Theor Appl Genet (2003) 106:539–549
DOI 10.1007/s00122-002-1045-7

T. G. Sjakste · I. Rashal · M. S. Röder

Inheritance of microsatellite alleles in pedigrees of Latvian barley varieties and related European ancestors

Received: 5 March 2002 / Accepted: 21 May 2002 / Published online: 22 August 2002

Abstract Genetic diversity and inheritance of 65 microsatellite (SSR) loci were studied in a set of 37 barley varieties involved in the pedigrees of seven Latvian barley varieties: Abava, Agra, Balga, Imula, Linga, Priekulu 1 and Stendes. Cluster analysis divided all the varieties into two large groups according to their geographic distribution. Moravian, Swedish and Danish varieties clustered separately from varieties from Norway and Finland. The pattern of subgroups of both European and Latvian varieties was in accordance with their pedigree information. Graphical genotypes of microsatellite alleles of all seven barley chromosomes were determined for all the 37 varieties studied. Parental inheritance and transmission of microsatellite alleles through the generations of the pedigrees were analysed. The results confirmed the importance and informative value of microsatellite markers for genetic studies in barley and their utility for barley breeding and other applications in fundamental and applied barley genetics.

Keywords Barley · Microsatellite markers · Inheritance · Pedigree · Genotyping

Introduction

The development of new improved genotypes requires the characterization of genetic diversity in breeding material, the measurement of genetic similarity or genetic distance among parents and the estimation of expected genetic variance in segregating progenies derived from different crosses. The determination of linkage between agronomical useful traits and molecular markers also can support breeding efforts. Barley, as one of the major crops in Europe, attracts special attention for the introduction of molecular markers to resolve these problems. Moreover, barley as a self-pollinated crop has become an important object for modern approaches in plant genetics and genomics.

Nowadays the effectiveness and informative value of microsatellite markers in genetic studies has been demonstrated for all the major cereals (Yang et al. 1994; Smith et al. 1997; Röder et al. 1998; Donini et al. 2000). In barley more than 600 microsatellites were published, and established microsatellite maps for all seven barley chromosomes are now available for the public (Saghai-Maroof et al. 1994; Becker and Heun 1995; Liu et al. 1996; Struss and Plieske 1998; Ramsay et al. 2000). Microsatellites in barley were also used to study genetic diversity and trace the development of germplasm (Russell et al. 1997, 2000; Macaulay et al. 2001).

In the present study, we have examined the genetic diversity and inheritance of 65 microsatellite loci in a set of 37 barley varieties which are part of the pedigrees of the seven Latvian barley varieties Abava, Agra, Balga, Imula, Linga, Priekulu 1 and Stendes, and which include several well-known old European varieties. The main objective of this study was to perform microsatellite analysis in order to reach the following goals:

(1) To characterize the polymorphism and genetic diversity of the European barley gene pool involved in the development of the Latvian commercial varieties.

(2) To detect the level of similarity (or dissimilarity) of Latvian and European genotypes with their ancestors and to reveal the impact of parental inheritance.

(3) To investigate the transmittance of microsatellite alleles and whole linkage blocks through generations.

(4) To demonstrate graphically the utility of microsatellite marker analysis for the characterization of barley genotype inheritance and individuality, as well as perspectives for variety identification.

Communicated by G. Wenzel

T.G. Sjakste (✉) · I. Rashal
Institute of Biology, Miera str. 3, LV 2169, Salaspils, Latvia
e-mail: tanja@email.lubi.edu.lv
Tel.: +371 7945435, Fax: +371 7944986

M.S. Röder
Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Correnstrasse 3, D-06466, Gatersleben, Germany

Materials and methods

Plant material and DNA isolation

Thirty seven spring barley varieties used in the current study, with their pedigrees, origin and year of release are given in Table 1. Seven commercial Latvian varieties (Abava, Agra, Balga, Imula, Linga, Priekulu 1 and Stendes) and their ancestors according to pedigree information (Gaike and Gaike 1997) are included in this set. The seeds of the varieties were obtained from the Latvian Genebank of Cultivated Plants in Salaspils, Latvia, the Nordic Gene Bank in Alnarp, Sweden, and the Gene Bank at the Institute for Plant Genetics and Crop Plant Research (IPK) in Gatersleben, Germany.

Bulk genomic DNA was extracted according to the previously described procedure (Plaschke et al. 1995) using leaves of 5–6 plants or ten embryos dissected from grains for each accession.

Microsatellite markers and PCR amplification

Sixty five primer pairs representing barley microsatellites were chosen for the analysis. Primer sequences and locations of the amplified loci on the barley genetic map were published previously for HVM, Bmac, EBmac, Bmag and EBmag markers (Liu et al. 1996; Ramsay et al. 2000). GBMS (Gatersleben barley microsatellite) markers were developed and mapped at IPK (Sjakste, Li and Ganal, unpublished results). The chosen microsatellite markers covered all seven barley chromosomes. The chromosomal locations of the microsatellite markers, the number of alleles per locus, the range of sizes of the amplified products and the polymorphism information content value (PIC value) of each locus are presented in Table 2.

PCR reactions and fragment detection were performed as described by Röder et al. (1998) using CY-5-labelled primer pairs. Fragment analysis was carried out on an automated laser ALF express sequencer (Pharmacia); fragment sizes were calculated using the computer program Fragment Analyser 1.02 (Pharmacia) by comparing the internal size standards added to each lane in the loading buffer.

Data analysis

The allelic data generated at each locus were used to construct graphical genotypes of the barley varieties. Allele frequencies, PIC values, heterogeneity (HG) by accession and by marker, parental inheritance and allele transmittance through the generations were analysed in the computer package GeneFlow v5.0.3. The microsatellite allele data were converted into a 1/0 matrix, and a dendrogram based on genetic distance (percentage difference) was constructed using the NTSYS-pc package (Rohlf 1992) based on the unweighted pair-group method with an arithmetic average (UPGMA).

Table 1 Description of the investigated varieties including year of release, origin, pedigree, form of spike and percentage of heterogeneity (HG) based on 65 microsatellite loci

Cultivars	Year	Origin	Pedigree	Form	HG (%)
Abava	1978	Latvia	Mari/Elsa//Domen	2	6.2
Agra	1984	Latvia	Priekulu 1/Otra	6	0
Akka	1969	Sweden	Monte Cristo/Arla	2	6.2
Arla	1964	Sweden	Tammi/Maja//Hanna/Svanhals/3/Opal	2	9.2
Asplund		Norway	Selection from a mixed pul (Norway or Sweden)	6	7.7
Balga	1990	Latvia	Gunilla/KM 1192	2	0
Binder	1916	Denmark	Selection from Hanna	2	6.2
Birgitta	1966	Sweden	Opal/Vega//Maja	2	0
Bonus	1950	Sweden	Maja//Seger/Opal	2	0
Denso		Denmark	Selection from Rigel [or Rigel mutant (short straw)]	2	0
Domen	1952	Norway	Opal B/Maskin	2	1.5
Drost	1954	Denmark	Maja/Kenia	2	0
Edda	1945	Sweden	Asplund/Vega	6	0
Edda II	1951	Sweden	Selection from Edda	6	3.1
Elsa	1953	Germany	Haha/Weihenstephan Mehltauresistente II	2	12.3
Gull	1913	Sweden	Selection from landrace from Gotland	2	1.5
Gunilla	1973	Sweden	Birgitta/Gull mutant	2	3.1
Hannchen		Sweden	Selection from Hanna	2	3.1
Imula	1985	Latvia	Abava/2*Akka	2	3.1
Kenia	1931	Denmark	Binder/Gull	2	0
KM 1192		Czech Rep.	Denso/Rg Slovensky 802	2	10.8
Linga	1985	Latvia	Gunilla/KM 1192	2	6.2
Maja	1934	Denmark	Binder/Gull	2	0
Mari	1962	Sweden	X-ray mutant of Bonus	2	0
Maskin	1918	Norway	Selection from Bjoernebybygg (east Norway)	6	0
Monte Cristo	1970	India	Landrace (Bihar, India), disease resistance selection	6	27.7
Olli	1927	Finland	Selection from Finnish landrace	6	0
Opal	1922	Denmark	Binder/Gull	2	9.2
Otra	1959	Finland	Edda/Tammi	6	1.5
Priekulu 1	1954	Latvia	Selection from Norwegian local varieties	2	3.1
Rg Slovensky 802		Czech Rep.	Selection from Slovensky	2	4.6
Rigel	1944	Denmark	Maja/Kenia	2	1.5
Slovensky 802	1946	Czech Rep.	Selection from KM9-13 Opavsky	2	0
Stendes	1972	Latvia	Drost/Maja	2	23.1
Svanhals		Sweden	Selection from Bestehorn Diamant or from PI 5474	2	0
Tammi		Finland	Olli/Asplund	6	10.8
Vega	1920	Sweden	Selection from landrace originating in Nordboten	6	1.5

Table 2 Description of 65 barley microsatellites used, including chromosomal location, range of product sizes, genetic diversity (PIC value) and percentage of heterogeneity (HG)

Chromosome	Locus	Alleles (N)	Product size (bp)	PIC	HG (%)
1H	Bmac0032	6	210–235	0.61	5.4
	Bmac0090	8	188–229	0.68	2.7
	Bmag0211	7	177–194	0.71	2.7
	Bmac0504	4	153–166	0.67	8.1
	Bmac0154	5	112–159	0.67	0
	Bmag0382	4	94–114	0.66	10.8
	Bmag0718	5	149–165	0.71	24.3
	GBMS0184	5	137–151	0.68	8.1
	Bmag0579	5	90–133	0.58	2.7
Total/1H	9	49			
Average/1H				0.64	
2H	Bmac0134	5	100–145	0.65	13.5
	GBMS0247	6	230–254	0.73	5.4
	HVM 36	5	109–121	0.68	2.7
	Bmag0140	4	152–161	0.51	2.7
	Bmag0518	5	162–172	0.71	0
	EBmac0715	7	95–175	0.72	13.5
	GBMS0160	4	198–206	0.51	0
	HVM 36	5	109–121	0.68	2.7
	HVM 54	5	148–164	0.55	8.1
Total/2H	8	46			
Average/2H				0.63	
3H	EBmac0705	3	146–165	0.05	2.7
	Bmag0603	7	112–152	0.61	2.7
	Bmag0225	7	142–164	0.72	10.8
	GBMS0189	6	116–140	0.59	2.7
	GBMS0212	2	140–151	0.05	0
	Bmag0013	7	143–169	0.74	5.4
	GBMS0074	4	118–146, null	0.65	2.7
	HVM 62	4	235–263	0.21	0
	EBmac0708	7	123–143, null	0.56	2.7
	EBmac0541	4	103–116	0.44	2.7
Total/3H	10	51			
Average/3H				0.46	
4H	HVM 40	3	144–160	0.34	18.9
	EBmac0906	4	154–160	0.55	8.1
	Bmac0310	4	140–175	0.67	8.1
	GBMS0087	3	148–167	0.24	2.7
	Bmac0701	7	114–148	0.77	5.4
	EBmac0635	7	81–114	0.77	8.1
	EBmac0788	7	135–173	0.78	2.7
	HVM 67	4	112–118	0.57	2.7
	GBMS0133	3	182–187	0.52	0
Total/4H	9	42			
Average/4H				0.58	
	Bmag0223	6	160–174	0.74	0
	EBmac0824	2	331–334	0.11	0
	GBMS0119	6	98–115	0,56	0
	Bmag0222	2	150, null	0.48	0
Total/5H	7	25			
Average/5H				0.46	
6H	Bmac0316	5	127–164	0.63	10.8
	Bmag0500	4	165–185	0.66	2.7
	GBMS0083	6	131–145	0.64	0
	GBMS0125	2	129–131	0.15	0
	EBmac0674	2	147–152	0.05	0
	EBmac0602	5	196–219	0.62	2.7
	Bmag0613	9	166–193	0.83	13.5
	Bmac0040	6	198–231	0.61	2.7
Total/6H	8	39			
Average/6H				0.52	
7H	EBmac0713	5	157–165, null	0.61	0
	GBMS0192	4	188–195	0.51	5.4
	Bmag0767	5	134–159	0.57	0

Table 2 (continued)

Chromosome	Locus	Alleles (N)	Product size (bp)	PIC	HG (%)
	Bmag0206	7	255–278	0.71	0
	GBMS0139	6	141–149, null	0.71	0
	GBMS0035	3	141–145	0.63	8.1
	EBmac0603	4	138–173	0.71	5.4
	Bmag0914	5	84–186	0.46	5.4
	Bmag0341	7	77–227	0.71	0
	Bmag0507	7	115–151	0.72	0
	Bmag0120	7	228–258	0.72	5.4
	EBmac0755	5	138–147	0.58	2.7
	HVM 49	7	102–196	0.66	10.8
	GBMS0183	2	192–198	0.49	2.7
Total/7H	14	74			
Average/7H				0.62	
Total	65	322			
Average		4.9		0.57	

Results

Microsatellite analysis

A total of 322 alleles were detected for 65 primer pairs in the set of barley varieties investigated (Table 2). Different alleles represented different sizes of the amplification products. Null alleles were detected in our study for five loci (GBMS0074 and EBmac0541 on 3H, Bmag0222 on 5H, EBmac0713 and GBMS0139 on 7H). A null allele can arise from a point mutation(s) in one or both of the primer sequences that result in the absence of the amplification product. According to our data the presence of most null alleles was in correlation with the pedigree information of the studied varieties (Table 1, Figs. 1 and 2). For example, the null allele at EBmac0713 on chromosome 7H was detected only in varieties of the first group of the dendrogram except Stendes, Drost and Elsa. By contrast, the null allele at Bmag0222 (chromosome 5H) characterised all genotypes of the varieties clustered in group 2 of the dendrogram, together with Monte Cristo, Svanhals and Slovensky 802. The null mutation at locus GBMS0074 on chromosome 3H was transmitted from Akka to Imula and from Kenia to Drost (Fig. 2).

The number of alleles per locus varied from 2 to 9 with an average of 4.9. The microsatellite markers used showed a different level of gene diversity; the PIC values varied from 0.05 to 0.83 (Bmag0613 on chromosome 6H) with 0.57 on average for all markers. Markers EBmac0705 and GBMS0212 on 3H, as well as EBmac0674 on chromosome 6H, amplified the same alleles for all varieties except Monte Cristo. The average PIC values on different chromosomes were comparable and varied from 0.46 (chromosomes 3H and 5H) to 0.64 (chromosome 1H) (Table 2). The location of the markers on the distal or centromeric parts of the chromosomes had no obvious correlation to the PIC values.

Twenty markers revealed one allele per locus in all varieties studied, thus their heterogeneity (HG) level was equal to zero. The remaining 45 markers had different levels of heterogeneity based on all variety/marker combinations (Table 2). The corresponding information on the percentage of heterogeneity observed in each variety is summarized in Table 1. Only 14 of all the varieties studied were homogeneous. Twenty three varieties revealed two alleles per locus at least once, with a minimum level of heterogeneity in Domen and Vega (HG = 1.5%) where only one locus was heterogeneous, respectively. Maximal heterogeneity was observed in the Latvian variety Stendes (HG = 23.1%) and the Indian variety Monte Cristo (HG = 27.7%). The observed heterogeneity in marker alleles most likely reflected heterogeneity or heterozygosity in the seeds of the varieties since bulk DNA from several plants was used for the analysis.

Genetic diversity

Pairwise comparisons were made between all varieties and the average dissimilarity values were calculated based on the microsatellite-derived data. The dendrogram discriminated all the varieties (Fig. 1). The smallest genetic distances were observed between varieties Bonus and Mari, Binder and Maja (less than 0.04), Denso and Rigel, Balga and Linga, Edda and Edda II, and Akka and Arla (less than 0.08). The calculated small distances corresponded to the pedigrees of these cultivars: Mari, Denso and Edda II were developed by selection from Bonus, Rigel and Edda respectively. Linga and Balga were derived from two common parents. A well-known Danish variety Maja showed practically the same genotype as one of its parents, Binder (Figs. 1 and 2). Monte-Cristo seemed to be the genetically most-distant variety with a genetic distance of 0.82 in relation to the other varieties. This variety clustered separately in the dendrogram.

The remaining varieties clustered into two large groups. Group 1 consisted of 24 varieties from Abava to Svanhals in the dendrogram, including four Latvian vari-

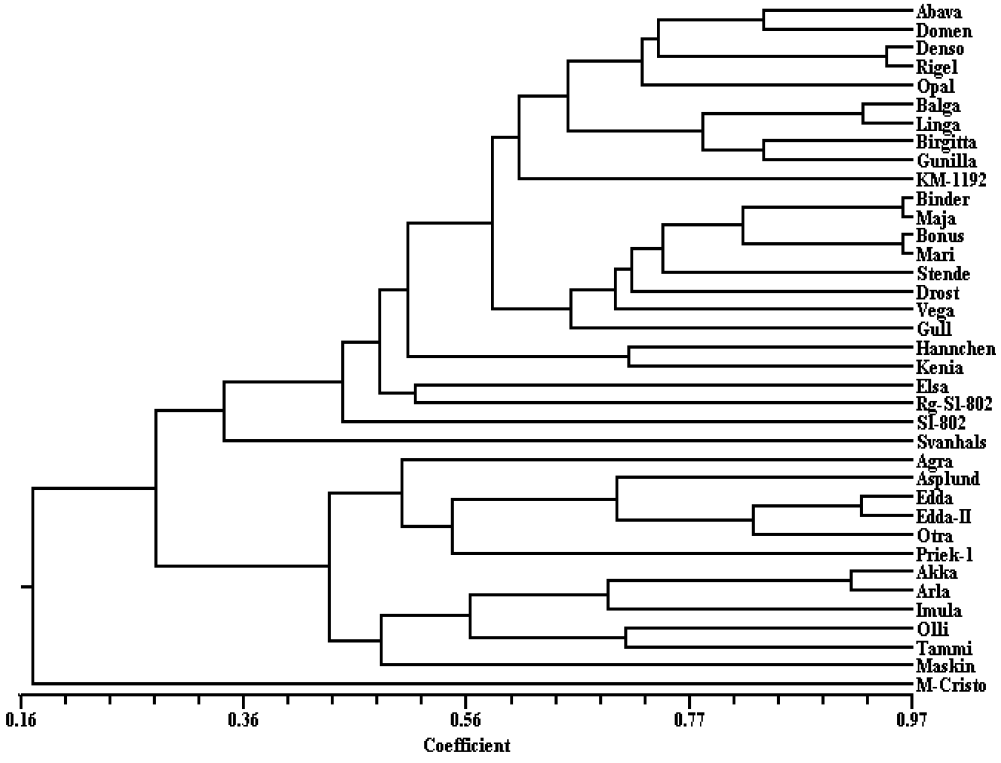


Fig. 1 Dendrogram reflecting the genetic distances among 37 barley varieties based on the analysis of 65 microsatellite loci

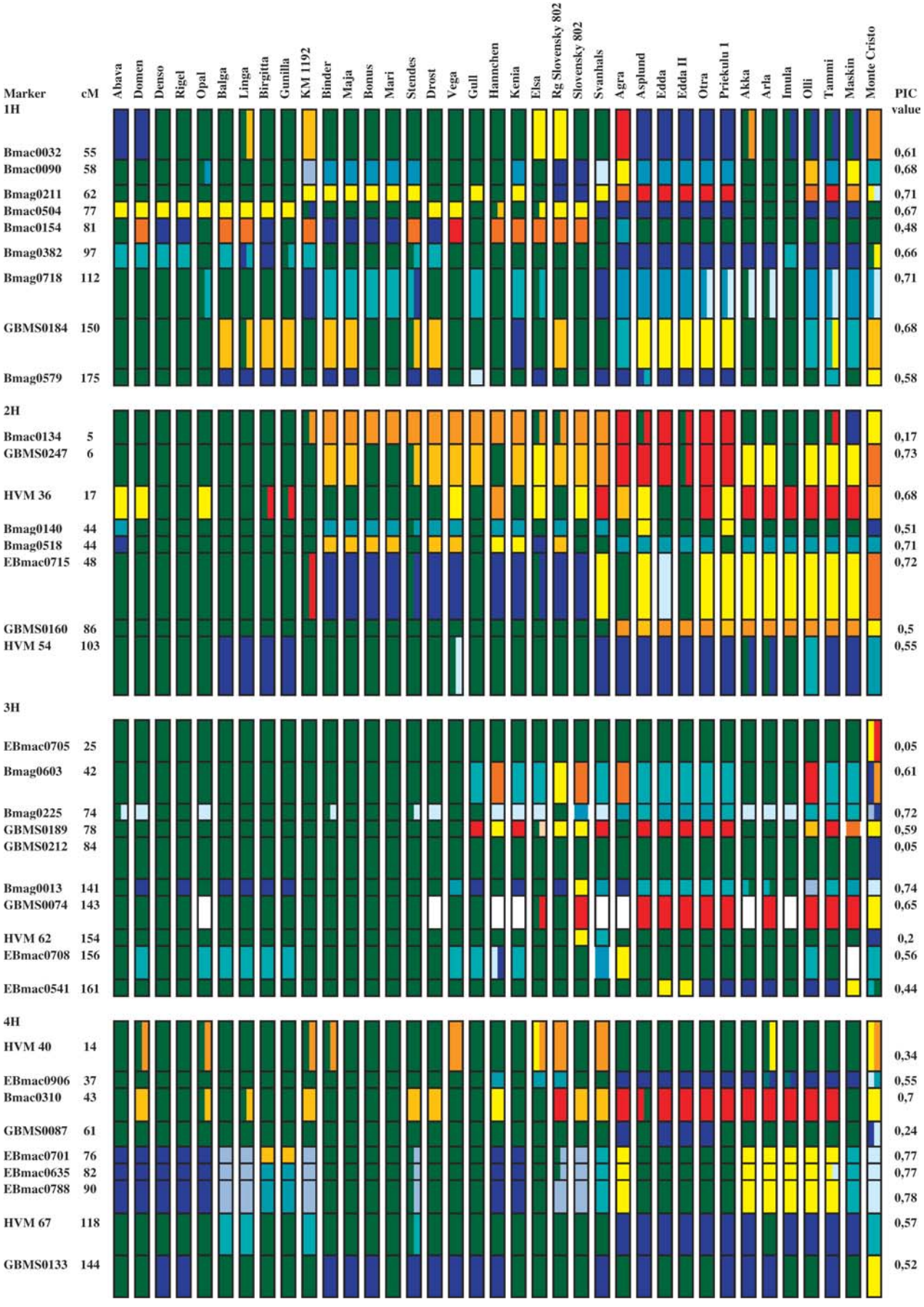
eties (Abava, Balga, Linga and Stendes), eight Swedish varieties (Birgitta, Bonus, Gull, Gunilla, Hannchen, Mari, Svanhals, and Vega), seven Danish varieties (Binder, Denso, Drost, Kenia, Maja, Opal, and Rigel), one Norwegian variety (Domen), three Moravian varieties (KM 1192, Slovensky 802 and RG Slovensky 602) and one German variety (Elsa). Group 2 consisted of 12 varieties from Agra to Maskin in the dendrogram, and included three Latvian varieties (Agra, Priekulu 1 and Imula), four Swedish varieties (Akka, Arla, Edda and EddaII), two Norwegian varieties (Asplund and Maskin) and three Finnish varieties (Olli, Otra and Tammi). The Latvian varieties Agra and Imula, as well as the varieties from Sweden Akka and Arla, have the Finnish varieties among their ancestors. The Swedish varieties Edda and EddaII have the Norwegian variety Asplund in their pedigrees. The remaining varieties of group 2 (Priekulu 1, Asplund and Maskin) were developed from Norwegian accessions or landraces. All the Latvian and other European varieties used clustered according to their pedigrees (Fishbeck 1992; Gaike and Gaike 1997). The Latvian varieties were grouped close to their immediate ancestors that gave the most important impact on their inheritance.

Graphical genotypes

Most of the microsatellite markers employed were represented by frequent alleles as well as by rare or unique alleles found in only one variety. Figure 2 shows the graphical genotypes of 37 barley varieties based on allele frequencies of 65 microsatellite markers. Each allele was coded by a different colour, and the loci were ordered into linkage groups. The varieties were given in the same order as they were clustered in the dendrogram. The graphs of the chromosomes clearly demonstrated the genomic regions of similarity and dissimilarity between the genotypes and revealed conserved linkage blocks transmitted through the generations. Regions of variability are responsible for the genetic distance between the varieties.

The differences in the representation of alleles for all seven barley chromosomes were evident between varieties of group 1 and group 2 of the dendrogram. Both groups had a similar genetic diversity and their PIC values equalled 0.42. Fifty five microsatellite markers revealed a quite different allelic spectrum for these two subclusters. Group 1 united all varieties originating from Moravia, Sweden, Denmark, Germany and those Latvian varieties that have no Finnish ancestors in their pedigrees. The genotype of the only Norwegian variety, Domen, resembled the Danish variety Opal rather than its other parent from Norway, Maskin, which lead to the clustering of Domen in group 1. All other Norwegian and Finnish varieties, as well as Swedish and Latvian varieties with Finnish and Norwegian ancestors in the pedigrees, belonged to group 2 in the dendrogram and revealed very similar graphical genotypes.

About 50% of all markers revealed unique or very rare alleles in the case of the variety Monte Christo of Indian origin. Four varieties (Mari, Denso, Edda II, and Rg Slovensky 802) had been developed by selection from the parental variety (Bonus, Rigel Edda and Slovensky 802 correspondingly) with an average impact


Marker
cM
Abava
Domen
Denso
Rigel
Opal
Balga
Linga
Birgitta
Gunilla
KM 1192
Binder
Maja
Bonus
Mari
Stendes
Drost
Vega
Gull
Hannchen
Kenia
Elsa
Rg Slovensky 802
Slovensky 802
Svanhals
Agra
Asplund
Edda
Edda II
Otra
Priekulu 1
Akka
Arla
Imula
Olli
Tammi
Maskin
Monte Cristo
PIC value
1H
Bmac0032 55 0,61
Bmac0090 58 0,68
Bmag0211 62 0,71
Bmac0504 77 0,67
Bmac0154 81 0,48
Bmag0382 97 0,66
Bmag0718 112 0,71
GBMS0184 150 0,68
Bmag0579 175 0,58
2H
Bmac0134 5 0,17
GBMS0247 6 0,73
HVM 36 17 0,68
Bmag0140 44 0,51
Bmag0518 44 0,71
EBmac0715 48 0,72
GBMS0160 86 0,5
HVM 54 103 0,55
3H
EBmac0705 25 0,05
Bmag0603 42 0,61
Bmag0225 74 0,72
GBMS0189 78 0,59
GBMS0212 84 0,05
Bmag0013 141 0,74
GBMS0074 143 0,65
HVM 62 154 0,2
EBmac0708 156 0,56
EBmac0541 161 0,44
4H
HVM 40 14 0,34
EBmac0906 37 0,55
Bmac0310 43 0,7
GBMS0087 61 0,24
EBmac0701 76 0,77
EBmac0635 82 0,77
EBmac0788 90 0,78
HVM 67 118 0,57
GBMS0133 144 0,52

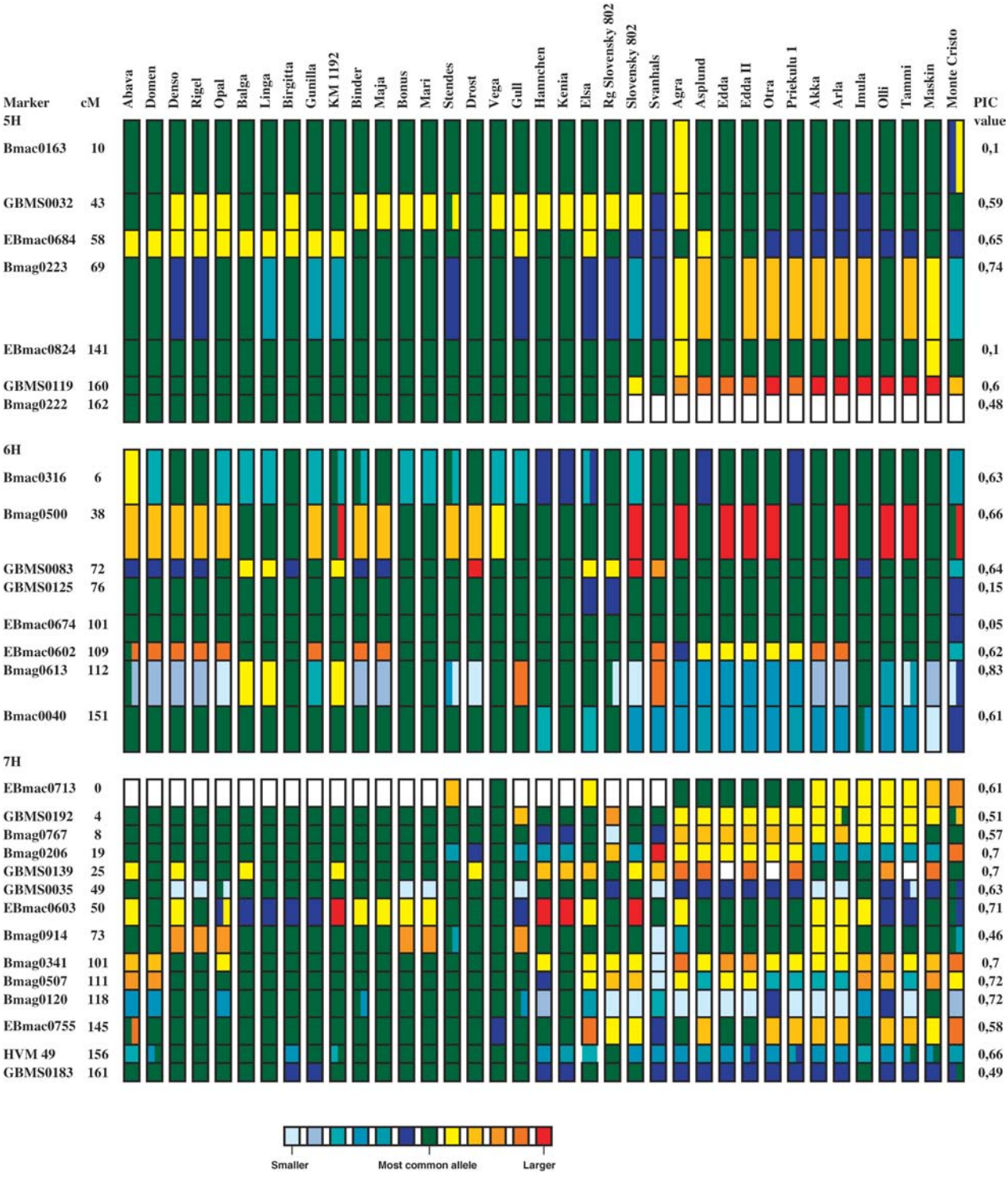


Fig. 2 Graphical genotypes of 37 barley varieties. The chromosomes are presented in columns, with microsatellite loci, their map positions and PIC values. The chromosomal regions represented by microsatellite markers are *coloured* according to the scale presented at the bottom of the figure. The most common allele at each locus is *coloured green* with *yellow and blue* representing the most-closely sized larger or smaller alleles, respectively. Heterogeneous loci are *coloured for both alleles*, null alleles are uncoloured

of inheritance of the parent of 83.46% (Table 3). The maximum transmission of alleles from one parent was observed for Mari/Bonus, with 98.46% representing only one difference in locus GBMS0133 on chromosome 4H. Variety Denso differed from its ancestor Rigel at three loci (Bmag0013 on 3H, GBMS0139 and Ebmac0603 on 7H). Edda II showed six loci deviating from Edda; however, only three of them were completely different (EBmac0715 on 2H, Bmag0223 on 5H and GBMS0139 on

Table 3 Parental inheritance in accessions developed by direct selection from a parent genotype

Accession (parent)	Inheritance[a]	
	Parent	Not parent
Mari (Bonus)	64	1
Denso (Rigel)	62	3
Edda II (Edda)	59	6
Rg Slovensky 802 (Slov. 802)	32	33
Total	217	43
Percentage	83.46%	16.54%

[a] Parental inheritance is given as the number of corresponding loci

7H), while the other three alleles appeared to be heterogeneous (Bmac0134 and GBMS0247 on 2H, HVM 49 on 7H). The maximal differences between the ancestor and its inbred progeny were observed between Slovensky 802 and Rg Slovensky 802, with 33 inconsistent alleles of which four were heterogeneous. In this pair of varieties identical linkage blocks flanked by microsatellite markers could be easily determined from the graphical genotypes (regions from Bmac 0090 to Bmag0718 on 1H, from EBmac0715 to HVM 54 on 2H, from Bmag0914 to Ebmac0755 on 7H and several blocks on other chromosomes).

Parental inheritance

The presence of hybrid lines, intermediate crosses in the pedigrees, as well as the absence of accessions of interest in barley collections, lead to difficulties in studying the inheritance of alleles in pedigrees due to incomplete sets of ancestors available. We were able to study the allelic transmittance for 16 varieties from their immediate parents (Fig. 3 and Table 4). Alleles were found to be identical in both parents in 44.9% of all cases and it was not possible to trace the inheritance of these marker alleles. The parents of Domen (Opal and Maskin) and of Akka (Monte Cristo and Arla) share a small number of identical alleles (11 and 8 out of 65, respectively). Presence of the identical alleles in both parents is the main obstacle for the analysis of parental inheritance. Another difficulty poses the occurrence of inconsistent alleles, which were not found in both parents. In total 145 out of 1,040 monitored cases (13.9%) did not match either of

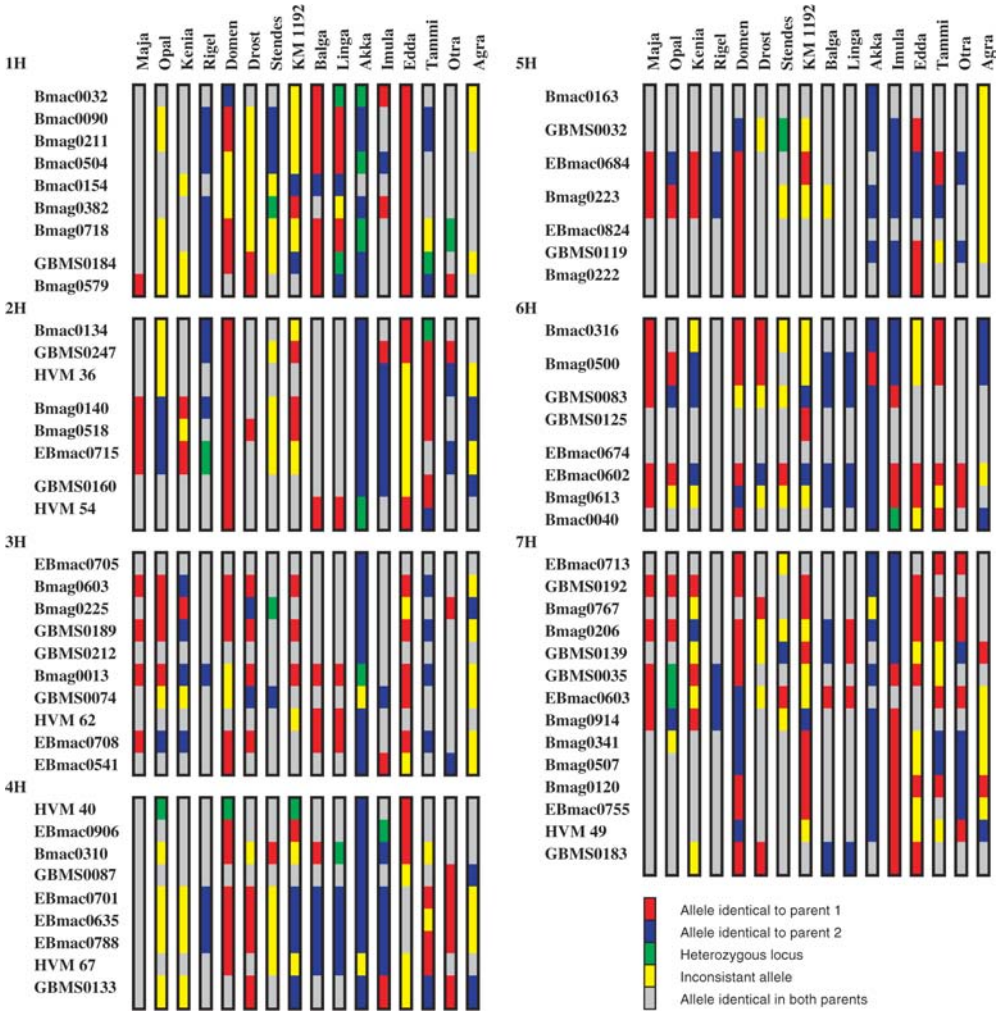


Fig. 3 Graphical presentation of the parental inheritance of 16 barley varieties based on the genotypes of 65 microsatellite loci in comparison to their immediate parents. Marker boxes are *coloured* according to the scale presented at the bottom of the figure. The alleles coloured as *red and blue* represent parent 1 and parent 2 inheritance, alleles identical in both parents are coloured *as gray*; heterozygous and inconsistent alleles are coloured as *green and yellow* correspondingly

Table 4 Inheritance of the 65 microsatellite loci in 16 varieties from their immediate parents. Alleles not found in both parents are designated as inconsistent

Accession with parents	Number of corresponding loci				
	Parent 1	Parent 2	Hetero	Same	Inconsistent
Maja (Binder/Gull)	20	0	0	45	0
Opal (Binder/Gull)	9	7	3	30	16
Kenia (Binder/Gull)	8	8	0	34	15
Rigel (Maja/Kenia)	0	19	1	45	0
Domen (Opal/Maskin)	40	8	1	11	6
Drost (Maja/Kenia)	15	3	0	34	13
Stendes (Drost/Maja)	3	5	3	36	18
KM 1192 (Rg Sl.802/Denso)	18	8	1	19	19
Balga (Gunilla/KM 1192)	13	12	0	39	1
Linga (Gunilla/KM 1192)	12	10	3	39	1
Akka (Monte Cristo/Arla)	1	49	4	8	3
Imula (Abava/2*Akka)	16	23	2	24	0
Edda (Asplund/Vega)	31	2	0	14	18
Tammi (Olli/Asplund)	18	12	2	25	8
Otra (Edda II/Tammi)	15	10	1	39	0
Agra (Priekulu 1/Otra)	2	10	0	26	27
Total	221	186	21	468	145
Percentage	21.23%	17.87%	2.01%	44.96%	13.93%

the parental alleles. Most inconsistencies were found for the Latvian variety Agra (42%). Despite these difficulties approximately 40% of all loci were informative for determining the parental inheritance and indicated two situations: either a predominant impact of one of the parents was observed in the developed genotype (Binder in Maja, Kenia in Rigel, Opal in Domen, Maja in Drost, Rg Slovensky 802 in KM 1192, Arla in Akka, Akka in Imula, Asplund in Edda, Otra in Agra). Alternatively, in some cases alleles have been transmitted to the new genotype from both parents in equal, or close to equal, ratios (Opal, Kenia, Balga, Linga, Tammi, Otra). It is impossible to give any conclusion about the parental inheritance of the Latvian variety Stendes, because only a small number of alleles were informative (8 from 65 studied). In Fig. 3 it is obvious that whole linkage blocks of microsatellite alleles were transmitted from the same parent, revealing the number and location of recombination events leading to the filial genotypes.

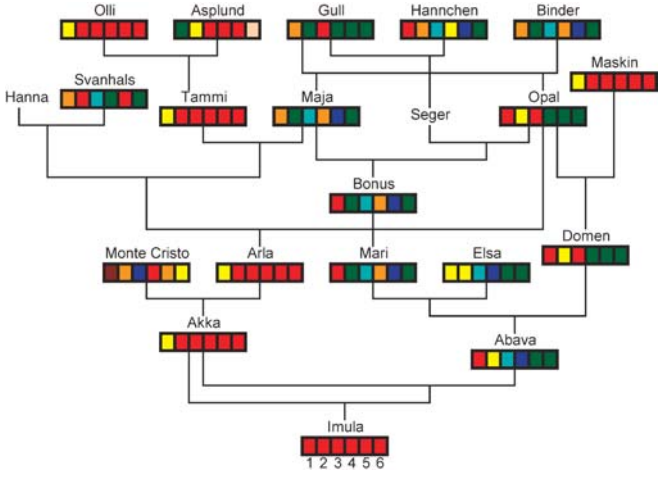


Fig. 4 Allelic pedigree of the Latvian barley varieties Abava and Imula on chromosome 2H. Microsatellite loci are presented in the following sequence from 1 to 6: GBMS0247, HVM 36, Bmag0140, Bmag0518, Bmag0715, GBMS0160. Alleles revealed in Imula are coloured *red*, other alleles are coloured according to the strategy of Fig. 2, identical alleles have the same colour. The genotypes Hanna and Seger were not analysed

GeneFlow analysis of pedigree

A pedigree analysis of all Latvian and European varieties under study was performed using the computer package GeneFlow. As example, Fig. 4 shows the double pedigree of two Latvian varieties, Abava and Imula, and presents the inheritance of six microsatellite alleles of chromosome 2H. The fate of the alleles could be traced from the earliest of the northern European varieties, Binder, Gull, Hannchen, Asplund, Olli and Maskin, through well known European genotypes developed in the middle of the last century, such as Maja and the local Latvian varieties Abava and Imula. Figure 4 stresses the differences in microsatellite alleles between two groups of ancestors: Olli and Asplund from one side, and Gull, Hannchen and Binder from the other side. Maja revealed most similarity with Binder. Bonus had one allele (GBMS0247) from Opal, and the whole block of four alleles (HVM36, Bmac0140, Bmag0518, EBmac0715) from Maja. Mari showed high similarity to Bonus, but due to the similarity of the alleles between Mari, Elsa and Domen it was impossible to reveal the sources of parental inheritance from Abava for this part of the genome. Quite a different conclusion could be drawn for Imula. Olli transmitted its alleles (GBMS0247, HVM36, GBMS0160) to Tammi, which gave the strongest impact

on the inheritance of Arla, Akka and Imula in sequence. Imula inherited only one allele (GBMS0247) from Abava for this set of six loci. No transmission of alleles was observed from Monte Christo to Akka for chromosome 2H.

Discussion

The presentation of microsatellite data as a pedigree scheme revealed the transmittance of alleles and linkage blocks through the generations. During such an analysis many questions of the breeding process could be addressed. The inheritance of genetic material from all parts of the genome and of different chromosomes could be evaluated from the point of view of allele flow in every step of the breeding process. It was possible to draw conclusions about the variability and purity of the ancestors, as well as the influence of selection on the genetic composition. Preferential or equal parental inheritance stable selection of specific alleles and the occurrence of genomic regions with high frequency of recombination could be identified.

Studies on the visualization of the flow of alleles through the ancestral lineage are very rare in crops in general and in barley in particular. The introduction of highly polymorphic microsatellite markers representing one locus opened the possibility to approach molecular pedigree analysis. A retrospective SSR analysis of the pedigree was presented for the European variety Cooper, and the fate of the alleles was illustrated for four alleles on chromosomes 3H and 5H (Russell et al. 2000).

Genetic variation among parents is necessary to derive improved progeny from crossing and selection. However, crosses are often performed among elite genotypes of limited number with similar agronomic and end-use characteristics. Such breeding programmes can lead to narrowing the genetic diversity of the barley germplasm. According to our data there is high similarity between some varieties according to their origin and pedigree, and a preference to one-parental inheritance in certain cases. In spite of these results, we did not detect a significant difference in the level of genetic diversity between older and more modern genotypes. The PIC values were determined as 0.581 and 0.514 for cultivars developed before and during the fifties, and during the following decennia of the last century. This could suggest a slow decrease of genetic diversity. Similar levels of diversity of 0.597 in the "foundation genotypes" of North European spring barley were shown for 28 barley SSRs in comparison to 0.484 within modern cultivars (Russell et al. 2000). In the mentioned study, differences in the allelic composition and allelic frequencies were also revealed between cultivars of the "foundation group" and post-1985 genotypes. However, we did not detect such differences between older and younger varieties belonging to one group of the dendrogram. Strong differences in the allelic composition and allelic frequencies revealed between two large groups of varieties reflected the distinction of the barley germplasm according to the origin and the consequences of barley breeding programmes.

Additional germplasm used by breeding programmes should provide increased genetic variability to permit the continued progress in developing high yield and high end-use quality cultivars. However, efforts to introduce new genes for special traits such as disease resistance may not lead to an overall increase of genetic variability due to a predominant selection for the phenotypic similarity of one of the parents. For example, according to our microsatellite analysis, Monte Cristo, which was used in breeding programmes as a donor of disease resistance, possessed many rare and unique microsatellite alleles. However, this variety did not transmit much of its genetic material to its progeny. The genetic distance between Monte Cristo and Akka equalled 0.842. Similar results were determined for Maskin and Domen (GD = 0.649), Vega and Edda (GD = 0.756) or for Priekulu 1 and Agra (GD = 0.541). In most cases the alleles determined in these pairs of varieties were inherited from the other parent of the respective cross. Rare or unique alleles from Monte Cristo, Maskin, Vega and Priekulu 1 were practically not transmitted to their progeny.

From the old varieties investigated, the ancestors for the varieties of the two groups of the dendrogram were Binder and Gull for the first group and Asplund and Olli for the second subgroup, underlining the essential impact of these genotypes in the creation of the modern North European and Latvian varieties studied.

Acknowledgements Research grants from the Deutsche Forschungsgemeinschaft and support from the Nordic Gene Bank are gratefully appreciated. We thank Gunta Marga for assistance in collection of the barley germplasm and Dr. X. Huang for help with the construction of the dendrogram.

References

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

Donini P, Law JR, Koebner RMD, Reeves JC, Cooke RJ (2000) Temporal trends in the diversity of UK wheat. Theor Appl Genet 100:912–917

Fischbeck G (1992) Barley cultivar development in Europe – success in the past and possible changes in the future. Barley Genet 6:885–901

Gaike V, Gaike M (1997) The genetic origin of Latvian spring barley varieties. In: Ruzgas V (ed) Plant breeding: theories, achievements and problems. Proc Int Con, Dotnuva-Akademija, Lithuania, 14–16 July, 1997. Dotnuva-Akademija, Lithuanian Institute of Agriculture, pp 52–57

Liu Z-W, Biyashev RM, Saghai Maroof MA (1996) Development of simple sequence repeat markers and their integration into barley linkage map. Theor Appl Genet 93:867–876

Macaulay M, Ramsay L, Powell W, Waugh R (2001) A representative, highly informative 'genotyping set' of barley SSRs. Theor Appl Genet 102:801–809

Plaschke J, Ganal MW, Röder MS (1995) Detection of genetic diversity in closely related bread wheat using microsatellite markers. Theor Appl Genet 91:1001–1007

Ramsay L, Macaulay M, degli Ivanissivich S, MacLean K, Cardle L, Fuller J, Edwards K, Tuvesson S, Morgante M, Massari A, Maesti E, Marmiroli N, Sjakste T, Ganal M, Powell W, Waugh R

(2000) A simple sequence repeat-based linkage map of barley. Genetics 156:1997–2005

Rohlf FJ (1992) NTSYS-PC: numerical taxonomy and multivariate analysis system. Exeter Software, New York

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

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

Russell JR, Ellis RP, Thomas WTB, Waugh R, Provan J, Booth A, Fuller J, Lawrence P, Young G, Powell W (2000) A retospective analysis of spring barley germplasm development from 'foundation genotypes' to currently successful cultivars. Mol Breed 6:553–568

Saghai-Maroof MA, Bijashev RM, Yang GP, Zhang Q, Allard RW (1994) Extraordinarily polymorphic microsatellite DNA in barley: species diversity, chromosomal locations, population dynamics. Proc Natl Acad Sci USA 91:5466–5470

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S, Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet 95:163–173

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

Yang GP, Maroof MAS, Xu CG, Zhang QF, Bjyashev RM (1994) Comparative analysis of microsatellite DNA polymorphism in landraces and cultivars of rice. Mol Gen Genet 245:187–194