density map could be powerful tools for marker-assisted selection. For this reason the possible association between mapped S-SAP markers and QTLs was investigated. Twenty S-SAP markers were located around different QTLs neighbouring area (distance ≤ 3.4 cM; Table 3). Since some QTL peaks were common to different traits, some of the S-SAP markers were adjacent to more than one QTL (e.g. yield, lodging and grain protein on chromosome 3; Table 3). In total 10 S-SAP markers were found to be significantly associated ($p \leq 0.01$) with phenotypic trait means (Table 3).

Table 6. Distribution of S-SAP loci (and markers) over barley genome.

Primer combination	Chromosomes							No. of loci (markers)	No. of covered chromosomes
	1(7H)	2(2H)	3(3H)	4(4H)	5(1H)	6(6H)	7(5H)		
NikM62	1	3	2	2	0	2 (2)	1	11 (12)	6
SukEM49	1 (2)[a]	2	2	1	1	3	1	11 (12)	7
BAGY2CM61	5 (6)	3 (4)	5 (6)	0	1	3 (4)	6	23 (27)	6
Suk9M49	1	3	2	1	2	2	2	13 (13)	7
SukEM59	3	3 (4)	1	2	3	2	1 (2)	15 (17)	7
BARE1-5M59	2	4	5	1	2	0	2	16 (16)	6
No. of loci (markers)	13 (15)	18 (20)	17 (18)	7 (7)	9 (9)	12 (14)	13 (14)	89 (97)	

[a]Numbers between brackets indicate the total number of mapped markers.

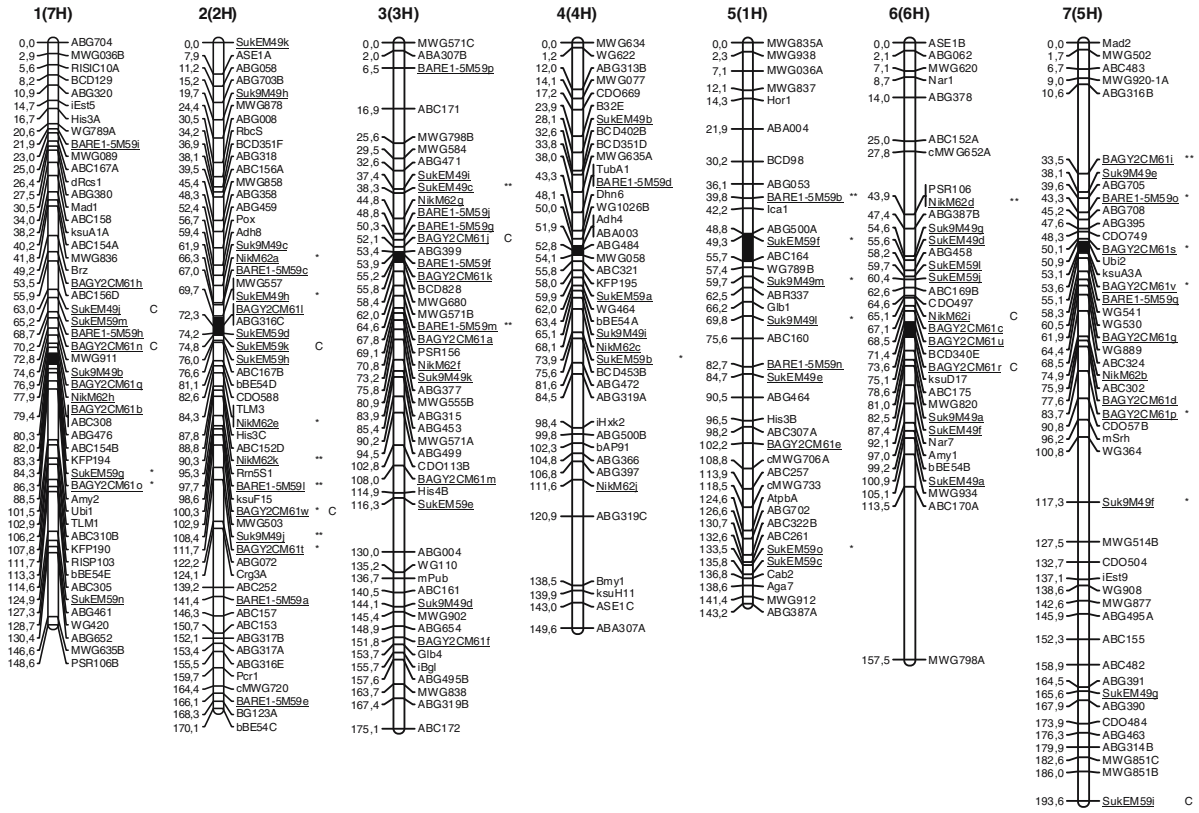

Figure 2. Genetic linkage map including S-SAP markers and RFLP markers derived from the barley S×M DH population. The underlined loci are the S-SAP markers. The skewed loci are indicated with one ($p \leq 0.05$) or two ($p \leq 0.01$) asterisks. Hypothetic co-dominant loci are indicated by the C letter after the marker name. Regions in black show the inferred centromeric positions.

Multiscore

Band scoring in complex fingerprints, such as S-SAP profiles, may not be straightforward depending on the exact amplification conditions, electrophoresis method and detection system as well as the presence of similar sized bands in a given background. For this reason, we wished to present a method by which each polymorphism is recognized by a unique multiindividual score (hereafter called the multiscore), not shared with any other band generated from the same primer combination. To this end, we have identified 8 DHLs presenting (not including the parents) a different multiscore for each S-SAP locus within each primer combination. Considering all the 90

loci (89 mapped and 1 un-mapped) we identified 61 unique multiscores, 9 shared by two and 3 shared by 3 fragments from different primer combinations. Including the parents, the average number of presence of the fragments in each multiscore was 5.13, ranging from 2 (2 loci) to 8 (9 loci). Thus, each multiscore was characterized by the presence of the fragment in at least two genotypes (see Appendix 1). The whole dataset can be obtained from supplemental web information (GrainGenes website: http://wheat.pw.usda.gov/).

Discussion

S-SAP polymorphism

In barley, the presence of *BARE*-1 together with other retrotransposon families can determine important changes to the genome structure (Hirochika et al. 1996; Kumar and Bennetzen 1999; Kalendar et al. 2000; Shirasu et al. 2000).

The presence of retrotransposons and their activities has been efficiently highlighted in the S×M population by the relatively high polymorphism (30.6% of polymorphic bands and 28.1% of polymorphic loci; Table 4), detected using the S-SAP technique. The mean polymorphism value observed in our research was similar to the one obtained by Leigh et al. (2003), using the same primer combinations, on three different barley varieties. Similar studies by other authors, conducted on the retrotransposons distribution on barley and wheat genomes, are in line with our results (Gribbon et al. 1999; Kalendar et al. 2000).

Moreover, the average polymorphic markers obtained in this study (30.6%) with six primer combinations was slightly higher than that obtained by Waugh et al. (1997) on the Blenheim × E224/3 barley population (26%), using 10 primer combinations derived exclusively from the *BARE*-1 retrotransposon family. However, if we consider LTR sequences from different retrotransposon families than *BARE*-1, the level of polymorphic bands was the 29.6%.

Marker segregation and mapping

The segregation analysis of the S-SAP polymorphic markers showed 9.2% of distorted markers for a p value ≤ 0.01 (Table 5). Distortion of loci was already described by RFLPs in barley (10% by Heun et al. 1991) and rice (22 and 32% by Xu et al. 1997), and with AFLP markers in barley (6% by Becker et al. 1995) and tomato (18% by Saliba-Colombani et al. 2000). Kleinhofs et al. (1993) also observed segregation distortion of RFLP markers on the same S×M population, showing the percentage of 3% with $p \leq 0.01$ and 14% with $p \leq 0.05$.

The polymorphic S-SAP bands obtained in this experiment were scored as dominant markers. However, 16.3% of the polymorphic markers were assumed to be co-dominant. We have not found any previous reports of co-dominant S-SAP loci (Waugh et al. 1997; Ellis et al. 1998; Yu and Wise 2000; Porceddu et al. 2002) though the presence of co-dominant markers was already showed for other dominant markers as AFLP and SRAP. Putative co-dominant markers were actually found by Maheswaran et al. (1997), Alonso-Blanco et al. (1998) and Saliba-Colombani et al. (2000) who have described in rice, arabidopsis and tomato, respectively the 21.2, 33.4 and 12.1% of putative co-dominant AFLP markers. Alonso-Blanco et al. (1998) assumed co-segregating and co-mapping bands to be the same locus, while Saliba-Colombani et al. (2000) have only re-coded co-segregating bands found in the same primer combination as being co-dominant. Li and Quiros (2001) using SRAP markers on *Brassica* have found that the 20% of the polymorphic markers were co-dominant and they have confirmed it by sequencing.

When genetic relationship between individuals and populations is studied using dominant random markers such as S-SAP, all the fragments are usually assumed to represent different loci. In such a context the occurrence of co-segregating markers violate this assumption (Bonnin et al. 1996). On the other hand, for different types of studies a high level of co-dominant markers may be useful (e.g. developing STS for MAS). For this reason, it is of particular importance to estimate the level of co-dominance occurring among assumed dominant markers. Here we show that S-SAP present a similar level of putative co-dominant markers as found for analogous methods such as AFLP.

Marker distribution

The addition of the 89 S-SAP loci into the RFLP map resulted in minor variations in the original map length (from 1214 to 1138 cM). Furthermore, no relevant changes were observed between the S-SAP-RFLP map and the S × M bin map taken from the NABGP website (1156 cM; data not shown).

Sequence specific amplified polymorphism markers were present on both arms of each chromosome and they were distributed along the RFLP framework (average distance between S-SAPs of 13.8 cM). However, clusters of S-SAP markers were observed, as shown in five of the seven chromosomes (Figure 2). Clustering of S-SAP markers may be related to the high copy number of the retroelements on barley genome and the nested insertion of some of the retrotransposons one into each other (Vicient et al. 1999, 2000; Shirasu et al. 2000; Schulman et al. 2004). However, the degree of clustering was much lower than that described by Manninen et al. (2000), who used primers from the same retroelements for REMAP and IRAP whilst mapping a net blotch resistance gene in barley.

Linkage between QTLs and S-SAP markers

The analysis of linkage between S-SAP markers and phenotypic traits has shown that a tight association exists between QTLs and 20 mapped markers. These QTLs flanking markers could be immediately available for marker assisted selection in barley breeding and further genetic studies, such as genetic diversity studies. Actually, MAS for QTLs has the potential to make traditional breeding strategies for crop improvement more efficient, and reduce the time needed for developing improved populations or cultivars (Romagosa et al. 1999; Igartua et al. 2000; Ayoub et al. 2003; Hori et al. 2003). S-SAP markers seem particularly useful to reach these goals because of the high number of polymorphic bands per assay detected, and the distribution of mapped markers on all of the barley chromosomes.

Moreover, those markers may be useful at evaluating the genetic diversity of different populations. Actually, when some of the polymorphic bands present in a segregating mapping population may be reproducibly scored in a diverse set of materials, the marker polymorphisms in different barley populations may be attributed to specific genetic map positions (e.g. landraces or varieties; Kraakman et al. 2004).

However, their dominant and multilocus characteristics may limit their use because different fragments number can be visualized by diverse protocols and systems for electrophoresis, making it difficult to distinguish between fragments of similar molecular weight. Therefore, knowledge of the molecular weight of each mapped fragment could be useful information for other labs, facilitating the identification of a given marker, but will not assure reproducibility. Hence, here we have developed a method to unambiguously identify each fragment, combining the information on their molecular weight and scoring. To this end, multiscores were identified for each polymorphism, which were unique within each primer combination in the same small subset (8 DHLs and the parents) of the mapping population. The same approach may also be used for other molecular markers types, such as AFLP or ISSR. Results of our analysis have shown that S-SAP markers are highly polymorphic and distributed over all the barley genome and that they are a reliable and useful method for a range of genetic analyses (Kumar and Hirochica 2001; Queen et al. 2004).

Moreover, their genetic characterization can offer useful information to allow the selection of the appropriate type of marker for various purposes, such as LD studies (Kraakman et al. 2004). Indeed, the careful choice of markers among the different types available may provide a highly polymorphic assay with a wide genome coverage yet requiring lowereffort.

Acknowledgements

The authors gratefully acknowledge Dr Domenico Rau for helpful discussion. M. Rodriguez was supported from the EC by a Marie-Curie Training Site Fellowship during her stay at NIAB Institute (Cambridge, UK).

Appendix

Appendix 1. Example of the scoring performed on 8 of the 150 S × M DHLs for the combinations Suk9M49 and BARE1-5M59a.

Marker code	M.W. (bp)	S × M line number										
		SM12	SM22	SM32	SM69	SM71	SM116	SM130	SM167	S	M	Multiscore
Suk9M49a	200	1	1	0	1	1	1	0	1	1	0	1101110110
Suk9M49b	238	1	1	1	1	1	1	0	0	1	0	1111110010
Suk9M49c	270	0	1	1	1	1	0	1	0	0	1	0111101001
Suk9M49d	310	1	0	1	0	0	0	0	0	1	0	1010000010
Suk9M49e	324	0	0	0	1	0	1	0	1	0	1	0001010101
Suk9M49f	482	1	1	1	1	0	0	1	0	0	1	1111001001
Suk9M49g	485	1	1	1	0	0	0	1	1	0	1	1110001101
Suk9M49h	500	0	1	0	1	1	0	1	1	1	0	0101101110
Suk9M49i	550	0	0	0	1	0	0	0	1	0	1	0001000101
Suk9M49j	572	0	1	1	1	1	0	0	1	0	1	0111100101
Suk9M49k	594	0	0	1	0	1	1	0	1	1	0	0010110110
Suk9M49l	624	0	1	0	1	0	1	0	1	1	0	0101010110
Suk9M49m	634	0	0	0	1	0	1	0	1	1	0	0001010110
BARE1-5M59a	74	1	1	1	1	1	0	0	1	0	1	1111100101
BARE1-5M59b	78	1	0	0	1	0	0	0	1	1	0	1001000110
BARE1-5M59c	90	1	0	1	0	1	1	0	1	1	0	1010110110
BARE1-5M59d	106	1	0	1	0	1	1	1	1	1	0	1010111110
BARE1-5M59e	114	1	1	1	1	1	0	1	1	0	1	1111101101
BARE1-5M59f	136	0	1	1	0	0	0	1	0	0	1	0110001001
BARE1-5M59g	150	1	0	0	1	1	1	1	1	1	0	1001111110
BARE1-5M59h	158	1	1	0	1	1	0	0	0	1	0	1101100010
BARE1-5M59i	167	0	0	0	0	0	1	1	1	0	1	0000011101
BARE1-5M59j	170	0	1	1	0	0	0	0	0	0	1	0110000001
BARE1-5M59k	173	0	0	1	0	0	0	0	0	0	1	0010000001
BARE1-5M59l	175	0	1	0	1	0	0	0	1	0	1	0101000101
BARE1-5M59m	218	0	0	0	0	1	1	0	1	1	0	0000110110
BARE1-5M59n	234	1	1	1	0	1	0	1	0	1	0	1110101010
BARE1-5M59o	250	1	1	1	1	1	0	1	1	1	0	1111101110
BARE1-5M59p	338	0	0	0	0	1	0	1	0	0	1	0000101001
BARE1-5M59q	344	1	1	1	0	1	0	1	1	1	0	1110101110

Marker codes and molecular weights (M.W.) are listed for the 90 polymorphic markers.

References

Alonso-Blanco C., Peeters A.J.M., Koornneef M., Lister C., Dean C., van den Bosch N., Pot J. and Kuiper M.T.R. 1998. Development of an AFLP based linkage map of L*er*, Col and Cvi *Arabidopsis thaliana* ecotypes and construction of a L*er*/Cvi recombinant line population. Plant J. 14: 259–271.

Ayoub M., Armstrong E., Bridger G., Fortin M.G. and Mather D.E. 2003. Marker-based selection in barley for a QTL region affecting α-amylase activity of malt. Crop Sci. 43: 556–561.

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

Boeke J.D. and Corces V.G. 1989. Transcription and reverse transcription of retrotransposons. Annu. Rev. Microbiol. 43: 403–434.

Bonnin I., Prosperi J.M. and Olivieri I. 1996. Genetic markers and quantitative genetic variation in *Medicago truncatula* (Leguminaosae): a comparative analysis of population structure. Genetics 143: 1795–1805.

Chen F. and Hayes P.M. 1989. A comparison of *Hordeum bulbosum*-mediated haploid production efficiency in barley using *in vitro* floret and tiller culture. Theor. Appl. Genet. 77: 701–704.

Ellis T.H.N., Poyser S.J., Knox M.R., Verschinin A.V. and Ambrose M.J. 1998. Polymorphism of insertions sites of *Ty1-copia* class retrotransposons and its use for linkage and diversity analysis in pea. Mol. Gen. Genet. 260: 9–19.

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

Graner A., Jahoor A., Schondelmaier J., Siedler H., Pillen K., Fishbeck G., Wenzel G. and Herrmann R.G. 1991. Construction of an RFLP map of barley. Theor. Appl. Genet. 83: 250–256.

Gribbon B.M., Pearce S.R., Kalendar R., Schulman A.H., Paulin L., Jack P., Kumar A. and Flavell A.J. 1999. Phylogeny and transpositional activity of *Ty*1-*copia* group retrotransposons in cereal genomes. Mol. Gen. Genet. 261: 883–891.

Hayes P.M., Liu B.H., Knapp S.J., Chen F., Jones B., Blake T., Franckowiak J., Rasmusson D., Sorrells M., Ullrich S.E., Wesenberg D. and Kleinhofs A. 1993. Quantitative trait locus effects and environmental interaction in a sample of North American barley germ plasm. Theor. Appl. Genet. 87: 392–401.

Heun M., Kennedy A.E., Anderson J.A., Lapitan N.L.V., Sorrells M.E. and Tanksley S.D. 1991. Construction of a restriction fragment lenght polymorphism map for barley (*Hordeum vulgare*). Genome 35: 1019–1025.

Hirochika H., Sugimoto K., Otsuki Y., Tsugawa H. and Kanda M. 1996. Retrotransposons of rice involved in mutations induced by tissue culture. Proc. Natl. Acad. Sci. USA 93: 7783–7788.

Hori K., Kobayashi T., Shimizu A., Sato K., Takeda K. and Kawasaki S. 2003. Efficient construction of high-density linkage map and its application to QTL analysis in barley. Theor. Appl. Genet. 107: 806–813.

Hull R. and Will H. 1989. Molecular biology of viral and nonviral retroelements. Trends Genet. 5: 357–359.

Igartua E., Edney M., Rossnagel B.G., Spaner D., Legge W.G., Scoles G.J., Eckstein P.E., Penner G.A., Tinker N.A., Briggs K.G., Falk D.E. and Mather D.E. 2000. Marker-based selection of QTL affecting grain and malt quality in two-row barley. Crop Sci. 40: 1426–1433.

Jääskeläinen J.M., Mykkänen A.H., Arna T., Vicient C., Suoniemi A., Kalendar R., Savilahti H. and Schulman A.H. 1999. Retrotransposon *BARE*-1: expression of encoded proteins and formation of virus-like particles in barley cells. Plant J. 20: 413–422.

Kalendar R., Tanskanen J., Immonen S., Nevo E. and Schulman A.H. 2000. Genome evolution of wild barley (*Hordeum spontaneum*) by *BARE*-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc. Natl. Acad. Sci. USA 97: 6603–6607.

Kalendar R., Vicient C.M., Pelag O., Anamthwat-Jonnson K., Bolshoy A. and Schulman A.H. 2004. Large retrotransposon derivitives: abundant, conserved but nonautonomous retroelements of barley and related genomes. Genetics 166: 1473–1450.

Kleinhofs A., Kilian A., Saghai-Maroof M.A., Biyashev R.M., Hayes P., Chen F.Q., Lapitan N., Fenwick A., Blake T.K., Kanazin V., Ananiev E., Dahleen L., Kudnra D., Bollinger J., Knapp S.J., Liu B., Sorrells M., Heun M., Franckowiak J.D., Hoffman D., Skadsen R. and Steffenson B.J. 1993. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86: 705–712.

Kosambi D.D 1944. The estimation of map distance from recombination values. Ann. Eugen. 12: 172–175.

Kraakman A.T.W., Niks R.E., Van den Berg P.M.M.M., Stam P. and Van Eeuwijk F.A. 2004. Linkage disequilibrium mapping of yield and yield stability in modern spring barley cultivars. Genetics 168: 435–446.

Kumar A. and Bennetzen J.L. 1999. Plant retrotransposons. Annu. Rev. Genet. 33: 479–532.

Kumar A. and Hirochika H. 2001. Applications of retrotransposons as genetic tools in plant biology. Trends Plant Sci. 6: 127–134.

Leigh F., Kalendar R., Lea V., Lee D., Donini P. and Schulman A.H. 2003. Comparison of the utility of barley retrotransposon families for genetic analysis by molecular marker techniques. Mol. Genet. Genomics 269: 464–474.

Li G. and Quiros C.F. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in *Brassica*. Theor. Appl. Genet. 103: 455–461.

Li J.Z., Sjakste T.G., Röder M.S. and Ganal M.W. 2003. Development and genetic mapping of 127 new microsatellite markers in barley. Theor. Appl. Genet. 107: 1021–1027.

Liu Z.W., Biyashev R.M. and Saghai Maroof M.A. 1996. Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor. Appl. Genet. 93: 869–876.

Maheswaran M., Subudhi P.K., Nandi S., Xu J.C., Parco A., Yang D.C. and Huang N. 1997. Polymorphism distribution and segregation of AFLP markers in a doubled haploid rice population. Theor. Appl. Genet. 94: 39–45.

Manninen I. and Schulman A.H. 1993. *BARE*-1, a copy-like retroelement in barley (*Hordeum vulgare* L.). Plant Mol. Biol. 22: 829–846.

Manninen O., Kalendar R., Robinson J. and Schulman A.H. 2000. Application of BARE-1 retrotransposon markers to the mapping of a major resistance gene for net blotch in barley. Mol. Gen. Genet. 264: 325–334.

Mohan M., Nair S., Bhagwat A., Krishna T.G., Yano M., Bhatia C.R. and Sasaki T. 1997. Genome mapping, molecular markers and marked-assisted selection in crop plants. Mol. Breed. 3: 87–103.

Porceddu A., Albertini E., Barcaccia G., Marconi G., Bertoli F.B. and Veronesi F. 2002. Development of S-SAP markers based on LTR-like sequence from *Medicago sativa* L. Mol. Genet. Genomics 267: 107–114.

Powell W., Thomas W.T.B., Baird E., Lawrence P., Booth A., Harrower B., McNicol J.W. and Waugh R. 1997. Analysis of quantitative traits in barley by the use of Amplified Fragment Length Polymorphisms. Heredity 79: 48–59.

Qi X., Stam P. and Lindhout P. 1996. Comparison and integration of four barley genetic maps. Genome 39: 379–394.

Queen R.A., Gribbon B.M., James C., Jack P. and Flavell A.J. 2004. Retrotransposon-based molecular markers for linkage and genetic diversity analysis in wheat. Mol. Gen. Genomics 271: 91–97.

Ramsay L., Macaulay M., degli Ivanissevich S., MacLean K., Cardle L., Fuller J., Edwards K.J., Tuvesson S., Morgante M., Massari A., Maestri E., Marmiroli N., Sjakste T., Ganal M., Powell W. and Waugh R. 2000. A simple sequence repeat-based linkage map of barley. Genetics 156: 1997–2005.

Romagosa I., Han F., Ullrich S.E., Hayes P.M. and Wesenberg D.M. 1999. Verification of yield QTL through realized molecular marker-assisted selection responses in a barley cross. Mol. Breed. 5: 143–152.

Saliba-Colombani V., Causse M., Gervais L. and Philouze J. 2000. Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. Genome 43: 29–40.

San Miguel P., Tikhonov A., Jin Y.K., Motchoulskaia N., Zakharov D., Melake-Berhan A., Springer P.S., Edwards K.J., Lee M., Avramova Z. and Bennetzen J.L. 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274: 765–768.

Sherman J.D., Fenwick A.L., Namuth D.M. and Lapitan N.L.V. 1995. A barley RFLP map: alignment of three barley maps and comparisons to Gramineae species. Theor. Appl. Genet. 91: 681–690.

Shirasu K., Schulman A.H., Lahaye T. and Schulze-Lefert P. 2000. A contiguous 66-kb barley DNA sequence provides evidence for reversible genome expansion. Genome Res. 10: 908–915.

Schulman A.H., Gupta P.K. and Varshney R.K. 2004. Organization of retrotransposons and microsatellites in cereal genomes. In: Gupta P.K. and Varshney R.K. (eds), Cereal Genomics. Kluwer Academic Press, Dordrecht, The Netherlands, 639 pp. ISBN 1-5882-9007-7, pp. 83–118.

Stam P. 1993. Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J. 3: 739–744.

Stam P. and Van Ooijen J.W. 1995. JoinMap Version 2.0: Software for the Calculation of Genetic Linkage Maps. CPRO-DLO, Wageningen.

Suoniemi A., Schmidt D. and Schulman A.H. 1997. BARE-1 insertion site preferences and evolutionary conservation of RNA and cDNA processing sites. Genetica 100: 219–230.

Vicient C.M., Suoniemi A., Anamthawat-Jónsson K., Tanskanen J., Beharav A., Nevo E. and Schulman A.H. 1999. Retrotransposon *BARE*-1 and its role in genome evolution in the genus *Hordeum*. Plant Cell 11: 1769–1784.

Vicient C.M., Kalendar R., Anamthawat-Jónsson K., Suoniemi A. and Schulman A.H. 2000. Structure, functionality, and evolution of the *BARE*-1 retrotransposon of barley. Genetica 107: 53–63.

Voorrips R.E. 2002. MapChart: software for the graphical presentation of linkage maps and QTLs. J. Hered. 93(1): 77–78.

Waugh R., McLean K., Flavell A.J., Pearce S.R., Kumar A., Thomas B.B.T. and Powell W. 1997. Genetic distribution of *BARE*-1-like retrotransposable elements in the barley genome revaled by sequence-specific amplification polymorphisms (S-SAP). Mol. Gen. Genet. 253: 687–694.

Xu Y., Zhu L., Xiao J., Huang N. and McCouch S.R. 1997. Chromosomal regions associated with segregation distortion of molecular markers in F2, backcross, doubled haploid, and recombinant inbred populations in rice (*Oryza sativa* L.). Mol. Gen. Genet. 253: 535–545.

Yu G.X. and Wise R.P. 2000. An anchored AFLP and retrotransposon-based map of diploid *Avena*. Genome 43: 736–749.