Theor Appl Genet (2000) 101:652–660

K. Pillen · A. Binder · B. Kreuzkam · L. Ramsay
R. Waugh · J. Förster · J. Léon

Mapping new EMBL-derived barley microsatellites and their use in differentiating German barley cultivars

Received: 6 December 1999 / Accepted: 23 February 2000

Abstract By searching the EMBL DNA sequence database, we were able to develop 39 new, database-derived barley microsatellites. Eighteen of these EMBL microsatellites were mapped either to the interspecific barley map Lerche×BGRC41936 (L×41), the Igri×Franka map (I×F, Graner et al. 1991), or to both maps simultaneously. In addition, all 39 EMBL microsatellites were assigned to individual barley chromosomes by PCR screening of wheat barley addition lines. Both studies verified a random distribution of the microsatellites within the barley genome. Subsequently, 22 EMBL microsatellites were used to assess the genetic similarity among a set of 28, mainly German, barley cultivars and two wild form accessions. Spring and winter cultivars could be easily differentiated using the first coordinate of a principal coordinate analysis. Whereas the group of spring barley cultivars appeared rather homogeneous, winter barley cultivars could be divided into three subgroups. Two *H. v.* ssp. *spontaneum* accessions were included in the assessment of genetic similarity. They were placed among the winter barley cultivars. Based on the assessment of the 30 barley cultivars and accessions, the polymorphism information content (PIC) of each EMBL microsatellite has been calculated. The average PIC value among the EMBL microsatellites was equal to 0.38, which ascertains the value of these microsatellites as a genetic tool in barley genome research projects.

Communicated by G. Wenzel

K. Pillen (✉) · A. Binder · B. Kreuzkam · J. Léon
Department of Crop Science and Plant Breeding,
University of Bonn, Katzenburgweg 5, D-53115 Bonn, Germany
e-mail: k.pillen@uni-bonn.de

L. Ramsay · R. Waugh
Scottish Crop Research Institute (SCRI), Invergowrie, Dundee,
DD2 5DA, Scotland, UK

J. Förster
Saaten-Union Resistenzlabor GmbH, Hovedisser Str. 92,
D-33818 Leopoldshöhe, Germany

Key words Barley · Microsatellites · Linkage map · Genetic similarity (GS) · Polymorphism information content (PIC)

Introduction

Microsatellites, also called simple sequence repeats (SSR), contain stretches of short, tandem-repeated mono-, di-, tri- or tetra-nucleotides and were initially described by Hamada et al. (1982). Soon after the introduction of polymerase chain reaction (PCR) technology, microsatellites were developed as a new class of DNA markers (Weber and May 1989). According to Powell et al. (1996), the advantages of microsatellites for application in molecular biology are their multi-allelic nature, codominant inheritance, ease of detection by PCR, relative abundance, extensive genome coverage and low requirement of sample DNA. Consequently, microsatellites have been applied to all areas, including the plant kingdom (Morgante and Olivieri 1993; Wang et al. 1994). Up to now, microsatellites have been found in almost all crop plants and wild species and have been mainly used for the construction of linkage maps, determination of genetic variation and marker-assisted breeding programs. Examples of microsatellite utilization include *Arabidopsis* (Bell and Ecker 1994; Innan et al. 1997), soybean (Akkaya et al. 1995), barley (Liu et al. 1996), potato (Provan et al. 1996), sorghum (Brown et al. 1996), maize (Taramino and Tingey 1996; Lübberstedt et al. 1998), tomato (Smulders et al. 1997), wheat (Röder et al. 1998; Stephenson et al. 1998), and rice (Cho et al. 1998). However, the relative labor-intensive and costly detection of microsatellites by means of sequencing repeat-containing genomic clones turns out to be a drawback for a broad application in molecular plant breeding. One approach to overcome this limitation is based on the selection of potential microsatellite repeats from the wealth of sequence information which is, by now, deposited in DNA sequence databases. Several investigators have demonstrated the feasibility of this strategy to detect and utilize microsatellites; for instance, Senior and Heun (1993) and

Chin et al. (1996) in maize, Becker and Heun (1995) in barley, Isagi and Suhandono (1997) in oak, Whitton et al. (1997) in sunflower, and Smulders et al. (1997) in tomato. When these reports are compared, it appears that the number of detectable microsatellites correlates with the number of available sequence entries in the database. Thus, a new or first microsatellite screen of the sequence databases appears promising whenever the number of entries has increased substantially. Taking into account that the current number of published microsatellites in barley is still limited to approximately 70 (Becker and Heun 1995; Liu et al. 1996; Russell et al. 1997a; Struss and Plieske 1998), we conclude that a further increase in this number through the addition of new database-derived microsatellites would be beneficial to the barley genetics community.

For a plant breeder, reliable knowledge of the genetic diversity of his breeding material is important in order to select parents for a new breeding cycle. Microsatellites are very useful in estimating genetic diversity due to their high degree of variability (Powell et al. 1996). So far, several studies on the estimation of genetic variation among accessions of a taxon or between cultivars by means of microsatellite mapping have been carried out; for example, by Smulders et al. (1997), Russell et al. (1997a), and Struss and Plieske (1998). In barley, these studies extend or refine older reports which were based on restriction fragment length polymorphism (RFLP) random amplified polymorphic DNA (RFLP), or amplified fragment length polymorphism (AFLP) markers (Zhang et al. 1993; Graner et al. 1994; Melchinger et al. 1994; Ordon et al. 1997; Schut et al. 1997; Russell et al. 1997b; Manninen et al. 1997; Casas et al. 1998; Demissie et al. 1998). However, a further characterization of the barley germplasm, for instance, the current German barley cultivar pool, would provide valuable information to barley breeders. In this paper, we report on (1) the selection of informative barley microsatellites derived from the EMBL database and (2) the application of these microsatellites, to the characterization of genetic variability among German winter and spring barley cultivars.

Materials and methods

Plant material

Two hundred and eighty-two F_2 individuals originating from the cross of the German spring barley cultivar Lerche and the wild form BGR41936 (*Hordeum vulgare* ssp. *spontaneum*) were used for linkage mapping. In addition, DNA from 71 double-haploid (DH) lines of the cross Igri×Franka (Graner et al. 1991) was kindly provided by Dr. A. Graner for reference mapping. For chromosomal localization, the wheat-barley addition lines 1HS and 2H through 7H were used (Islam et al. 1981; Islam 1983). For estimation of genetic similarities, 28 mainly German winter and spring barley cultivars were used. This set was supplemented by *Hordeum v. ssp. spontaneum* accessions ISR101-23 and ISR42-8 collected from Israel and kindly provided by Dr. G. Fischbeck. All plant DNA was extracted with CTAB from young leaves according to Saghai Maroof et al. (1984).

Selection of microsatellites and primer design

In order to identify potential barley microsatellites, 628 barley DNA sequences were downloaded from the EMBL database at the European Bioinformatics Institute (EBI) in August 1996. For this, the Sequence Retrieval System at *http://srs.ebi.ac.uk/* was used together with the search criterion *"Hordeum"*. After eliminating spaces and numbers from the raw text file, the remaining DNA sequences were filtered with a word processor using all possible mono-, di-, and trinucleotides as filter criteria. From this, unique genomic and cDNA sequences were selected which contain a repeat length of at least 8 nucleotides. Primers for 83 potential microsatellites were designed online using the software PRIMER 3.0 from S. Rozen and H.J. Skaletsky at the Whitehead Institute for Bio-medical Research (*http://www-genome.wi.mit.edu/cgi-bin/primer/primer3.cgi*). Primers flanking a repeat had an optimum length of 20 nucleotides (minimum 18 bp, maximum 27 bp), a GC-content of between 20% and 80% and a resulting PCR fragment size between 100 and 250 bp. The primers were synthesized by Amersham Pharmacia Biotech.

PCR amplification and separation of microsatellites

Microsatellites were checked for polymorphism using two PCR protocols. The low-stringent protocol (no. 14) included 35 cycles of, 1 min at 94°C, 1 min at 50°C, 2 min at 72°C, followed by a 5-min extension at 72°C. The high-stringent protocol (no. 20) included 10 cycles of 1 min at 94°C, 0.5 min starting at 64°C and decreasing 1°C per cycle, 1 min at 72°C, followed by 30 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, followed by a 5-min extension at 72°C. Each PCR reaction of 25 µl contained 50 m*M* KCl, 10 m*M* TRIS-HCl pH 9.0, 0.1% Triton X-100, 2.5 m*M* $MgCl_2$, 0.2 m*M* of each mono-nucleotide, 0.1 µ*M* of each primer, 0.25 U *Taq* polymerase (Promega) and approximately 100 ng of template DNA. The PCR amplifications were carried out in either the GeneAmp PCR system 9600 (Perkin Elmer Applied Biosystems) or the UNO II thermo cycler (Biometra).

PCR-amplified microsatellites were separated on manual sequencing gels containing 6% polyacrylamide (AA:BIS=19:1) and 7 *M* urea in 0.5×TBE buffer. For this, 2 µl PCR reaction plus 1 µl loading buffer (95% formamide, 10 m*M* NaOH, 0.05% (w/v) bromophenol blue, and 0.05% (w/v) xylene cyanol) were mixed, heated for 3 min at 94°C, and loaded on a pre-heated S2 sequencing apparatus (Life Technologies). The gel was subsequently run for approximately 2 h at 100 W.

After the run, the glass plate covering the gel was silver-stained according to the Promega manual in order to visualize the microsatellite bands. For this, the plate was fixed for 20 min in 10% acetic acid, rinsed three times in deionized water, stained for 30 min in 0.2% (w/v) silver nitrate, rinsed again for 10 s in deionized water, and developed for approximately 10 min until the bands became visible in 30 g/l sodium carbonate, 1.5 ml/l of 37% formaldehyde, and 2 mg/l thiosulfate. Finally, the developing process was arrested by rinsing the glass plate in 10% acetic acid.

The assessment of variability among barley cultivars/accessions was performed at the Saaten-Union Resistenzlabor GmbH on an automatic sequencer (ALF-Express II, Amersham-Pharmacia Biotech) according to the manufacturer.

Linkage mapping

Polymorphic microsatellites were genetically located using either the interspecific F_2 population Lerche×BGRC41936 (L×41) or the DH mapping population Igri×Franka (I×F, Graner et al. 1991). New EMBL-derived microsatellites were mapped in L×41 together with already published barley microsatellites. Linkage groups and map distances were calculated by means of the software package MAPMAKER (Lander et al. 1987). Linkage criteria were set to LOD 3.0 and a maximum distance of *theta*=0.40. The Kosambi function (Kosambi 1944) was applied to calculated cen-

tiMorgan (cM) distances. The I×F map locations of EMBL microsatellites were calculated with MAPMAKER relative to RFLP data from the I×F DH lines available from the Graingenes mirror website at *http://grain.jouy.inra.fr:80/ggpages/maps.html#barley.*

Statistical analysis

Each microsatellite fragment was scored as present (1) or absent (0) for each member of the set of 30 barley cultivars and accessions. Genetic similarity (GS) between each pair of barley cultivars and accessions was calculated according to Nei and Li (1979) with

$$GS = \frac{2N_{xy}}{N_x + N_y}$$

where N_{xy} is the number of shared fragments between two genotypes X and Y, and N_x and N_y are the total number of fragments present in lines X and Y, respectively.

Graphical representations of the estimated genetic similarities between cultivars and accessions were obtained by principle coordinate analysis (PCoA) from NTSYSPC 2.0 (Exeter Software, East Setauket, N.Y.) based on the above calculated similarity matrix as described by Gower (1966). The consecutive commands *Dcenter* using the GS matrix as input, *Eigen*, and *2Dplot* were used to generate the two-dimensional PCoA plot.

In order to measure the informativeness of a DNA marker, the polymorphic information content (PIC) for each microsatellite was calculated according to Weber (1990) as:

$$PIC = 1 - \left(\sum_{i=1}^{n} p_i^2\right) - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

where n is the total number of fragments (bands) detected for a microsatellite and p_i and p_j are the frequencies of the ith and jth fragment in the set of 30 barley cultivars and accessions investigated.

Results and discussion

Selection of database-derived microsatellites

Altogether 83 potential microsatellites were identified among the 628 barley DNA sequences that were downloaded from the EMBL DNA sequence database in August 1996. Based on a polymorphism survey between 3 spring barley cultivars and 3 wild barley accessions, 39 informative EMBL microsatellites (see Table 1) were selected for this study. The primer sequences can be requested from the authors. The set of EMBL microsatellites represents DNA sequences obtained from 21 cDNA clones and 18 genomic clones. Out of 43 repeats present among the selected microsatellites, 42% contained mono-, 26% di-, and 33% tri-nucleotide repeats, respectively. The number of T/A mono-nucleotide repeats clearly exceeded the number of G/C mono-nucleotide repeats (13 versus 5), which is in accordance with Becker and Heun (1995). However, the prevalence of mononucleotides is in contrast to the result of Becker and Heun (1995) who predominantly found useful di-nucleotide repeats (52%) in their survey of database-derived barley microsatellites. No tetra-nucleotide repeats could be detected in out study. The average repeat length within the set of EMBL microsatellites was 13 bp. Thus, these EMBL-derived microsatellites are shorter than previously published barley ones derived from sequence databases (17 bp, Becker and Heun 1995) or genomic libraries (19 bp, Liu et al. 1996; 31 bp, Russell et al. 1997a; 28 bp, Struss and Plieske 1998). This finding might reflect the reduced selection criteria of 8 bp for potential repeats in this study compared to an exclusion limit of 10 bp by Becker and Heun (1995).

Linkage maps for database-derived microsatellites

Initially, the interspecific F_2 population Lerche×BGRC41936 was used to identify polymorphisms among the 39 EMBL microsatellites (listed in Table 1) and the 100 additional barley microsatellites that are published or submitted for publication (Becker and Heun 1995; Liu et al. 1996; Russell et al. 1997a; Struss and Plieske 1998; Ramsay et al. submitted). In total, 60 microsatellites (43%) from different sources could be classified as polymorphic (Table 2). Fifteen EMBL microsatellites (39%) revealed a polymorphism between Lerche and BGRC41936. This rate of polymorphism is within the range of the published barley microsatellites which were also tested in the L×41 population (Table 2). Only the genomic microsatellites from Struss and Plieske (1998) exhibited, with 60%, a considerably higher rate of polymorphism. The L×41 map currently comprises 51 microsatellites that are distributed over 840.1 cM (Fig. 1). The coverage of the L×41 map is still incomplete compared to, for instance, the Igri×Franka or the Steptoe×Morex maps which both cover approximately 1200 cM (current status, see graingenes mirror at: *http://grain.jopuy.inra.fr:80/ggpages/maps.html#barley*). This finding is also reflected by the presence of 9 so far unliked markers in our study. However, our primary goal was to locate as many EMBL microsatellites as possible on our own mapping population in order to use the microsatellite data for subsequent quantitative trait locus (QTL) studies. The average interval length of the L×41 map corresponds to 21.0 cM, which is comparable high. This is due to the relatively low linkage exclusion limit of *theta*=0.40 that we had selected in order to link even distant EMBL microsatellites. For instance, due to the lax linkage exclusion limit, we could map *HvLTPPB*, *HdAMYB*, and *HvSS1* to the ends of chromosomes 3H, 4H and 7H, respectively (Fig. 1). In order to prevent false linkages being declared in our study, we verified the chromosomal location of all markers by means of wheat barley addition line mapping (see below). Altogether, the L×41 map includes 22 newly located microsatellites. These are 14 EMBL-derived microsatellites (Table 1) and 8 previously unmapped microsatellites from Struss and Plieske (1998). No clustering of EMBL microsatellites has been observed. On the contrary, *HvA22S* and *HvACL3* on chromosome 7H exhibited the closest linkage between two EMBL microsatellites with a distance of 7.6 cM. These data support the assumption that our procedure for selection of database-derived microsatellites inlcudes no bias and, thus, resulted in an evenly distributed sample of mapped genes. The genom-

Table 1 List of 39 new EMBL-derived microsatellites and data on PCR, chromosomal location, and polymorphism information content (PIC)[a]

Locus =EMBL ID	EMBL gene description	Origin	Repeat	PCR[c]	Size[d] (bp)	Lx41[e]	I×F[f]	Chromosomal assign.[g]	PIC[h]
HdAMYB	Gene for beta-amylase	g	(tg)10+(g)16)	14	219	4H		4H+5H	
Hv13GEIII	1,3-Glucan endohydrolyase III	g	(at)5	20	175			3H	0.35
Hv22951	Gamma-thionin (HTH3)	c	(gc)6	20	174			3H	0.43
Hv26916	Protoporphyrin IX Mg-chelatase precurs	g	(cct)8	14	105	2H		2H, 4H+5H	
Hv49505	Profilin (Hvpro1) mRNA	c	(a)8	14	152			3H	0.47
Hv52867	High-affinity sulfate transporter HvST1	c	(gt)4	20	235	4H		4H	0.33
HvA22S	Abscisic acid and stress inducible (A22) gene	g	(t)10	20	228	7H	7H	7H	0.49
HvABAIP	ABA-inducible protein	c	(acc)5	20	144			1HL	0.33
HvACL3	Acyl carrier protein III (Acl3) gene	g	(t)10	20	204	7H	7H	7H	0.36
HvALAAT	mRNA for alanine aminotransferase	c	(c)12+(tc)9	20	183	1H	1H	1HS	0.45
HvAQ	(clone ABG20) STS	c	(ttc)4	14	206			6H	
HvARHGN	*AR-h* gene for aldose reductase	g	(t)10	20	155			1HL	
HvBAMY	5´-Upsteam region of beta-amylase	g	(a)17	14	132			4H	0.57
HvBKIN12G	*BKIN12* gene for protein kinase	g	(t)8	20	205			3H	
HvBTAI3	Alpha-amylase inhibitor	c	(gcc)4	14	234			4H	0.35
HvC1	(clone ABC154) STS	c	(a)12	14	161	?		6H	0.30
HvCH126A	Chitinase (CHI26) gene	g	(ac)9	14	239			7H	0.17
HvCW21	Non-specific lipid transfer protein	c	(ag)6	20	153			3H	0.27
HvES1A	GA3-regulated cDNA from *H. v.* leaves	c	(atc)6	20	191	3H		3H	0.20
HvGLB2	1,3-1,4-Beta-D glucan 4-glucanohydrolase	g	(gt)5+(g)12	20	210		7H	7H	
HvGLUEND	(1,3;1,4)-Beta-glucanase	g	(t)11	20	262			1HL	
HvHEMH1	mRNA for ferrochelatase	c	(t)8	14	194	5H	5H	5H	0.44
HvITR1	*Itr1* gene trypsin inhibitor	g	(g)12	20	242			3H	
HvJASIP	Jasmonate-induced protein	c	(acg)4	14	197		4H	4H	0.40
HvKNOX3	Knox3 gene	g	(t)14	14	190			4H+7H	0.35
HvLOXC	Lipoxygenase 2 (LoxC)	c	(tg)8	14	182	5H		5H	0.19
HvLTPPB	Lipid transfer protein precursor	c	(ac)10	14	216	3H		3H	
HvMUB1	Ubiquitin (*mub1*) gene	g	(t)14	14	128			1HL	
HvMYB1G	Genomic-binding protein	g	(ttc)4	14	176			5H	
HvPAF93	*paf93* gene	c	(agg)4	20	216			6H	
HvPAZXG	*pazx* gene-encoding protein zx	g	(c)12+(a)17	20	177	4H		4H	0.78
HvPDIA	Disulfide isomerase (PDI)	c	(ggc)4	20	189			4H	0.55
HvPEPD1PR	Peptidase of D1 protein	c	(ag)7	14	189		3H	3H	
HvSS1	*Ss1* gene for sucrose synthase	g	(t)20	14	240	7H	7H	7H	
HvUDPGPP	UDP-glucose pyrophosphylase	c	(gcc)4	14	129		5H	4H+5H	
HvX	(clone ABC306) STS	c	(agg)4	14	220			2H, 4H+6H	
HvXYLISOG	Xylose isomerase gene	g	(tcc)4	14	170	2H		2H	0.14
S53707	Aleurone cell gene	c	(acg)4	14	250	1HS		1HS	0.38
TaCMD	Seed protein CMd	c	(gcc)4	14	209			4H	

[a] The primer sequences can be requested from the authors
[b] Origin of sequence: c, cDNA; g, genomic
[c] PCR profiles: 20=stringent; 14=less stringent (see Materials and methods)
[d] Expected fragment size in basepairs calculated from EBML sequence
[e] Map location derived from L×41 ? indicates no linkage detected as yet
[f] Map location derived from I×F
[g] Chromosomal assignment derived from wheat-barley addition lines
[h] The PIC as a measure of informativeness has been calculated for 22 microsatellites from a survey of 30 barley cultivars and accessions (see Fig. 3)

Table 2 Polymorphism rate between Lerche and BGRC41936 calculated from different sources of barley microsatellites

Source of microsatellites	Number of microsatellites tested	Number of polymorphic microsatellites	Polymorphism rate (%)
Becker and Heun (1995)	13	6	46.2
Liu et al. (1996)	38	15	39.5
Russell et al. (1997a)	7	3	42.8
Struss and Plieske (1998)	15	9	60.0
Ramsay et al. (submitted)	27	12	44.4
EMBL microsatellites (this publication)	39	15	38.5
Total	139	60	43.2

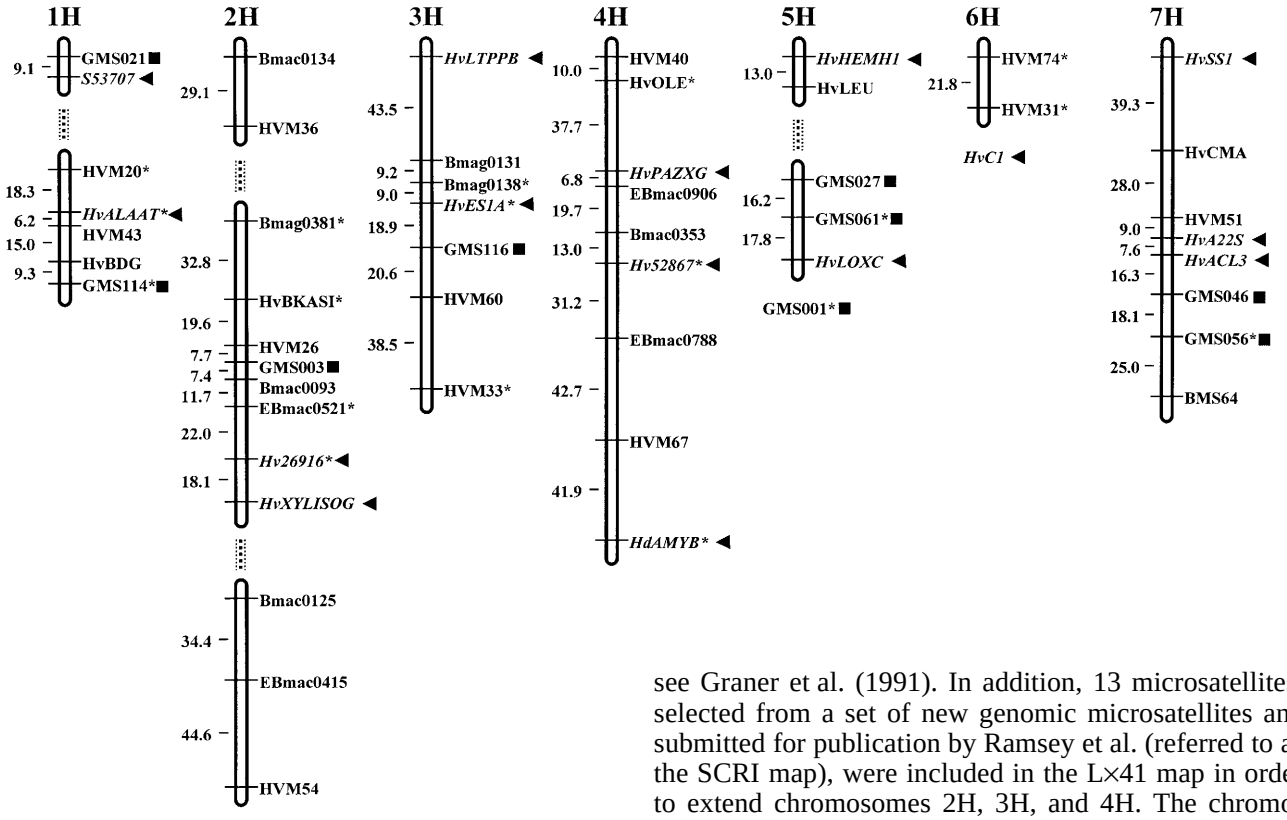

Fig. 1 First genetic location of 14 database-derived EMBL microsatellites (this study) and 8 genomic microsatellites from Struss and Plieske (1998) together with already published microsatellites on the interspecific L×41 map. EMBL micorsatellites are in *italics* and marked with *arrowheads*. Genomic microsatellites from Struss and Plieske (1998) are indicated by *boxes.* Microsatellites exhibiting distorted segregation at α=5% are denoted with an *asterisk.* Map distances (in cM) are printed to the *left* and chromosome numbers at the *top* of each chromosome. *Dotted lines* indicate gaps within the chromosome. GMS001 and *HvC1* could not be located on the L×41 map due to missing linkage to other markers. However, both microsatellites were assigned to chromosomes 5H and 6H, respectively, by means of the wheat-barley addition line assay

ic microsatellites from Struss and Plieske (1998) turned out to be very useful for our mapping purpose. They revealed the highest rate of polymorphism among all sources of barley microsatellites and, like the EMBL microsatellites, exhibited a random distribution over all chromosomes without any clustering. In order to further extend the L×41 map and to compare this map with already established maps, we considered the addition of published microsatellites as a framework to be beneficial. For this, polymorphic microsatellites from Becker and Heun (1995) and Liu et al. (1996) were included (Fig. 1). Linkages between these markers could be confirmed in the L×41 map, although the distances generally increased compared to the original mapping data which are based on intra-specific crosses. A likely explanation for this observation is the inter-specific nature of the L×41 cross. The inflating of map distances from intra- to inter-specific maps is an often-described phenomenon; see Graner et al. (1991). In addition, 13 microsatellites, selected from a set of new genomic microsatellites and submitted for publication by Ramsey et al. (referred to as the SCRI map), were included in the L×41 map in order to extend chromosomes 2H, 3H, and 4H. The chromosomal location and the detection of linkages among these microsatellites were consistent between both maps. Likewise, the linear order of microsatellites were in most cases in accordance. Only two inversions on chromosomes 2H (EBmac0415-HVM54) and 3H (HVM60-HVM33) were detected relative to the SCRI map. Interestingly, the distance between common microsatellites was, on average, approximately two times larger in the L×41 map than in the SCRI map (28.9 versus 14.4 cM per marker pair). For instance, the distance between Bmag0134 and HVM36 on chromosome 2H increased from 12 cM in the SCRI map to 29.1 cM in the L×41 map. Both maps are based on inter-specific crosses but differ with respect to the number of individuals (282 vs. 86) and generations investigated (F_2 vs. DH). A likely explanation for the increased average distance in the L×41 map is the existence of hot spots of recombination in one or both parents of the cross, a phenomenon which is reported in all kingdoms (Shenkar et al. 1991; Xu et al. 1995; Henry and Damerval 1997). Likewise, Nilsson and Säll (1995) found the overall recombination frequency as well as the chromosomal distribution of recombination events to be genetically controlled when they compared marker distances in four different barley crosses. Altogether 35% of all microsatellites of the L×41 map exhibited distorted segregation at α=5%. These markers were more or less evenly distributed (Fig. 1). Homozygous Lerche genotypes prevailed at 6 loci, whereas homozygous genotypes of BGRC41936 dominated at 3 loci. At 10 loci, heterozygous genotypes significantly exceeded the sum of both homozygous classes.

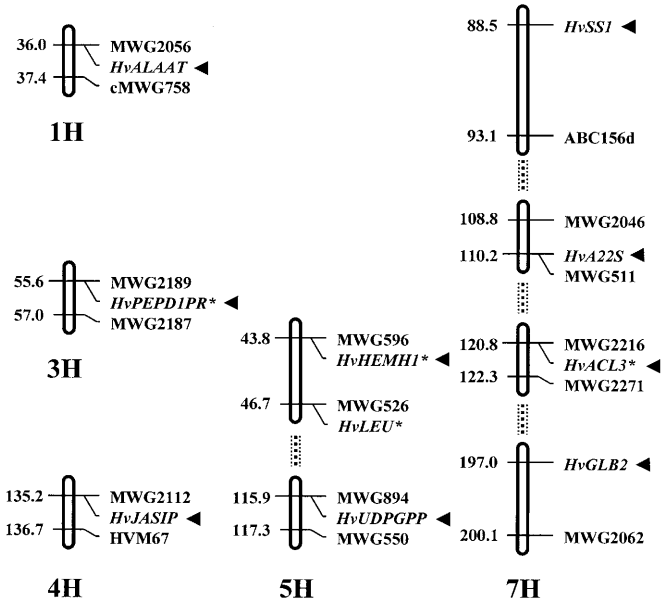

Fig. 2 Genetic location of 9 new database-derived EMBL microsatellites and *HvLEU* (Becker and Heun 1995) on the I×F map (Graner et al. 1991). EMBL microsatellites are in *italics* and marked with *arrowheads.* Microsatellites exhibiting distorted segregation at α=5% are denoted with an *asterisk.* Map positions (in cM) are printed to the *left*, nearest flanking and cosegregating markers to the *right*, and chromosome numbers at the *bottom* of each chromosome. *Dotted lines* indicate gaps within the chromosome

In order to further increase the number of mapped EMBL microsatellites, we detected 9 polymorphisms between barley cultivars Igri and Franka (23%). The relatively low polymorphism rate for EMBL microsatellites between Igri and Franka compared to the interspecific cross Lerche×BGRC41936 (38%) resembles the original findings of Graner et al. (1991). The authors reported only 26% polymorphism for RFLP between Igri and Franka but 76% polymorphism within the interspecific cross Vada×1B-87. All 9 polymorphic EMBL microsatellites were subsequently placed on the I×F map relative to a wealth of RFLP markers (Graner et al. 1991; Fig. 2 and Table 1). In all cases except for *HvSS1* and *HvGLB2* on chromosome 7H, the EMBL microsatellite cosegregated with at least one RFLP marker. Five EMBL microsatellites and *HvLEU* (Becker and Heun 1995) were mapped both in L×41 and I×F (Table 1). In all cases, the chromosomal locations were consistent between both maps. Linkage between common microsatellites was also verified. For instance, *HvHEMH1* and *HvLEU* on chromosome 5H were linked in both maps. The absolute distance was, however, larger in the L×41 map than in the I×F map (13.0 vs. 2.9 cM). This observation is in accordance with Graner et al. (1991) who found the same tendency for RFLP markers. In most cases the I×F map showed reduced interval sizes compared to the interspecific Vada×1B-87 map. Among 9 EMBL microsatellites mapped in I×F, 33% showed distorted segregation at α=5%. In all cases the Igri allele prevailed over the Franka allele (Fig. 2). The percentage of distorted segregation and the tendency of the Igri alleles to prevail are in accordance with Graner et al. (1991). These authors explained the latter observation by a selective advantage of Igri alleles because the parent Igri responded better to in vitro culture during the production of the DH population.

In summary, 18 new EMBL microsatellites (46%) were placed on either the L×41 map or the I×F map, or on both maps simultaneously. In addition, 8 previously unmapped barley micorsatelites from Struss and Plieske (1998) were included in the L×41 map. These microsatellites will be beneficial as a further increase of the number of useful markers available for applied and basic genome research in barley.

Chromosomal assignment of EMBL microsatellites

EMBL microsatellites were assigned to individual barley chromosomes by means of a PCR assay with a set of wheat barley addition lines (Islam et al. 1983 and Islam 1983). The set included six disomic addition lines for barley chromosomes 2H to 7H plus the ditelosomic addition line 1HS. The presence of the microsatellite fragment of the barley parent Betzes in one of the addition lines indicates its location on the respective barley chromosome. Applying this strategy, all 39 EMBL micro-satellites were assigned to individual chromosomes (Table 1). Four EMBL microsatellites revealed no signal in any addition line. Subsequently, these markers were assigned to the long arm of chromosome 1H since 1HL is the only segment not present in the set of addition lines. Whereas the majority of microsatellites could be exclusively assigned to one barley chromosome, 4 microsatellites exist in two or three copies. Interestingly, *HdAMYB*, *Hv26916*, and *HvUDPGPP* have a common characteristic in that two duplications are located on chromosomes 4H and 5H. In addition, *Hv26916* and *HvX* share duplications on chromosomes 4H and 2H. Whether these common duplications occurred independently during evolution or in tandem as a single duplicated segment cannot be determined yet since not all of the loci involved have been mapped so far. The number of assigned EMBL microsatellites ranges from 3 for chromosomes 2H to 10 for chromosome 4H, indicating that no hidden bias occurred during the selection of microsatellites from the EMBL database. Out of 39 database-derived microsatellites, *HvAQ*, *HvC1*, and *HvX* were previously mapped as RFLP probes (Table 1). The location of the respective RFLP probes ABG20, ABC154, and ABC306 on chromosomes 6H, 6H, and 2H coincided with the corresponding microsatellites. These examples demonstrate that RFLP probes containing short repeated sequences can be successfully transformed into PCR-based microsatellites.

Characterization of the genetic diversity among German barley cultivars

In order to demonstrate the usefulness of the EMBL micorsatellites, we assessed the genetic similarity among

25 mainly German winter and spring barley cultivars as well as the North American winter barley cultivars Pennco, Steptoe and Morex and two *H. v.* ssp. *spontaneum* accessions. For this study, a set of 22 EMBL microsatellites were used (listed in the PIC column of Table 1). At least 1 microsatellite originated from each barley chromosome although, due to availability, the majority of selected markers were located on chromosomes 3H and 4H (5 and 7 microsatellites, respectively). Altogether, the microsatellites produced 70 polymorphic bands (3.2 bands per microsatellite). As a measure of informativeness, the polymorphism information content (PIC value) was calculated for each microsatellite (Weber 1990). The PIC values for all 22 EMBL microsatellites ranged from 0.14 to 0.78, with an average value of 0.38. *HvPAYXG* revealed the highest PIC value (0.78), which coincide with the highest number of polymorphic bands (7) that were scored at this locus. The average PIC value of the EMBL microsatellites is in accordance with Bohn et al. (1999) who compared the usefulness of RFLPs, AFLPs and microsatellites for differentiating 11 winter wheat cultivars. All three marker types produced average PIC values between 0.30 and 0.33. Russell et al. (1997a) applied barley microsatellites to discriminate between 24 modern spring and winter barley cultivars. They found an average PIC value of 0.50 for the 11 microsatellites they used, a value slightly higher than the one we found. A clearly higher average PIC value was reported by Struss and Plieske (1998). The latter found an average gene diversity of 0.74 when they screened barley wild forms and landraces with 15 genomic microsatellites. Even among old barley cultivars, the authors found a mean gene diversity of 0.72. This finding might, at least partly, be explained by the fact that the wild forms, land races and old barley cultivars studied, which originated from completely different parts of the world, would be expected to be much more heterogeneous than the elite pool of, for instance, modern German barley cultivars and, thus, should result in elevated PIC values. However, the observation that the same microsatellites from Struss and Plieske (1998) also revealed at 60%, the highest rate of polymorphism in our L×41 mapping effort (Table 2), makes this set of 15 microsatellites a very useful tool for studies on genetic diversity in barley.

Based on the set of 22 EMBL microsatellites, genetic similarity estimates were calculated according to Nei and Li (1979) between each pair of 30 barley genotypes. The mean genetic similarity within the complete set was equal to 51.8%. Minimum and maximum genetic similarities were found for Krimhild/Scarlett and Krimhild/Maresi (13.3% each) and Alexis/Escada (100%), respectively. Spring cultivars exhibited a higher mean genetic similarity (67.7%) than winter forms (56.1%). As expected, the mean genetic similarity between winter and spring cultivars was lower, at 44.7%.

Based on the genetic similarity assay, all barley cultivars and accessions could be distinguished except for the 2 spring cultivars Alexis and Escada. The genetic conformity between Alexis and Escada in our study is only partly reflected by pedigree data since Alexis is a grandparent of Escada. Only the inclusion of additional microsatellites resulted in a separation of both cultivars, although they remained very closely related (data unpublished). The discordance between pedigree data and genetic similarity values based on marker data is a frequently described phenomenon (e.g. Graner et al. 1994; Casas et al. 1997; Schut et al. 1997; Manninen et al. 1997). A very low correlation in barley between these conflicting parameters have been reported by Graner et al. (1994). The authors found rank correlations between genetic similarity and coancestry of only 0.21 and 0.42 among winter and spring barley cultivars, respectively. Besides the risk of wrong pedigree information, they explained the finding with a selective bias in offspring populations due to breeder's selection and genetic drift. In order to quantify and illustrate the relatedness between the 30 barley genotypes, we applied a principle coordinate analysis (PCoA, Gower 1966) to the genetic similarity data. A two-dimensional plot of the PCoA is presented in Fig. 3. The first two principal coordinates accounted for 23.3% and 13.4% of the total variation, respectively. The first coordinate clearly differentiated winter (I. and II. quadrant) and spring barley forms (III and IV quadrants). The same distinction was found in a dendrogram which was based on the calculated genetic similarity values and the UPGMA clustering procedure (data not shown). This result is in accordance with Melchinger et al. (1994) and Russell et al. (1994) and Russell et al. (1997a) who could also separate winter and spring types of barley by means of the first principle co-

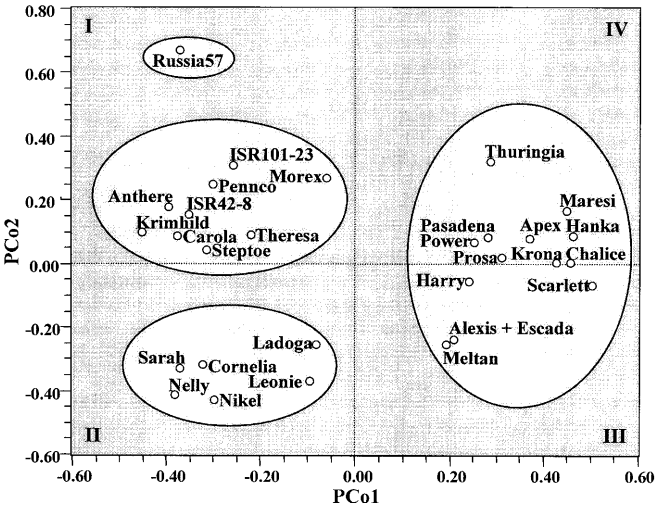

Fig. 3 Two-dimensional principal coordinate analysis (PCoA) plot of 28 barley cultivars and 2 *H. v.* ssp. *spontaneum* accessions based on genetic similarity (GS) values. The GS values and the PCoA plot were deduced from 22 database-derived barley microsatellites (listed in Table 1) and calculated with the software package *NTSYSpc* (Exeter Software, East Setauket, N.Y.). The first principle coordinate differentiates winter barley cultivars and wild-form accessions (*left side*) from spring barley cultivars (*right side*)

ordinate. To achieve this, the former authors scored 136 RFLP bands among 48 European barley cultivars, whereas the latter group applied 48 microsatellite bands to 24 barley cultivars. Among the spring barleys, only Thuringia to one side and the trio Alexis, Escada, and Meltan deviated to some extent from the center. Within the set of winter barleys and wild forms three subgroups could be distinguished. The first subgroup contained only the cultivar Russia 57 (top of I quadrant). The second subgroup comprised the majority of the winter barley cultivars including the North American winter forms Pennco, Steptoe, and Morex and the two wild forms ISR101-23 and ISR42-8 (I quadrant). In this subgroup, Morex was placed closest to the spring barley group. Morex is used as a malting barley and its placement in the PCoA plot might reflect some common ancestors between Morex and the bulk of the spring malting barleys. Interestingly, the two *H v.* ssp. *spontaneum* accessions ISR101-23 and ISR42-8 grouped with the winter barley cultivars. This result confirms the results of Dávila et al. (1998) who also found that *H. v.* ssp. *spontaneum* showed the smallest genetic distance with six-rowed winter barley cultivars. However, in their study the wild-form accessions could be clearly separated from all of the cultivated barley forms. The third subgroup included 6 winter barley cultivars (II quadrant). This subgroup was dominated by cultivars of the NORDSAAT breeder. In total, winter barleys were more widely spread out than spring forms (Fig. 3), this is in contrast to the results of Melchinger et al. (1994) who found that the spring cultivars were more widely dispersed. One reason for this difference might be the inclusion of geographically more distant genotypes like Russia 57 or Steptoe and Morex in our study of winter barley. However, the occurrence of a third subgroup in the II quadrant might also indicate that breeders have introduced a new and more distant genetic background in modern winter barley cultivars which did not yet exist when Melchinger et al. (1994) made their survey. A broader study of genetic relationships including more cultivars and a larger set of microsatellites would be helpful in shedding more light on the genetic basis of our past and present barley gene pool. The EMBL microsatellite introduced in this study will be a helpful tool towards this objective as well as for other genome research applications.

Acknowledgments The authors with to thank Dr. C. Knaak for helpful suggestions and valuable assistance on the assessment of genetic diversity and Hedda von Quistorp for excellent technical assistance. We are indebted to Drs. Dambroth, FAL-Braunschweig, and G. Fischbeck, Technical University, München, for providing the wild barley accessions BGRC41936, and ISR101-23 and ISR42-8, respectively. We thank Drs. A. Graner for DNA samples and mapping data of the Igri×Franka DH population and R. Islam for providing the wheat-barley addition lines. This study was supported by the Deutsche Forschungsgemeinschaft (DFG grant nos. Le813/5-2 and Pi339/1-1).

References

Akkaya MS, Shoemaker RC, Specht JE, Bhagwat AA, Cregan PB (1995) Integration of simple sequence DNA markers into a soybean linkage map. Crop Sci 35:1439–1445

Becker J, Heun M (1995) Barley microsatellites: allele variation and mapping. Plant Mol Biol 27:835–845

Bell CJ, Ecker JR (1994) Assigment of 30 microsatellite loci to the linkage map of *Arabidopsis*. Genomics 19:137–144

Bohn M, Utz HF, Melchinger AE (1999) Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs and SSRs and their use for predicting progeny variance. Crop Sci 39:228–237

Brown SM, Hopkins MS, Mitchell SE, Senior ML, Wang TY, Duncan RR, Gonzalez Candelas F, Kresovich S (1996) Multiple methods for the identification of polymorphic simple sequence repeats (SSRs) in sorghum (*Sorghum bicolor* (L.) Moench). Theor Appl Genet 93:190–198

Casas AM, Igartua E, Valles MP, Molina-Cano JL (1998) Genetic diversity of barley cultivars grown in Spain, estimated by RFLP, similarity and coancestry coefficients. Plant Breed 117:429–435

Chin ECL, Senior ML, Shu H, Smith JSC (1996) Maize simple repetitive DNA sequences: abundance and allele variation. Genome 39:866–873

Cho YG, McCouch SR, Kuiper M, Kang MR, Pot J, Groenen JTM, Eun MY (1998) Integrated map of AFLP, SSLP and RFLP markers using a recombinant inbred population of rice (*Oryza sativa* L.). Theor Appl Genet 97:370–380

Dávila JA, Sanchez de la Hoz MP, Loarce Y, Ferrer E (1998) The use of random amplified microsatellite polymorphic DNA and coefficients of parentage to determine genetic relationships in barley. Genome 41:477–486

Demissie A, Bjornstad A, Kleinhofs A (1998) Restriction fragment length polymorphisms in landrace barleys from Ethiopia in relation to geographic, altitude, and agro-ecological factors. Crop Sci 38:237–243

Gower JC (1966) Some distance properties of latent root and vector methods used in multivariate analysis. Biometrika 53: 325–338

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

Graner A, Ludwig WF, Melchinger AE (1994) Relationships among European barley germplasm: II. comparison of RFLP and pedigree data. Crop Sci 34:1199–1205

Hamada H, Petrino MG, Kakunaga T (1982) A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proc Natl Acad Sci USA 79:6465–6469

Henry AM, Damerval C (1997) High rates of polymorphism and recombination at the *Opaque-2* locus in cultivated maize. Mol Gen Genet 256:147–157

Innan H, Terauchi R, Miyashita NT (1997) Microsatellite polymorphism in natural populations of the wild plant *Arabidopsis thaliana*. Genetics 146:1441–1452

Isagi Y, Suhandono S (1997) PCR primers amplifying microsatellite loci of *Quercus myrsinifolia* Blume and their conservation between oak species. Mol Ecol 6:897–899

Islam AKMR (1983) Ditelosomic additions of barley chromosomes to wheat. In: Sakamoto S (ed) Proc 6th Int Wheat Genet Symp Kyoto University Press, Kyoto, pp 233–238

Islam AKMR, Shepard KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome additon lines. Heredity 46:161–174

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly M, Lincoln S, Newburg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174–181

Liu ZW, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat DNA markers and their integration into a barley linkage map. Theor Appl Genet 93:869–876

Lübberstedt T, Dussle C, Melchinger AE (1998) Application of microsatellites from maize to teosinte and other relatives of maize. Plant Breed 117:447–450

Manninen O, Nissila E, Jaccard P (1997) Genetic diversity among Finnish six-rowed barley cultivars based on pedigree information and DNA markers. Hereditas 126:87–93

Melchinger AE, Graner A, Singh M, Messmer MM (1994) Relationships among European barley germplasm. I. Genetic diversity among winter and spring cultivars revealed by RFLPs. Crop Sci 34:1191–1199

Morgante M, Olivieri AM (1993) PCR-amplified microsatellites as markers in plant genetics. Plant J 3:175–182

Nei M, Li WH (1979) Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 76:5269–5273

Nilsson NO, Säll T (1995) Distributive control of recombination in barley: variation in linkage in chromosome 1. Hereditas 122:143–151

Ordon F, Schiemann A, Friedt W (1997) Assessment of the genetic relatedness of barley accessions resistant to soil-borne mosaic-inducing viruses (BaMMV, BaYMV, BaYMV-2) using RPDS. Theor Appl Genet 94:325–330

Powell W, Machray GC, Provan J (1996) Polymorphism revealed by simple sequence repeats. Trends Plant Sci 1:215–222

Provan J, Powell W, Waugh R (1996) Microsatellite analysis of relationships within cultivated potato (*Solanum tubercosum*). Theor Appl Genet 92:1078–1084

Röder MS, Korzun V, Wendehake K, Plaschke J, Tixier MH, Leroy P, Ganal MW (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Russell J, Fuller JD, Young G, Thomas B, Taramino G, Macaulay M, Waugh R, Powell W (1997a) Discriminating between barley genotypes using microsatellite markers. Genome 40:442–450

Russell JR, Fuller JD, Macaulay M, Hatz BG, Jahoor A, Powell W, Waugh R (1997b) Direct comparison of levels of genetic variation among barley accessions detected by RFLPs, AFLPs, SSRs and RAPDs. Theor Appl Genet 95:714–722

Saghai Maroof MA, Soliman KM, Jorgenson 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

Schut JW, Qi X, Stam P (1997) Association between relationship measures based on AFLP markers, pedigree data and morphological traits in barley. Theor Appl Genet 95:1161–1168

Senior ML, Heun M (1993) Mapping maize microsatellites and polymerase chain reaction confirmation of the targeted repeats using a CT primer. Genome 36:884–889

Shenkar R, Shen M, Arnheim N (1991) DNAse I-hypersensitive sites and transcription factor-binding motifs within the mouse *Ebeta* meiotic recombination hot spot. Mol Cell Biol 11: 1813–1819

Smulders MJM, Bredemeijer G, Rus Kortekaas W, Arens P, Vosman B (1997) Use of short microsatellites from database sequences to generate polymorphisms among *Lycopersicon esculentum* cultivars and accessions of other *Lycopersicon* species. Theor Appl Genet 94:264–272

Stephenson P, Bryan G, Kirby J, Collins A, Devos K, Busso C, Gale MD (1998) Fifty new microsatellite loci for the wheat genetic map. Theor Appl Genet 97:946–949

Struss D, Plieske J (1998) The use of microsatellite markers for detection of genetic diversity in barley populations. Theor Appl Genet 97:308–315

Taramino G, Tingey S (1996) Simple sequence repeats of germplasm analysis and mapping in maize. Genome 39:277–287

Wang Z, Weber JL, Zhong G, Tanksley SD (1994) Survey of plant short tandem DNA repeats. Theor Appl Genet 88:1–6

Weber JL (1990) The informativeness of human (dC-dA)n. (dG-dT)n polymorphism. Genomics 7:524–530

Weber JL, May PE (1989) Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 44:388–396

Whitton J, Rieseberg LH, Ungerer MC (1997) Microsatellite loci are not conserved across the *Asteraceae*. Mol Biol Evol 14:204–209

Zhang QF, Saghai-Maroof MA, Kleinhofs A (1993) Comparative diversity analysis of RFLPs and isozymes within and among populations of *Hordeum vulgare* spp. *spontaneum*. Genetics 134:909–916

Xu XJ, Hsia AP, Zhang L, Nikolau BJ, Schnable PS (1995) Meiotic recombination break points resolve at high rates at the 5´ end of a maize coding sequence. Plant Cell 7:2151–2161