Genetic Resources of Barley and Oat Characterised by Microsatellites

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Abstract: Barley *(Hordeum vulgare* L.) and oat (*Avena sativa* L.) are important crop species. 1865 accessions of winter barley, 2707 accessions of spring barley and 1998 accessions of oat are maintained in RICP Gene bank. The expert core collection is used to be established as a tool for germplasm study, conservation of genetic variability and for the identification of useful genes. The main aim of this study was to evaluate genetic diversity of barley and oat genotypes within the expert core collections. Genetic variation of 176 barley accessions was analyzed using 26 microsatellite loci, covering all 6 chromosomes. 330 oat accessions were analyzed using 26 microsatellite loci that are mapped only into linkage groups. For 26 barley microsatellite loci, 328 alleles were detected. The average number of alleles per locus was 12.6. In oat, for 26 oat microsatellite loci, 353 alleles were detected. The average number of alleles per locus was 13.6. The average DI (diversity index) was 0.11 in barley and 0.09 in oat. Dendrogram and PCA (Principal Component Analysis) based on microsatellite data showed a different influence of the place of origin, age of variety and pedigree on grouping into clusters. PCA showed that the breeding process had a negative impact on the level of genetic diversity and therefore there is a necessity of barley and oat germplasm conservation.

Keywords: barley; oat; microsatellites; genetic diversity

The loss of genetic diversity has become a problem of not only natural plant and animal populations but also agriculturally important species. Old cultivars or landraces and wild relatives of domesticated species are being lost as modern varieties become adopted by farmers. This led to calls for the genetic conservation of crop germplasm (Frankel & Bennet 1970). Currently, a large number of materials from major crop species and their wild relatives are stored in gene banks, in "in situ" conservation sites and "on farm" programs of conservation. Because the large size of germplasm collections, together with limited funding, combine to restrict the characterisation of the material available and hinder their use for breeding purposes (Brown 1995), a necessary proposal for germplasm management is to construct smaller "core collections" representing the maximum of the genetic diversity contained in the larger collection. The construction of such a collection usually starts by stratifying the larger collection into a series of groups according to their genetic distances (Bataillon *et al.* 1996).

To obtain genetic distances, molecular markers that are highly polymorphic and easy to use are beneficial. Microsatellites are commonly used to study genetic relationships between genotypes within species because of their high level of polymorphism (Devos *et al.* 1995; Plaschke *et al.* 1995; Korzun *et al.* 1997). In addition, microsatellites exhibit a codominant inheritance (Hernández *et al.* 2002), which is essential for effective discrimination between closely related lines (Akkaya *et al.* 1992). Microsatellite markers are currently used to

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identify genotypes, quantitative trait loci (QTLs) and genetic diversity (Leišová & Ovesná 2001; Medini *et al.* 2005; Roussel *et al.* 2005).

Barley *(Hordeum vulgare* L.) and oat (*Avena sativa* L.) are important crop species. 1865 accessions of winter barley, 2707 accessions of spring barley and 1998 accessions of oat are maintained in RICP Gene Bank. Barley is a diploid ($2n$ = 14) self-pollinated species and it has a large genome H of approximately 5.3×10^9 bp/1C (Bennett & Smith 1976). Cultivated oat (*Avena sativa* L.) is a self-pollinated allohexaploid with the basic chromosome number of $n = 3x = 21$ that consists of three basic genomes A, C and D (Rajhathy & Thomas 1974). It is characterised by a large genome size of 1.4×10^{10} bp/1C (Bennett & Smith 1976).

The aim of our study was to evaluate the genetic diversity of the collection of barley and oat accessions originating from different countries in the world using microsatellite markers.

MATERIALS AND METHODS

Plant material. One hundred and seventy-six barley and three hundred and thirty oat accessions as an empiric core collection were chosen in 38 different countries (Table 1). Barley accessions were obtained from Gene Bank in Prague-Ruzyně and oat accessions from Agricultural Research Institute in Kroměříž. Plants were grown in greenhouse conditions and about 30 plants per accession were pooled and frozen at –80°C. Genomic DNA was extracted using a CTAB method according to Saghai-Maroof *et al.* (1984).

Microsatellite analysis. To study the collection of barley and oat accessions a set of 26 microsatellite markers per each crop were chosen from several publications (Liu *et al.* 1996; Russel *et al.* 1997; Ramsay *et al.* 2000; Li & Rossnagel 2000; Pal *et al.* 2002). They are listed in Table 2 including repeat motif, annealing temperature and number of detected alleles per each microsatellite

Table 1. Summary of barley and oat accessions and their country of origin

State	No. of accessions		State	No. of accessions	
	barley	oat		barley	oat
Argentina	0	3	Italy	2	3
Australia	1	7	Japan	4	1
Austria	7	7	Lithuania	0	2
Belgium	0	4	Netherlands	5	7
Brazil	0	1	New Zealand	0	2
Bulgaria	7	3	Norway	0	1
Canada	3	6	Poland	7	13
Czechoslovakia	12	63	Portugal		3
Denmark	1	5	Romania	5	1
Estonia	0	1	Spain	1	0
Ethiopia	1	0	Sweden	2	14
Finland	0	1	Turkey	2	0
France	11	15	U.S.S.R.	27	14
Germany	42	43	United Kingdom	4	12
Greece	1	0	Uruguay	0	1
Hungary	7	7	USA	15	24
China	1	2	Yugoslavia	3	2
Ireland	1	1	Algeria	1	0
Israel	0	1	Korea	3	0
			unknown	0	60
Total			38	176	330

locus. PCRs with fluorescently labelled primers (6-fam, vic, ned and pet) were performed in a reaction volume of 15 µl according to the optimised protocol. Reactions were run in the cycler UNO II (Biometra). The analysis of PCR products was performed using the method of capillary electrophoresis on the sequencer ABI PRISM 3130 (Applied Biosystems). A multiplexed configuration of four reactions was used in one analysis. As to the interne size standard LIZ500 (Applied Biosystems) was used. Electrophoretograms were processed by GeneMapper software.

Data analysis. For each locus, the presence or absence of bands in each size category through all genotypes was scored. Data were set in a binary matrix. Genetic similarities were calculated using the Hamman coefficient and dendrogram as well as PCA (Principal Component Analysis) was obtained by clustering according to the UPGMA method using Statistica for Windows software (StatSoft, Inc., 2005).

The genetic diversity index (DI) based on Gini-Simpson index (Gini 1912):

$$DI = 1 - \Sigma p_i^2$$

where:

p_i – frequency of the i^{th} allele or the frequency of i^{th} pattern

was used as the measure of marker or locus and/or information content.

RESULTS AND DISCUSSION

Cultivars were sampled in 38 countries all over the world. They were chosen as expert core collections from Gene Bank accessions so all distinct genotypes of barley and oat were represented. Leaves from about 30 plants in the stage of 2 leaves were used for DNA extraction and microsatellite analysis. In barley, at least three microsatellite markers were chosen per chromosome. In oat

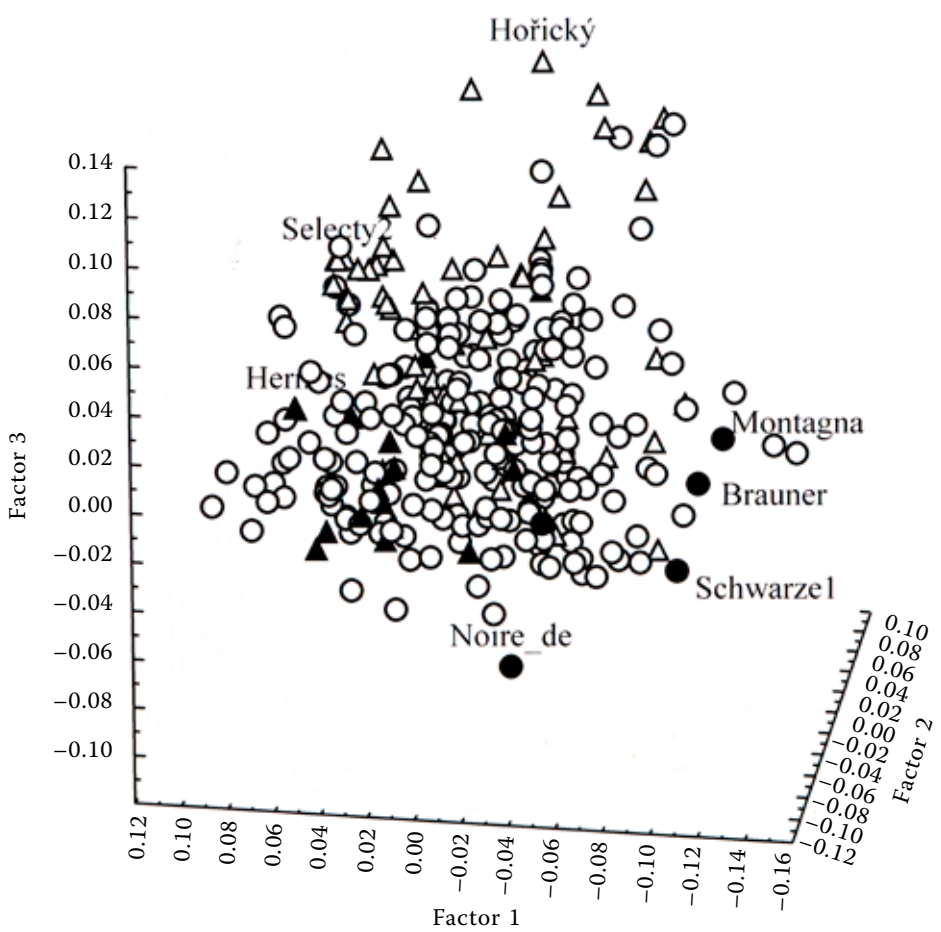

Figure 1. Three-dimensional principle component analysis (PCA) plot of oat genotypes based on the unweighted pair-group method (UPGMA) clustering analysis of microsatellite data; ▲ – modern Czech varieties, △ – Czech landraces, ● – non-Czech outliers, for example Noire de l'Aube, ○ – non-Czech other oat varieties

Table 2. A list of barley and oat microsatellites used in this study

Barley SSR primer-label	Chro-mosome	Motif type	Annealing temperature (°C)	Product sizes (bp)	No. of alleles	DI_P*
BMS02-6FAM	1H	(CA)21	60	190–228	11	0.511
BMS32-VIC	1H	(AT)5(CA)22	60	192–276	28	0.885
BMS90-NED	1H	(GA)8(AT)9(CA)20	60	187–237	24	0.886
HVM36-PET	2H	(GA)13	60	104–140	9	0.835
HVM54-6FAM	2H	(GA)14	60	107–165	12	0.660
HVBKASI-VIC	2H	(C)10(A)11	60	178–200	5	0.617
Bmag0136-NED	3H	(AG)6-(AG)10-(AG)6	60	184–202	3	0.155
HVM60-PET	3H	(AG)11,(GA)14	60	107–120	8	0.819
HVM62-6FAM	3H	(GA)11	60	235–265	9	0.669
HVM40-VIC	4H	(GA)6(GT)4(GA)7	55	131–161	10	0.612
HVM67-NED	4H	(GA)11	60	99–119	9	0.798
BLYRCAB-PET	4H	(AT)29	55	160–218	23	0.862
EBmac0906-6FAM	4H		55	131–157	6	0.765
Bmac0181-VIC	4H	(AC)20	55	158–184	8	0.671
Bmag0222-NED	5H	(AC)9(AG)17	50	148–184	8	0.621
Bmag0394-PET	5H	(AG)9CG(AG)4CG(AG)4	55	161–179	5	0.638
HVM30-6FAM	5H	(CA)8	60	135–155	7	0.684
Bmag0500-VIC	6H	(AG)7C(AG)30-(AG)6	60	139–209	26	0.921
BMS18-NED	6H	(CT)9(CA)12	60	123–141	10	0.748
BMS40-PET	6H	(CA)21(GA)21	60	177–237	19	0.752
Bmag0021-6FAM	7H	(CA)10AA(GA)28	55	99–169	16	0.744
Bmag0135-VIC	7H	(AG)10GG(AG)12	60	117–157	9	0.648
HVCMA-NED	7H	(AT)9	60	119–139	7	0.687
Bmag0496-6FAM	6H	(CT)20	60	170–227	23	0.910
Bmag0807-VIC	6H	(TC)18	55	95–113	8	0.723
HVM74-NED	6H	(GA)13	55	168–246	25	0.884
Total					328	0.719

where the microsatellites have not been mapped on chromosomes yet, the choice was random.

Most amplification products revealed only one allele per genotype. When two or three alleles were present at a single locus and genotype, they were all taken into account because especially landraces and old varieties had a character of populations with several different lines and some of them could carry agronomically interesting alleles. Two microsatellite loci by oat were extremely variable: AM4 and AM6 with the number of alleles 44 and 49 resp. and with up to four alleles per genotype. In total, 328 alleles were detected from the 26 amplified loci in barley and 353 alleles from the same number of microsatellite loci in oat. In barley, the total number of alleles per locus ranged from 3 to 28, with an average of 12.6 alleles per locus. In oat, the total number of alleles per locus ranged from 1 to 49, with an average of 13.6 alleles per locus. It is the same average as it was found in bread wheat (13.6 alleles per locus). It means that wheat and oat collections show a similar level of genetic variability. The level of barley collection genetic variability is lower.

Diversity index (DI) ranged from 0.02 to 0.18 with the average of 0.11 in barley. In oat, DI ranged from 0.01 to 0.17 with the average of 0.09. In comparison with DI of 0.65 found in wheat analysed

Table 2 continued

Oat SSR primer-label	Motif type	Annealing temperature (°C)	Product sizes (bp)	No. of alleles	DI_p*
AM1-6FAM	(AG)21(CAGAG)6	60	157–225	18	0.938
AM2-VIC	(AG)24	50	129–163	17	0.861
AM3-NED	(AG)35	60	243–325	32	0.943
AM4-PET	(AG)34	60	133–227	44	0.996
AM6-6FAM	(AG)20	60	205–329	49	0.988
AM13-VIC	(AG)15	46	182–206	8	0.166
AM14-NED	(AC)21	60	111–161	18	0.831
AM20-PET	(TG)10(CG)5	60	245–263	2	0.018
AM25-6FAM	(AC)8(AC)4(CT)4	60	215–233	4	0.539
AM40-VIC	(GAA)7	60	235–255	3	0.012
AM42-NED	(GAA)16	60	177–213	7	0.674
AM47-PET	(AC)14	60	255–273	7	0.592
AM53-6FAM	(AC)10	60	245–323	9	0.590
AM54-VIC	(AC)9	60	177–181	3	0.706
AM61-NED	(TTTC)4..(CCT)6	60	206	1	0.042
AM83-PET	(AC)11	46	187–197	4	0.490
AM84-6FAM	(AC)9	46	116–192	27	0.781
AM87-VIC	(AC)13	60	148–170	8	0.405
AM103-PET	(AC)16	46	189–193	2	0.226
AM92-6FAM	(AC)13	60	121–161	17	0.904
AM102-NED	(AC)9	60	201–251	8	0.483
AM115-6FAM	(AC)9	60	209–213	3	0.261
AM104-6FAM	(AG)36	60	185–223	5	0.181
AM107-VIC	(AC)3(AC)4	60	265–267	2	0.110
AM112-NED	(AG)3(AC)9(AT)8	60	231–297	17	0.479
AM114-PET	(AG)24(AC)14	60	175–267	38	0.955
Total/Average**				353	0.545

*DI_p – diversity index calculated for each microsatellite locus; it represents in what likelihood two analysed genotypes will differ in their patterns per locus

**the average is calculated for locus diversity index DI_p

with 42 microsatellite loci (Roussel *et al.* 2005), the collections of oat and barley showed a lower level of variability. Diversity index calculated per microsatellite loci (H) ranged from 0.155 to 0.921 with an average of 0.719 in barley and from 0.018 to 0.996 with an average of 0.545 in oat (Table 2). DI_p index represents the information content of each microsatellite locus and its values are dependent on the quality of each microsatellite locus and on the number of analysed genotypes. Some loci have a good information value, for example Bmag 0500 and Bmag 0496 in barley and AM4 and AM6 in oat, with regard to 176 and 330 analysed barley and oat genotypes, respectively. Some loci with low DI_p index could be left out in next analyses.

Cluster analysis could clearly identify the genetic relationship of barley and oat genotypes and demonstrates the potential and ability of microsatellite markers for genome analysis in both crops. In oat, the UPGMA cluster analysis established from the similarity data reflects mainly geographical origins of the accessions. Out of 14 main clusters, the

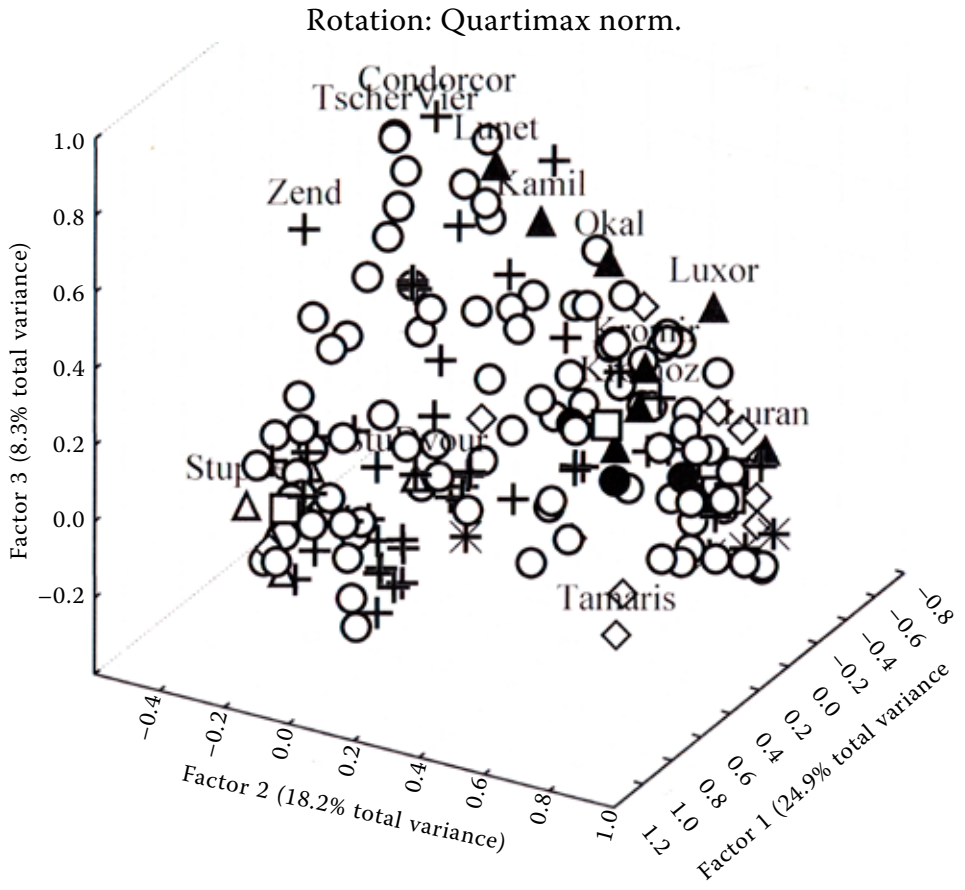

Figure 2. Three-dimensional principle component analysis (PCA) plot of barley genotypes based on the unweighted pair-group method (UPGMA) clustering analysis of microsatellite data; ▲ – Czech modern varieties, △ – Czech landraces, ● – European very modern barley varieties launched in the list of registered varieties in the Czech Republic, ✱ – varieties from the United Kingdom, ◇ – French varieties, □ – Dutch varieties, ○ – the rest of European varieties, + – varieties from former Soviet Union, America, Australia and from Asia

Czech lines/varieties are included particularly in five clusters. Varieties from America, Australia and Asia are involved in five clusters. The other clusters are formed mainly by European genotypes. Factors that also influenced the cluster composition are the year of variety release and its pedigree especially in modern varieties, for example Ardo, Athego and Radius. PCA (Figure 1) grouped all genotypes into two main groups that form together the shape of tetrahedron. Landraces and old varieties are present in one group, the other group consists of modern varieties. The older varieties or landraces are the more on the periphery of clusters they are found. Varieties having similar pedigrees are grouped very close together. So, the character of grouping, similarly like the low value of diversity index (DI), reveals a low variability of the oat genotype collection and shows negative impacts of oat breeding on the genetic diversity. Fu *et al.* (2003) who studied the diversity of 96 Canadian oat cultivars released from 1886 to 2001, detected a significant decrease of SSR alleles in cultivars released after 1970 and in some specific breeding programs. They concluded that allelic diversity was sensitive to oat breeding practices and eventually, the introgression of new alleles would be needed to overcome a possible "genetic ceiling" in oat improvement to avoid genetic vulnerability to biotic stresses and to widen crop adaptation to new environments (Fu *et al.* 2003). Our results confirm these findings and emphasize the necessity of oat germplasm conservation.

Cluster analyses based on microsatellite data of barley genotypes showed that diversity in the studied sets was not randomly distributed. Country of origin, age of variety and botanical characters influenced the distribution of barley varieties into clusters to a large extent. Out of all 14 clusters,

two are composed only of two-rowed varieties. The remaining two-rowed varieties occur in other 8 clusters. Czech modern varieties are grouped together in one cluster, the old Czech varieties and landraces are found in other two clusters. Varieties from America, Australia and Asia are found in the left part of the dendrogram, mainly in 5 clusters together with genotypes from the Soviet Union. The other Russian varieties occur in two clusters together with varieties from Germany and from the southern part of Europe. Varieties from Germany are in all clusters with the exception of four clusters in the left part of the dendrogram. None of the clusters is composed of genotypes originating from the same country, not even from the same continent. Struss and Plieske (1998) obtained the same results: they observed no correlation between geographical origin of cultivars/landraces and classification. The intensive exchange of seed material might have caused a misalignment of accessions to a particular origin of cultivars or advanced landraces.

PCA based on microsatellite data of barley genotypes showed two basic clusters (Figure 2). The older varieties and landraces are present in the smaller one, and the bigger one is composed of modern varieties. The most modern varieties and varieties with very good malting quality are found in the bottom part of the bigger cluster. This "trend" is followed also by Czech varieties: more modern varieties and varieties with better malting quality are situated closer to the bottom of the bigger cluster (Figure 2 – labelled varieties: Kamil, Lunet, Luran, etc.). Several genotypes are very close to each other, for example: Tschermaks Vierzeilige (Glatte) and Vogelsanger Gold. They are possibly the same variety or two varieties bred by selection from the same landrace.

The variability of both crops, barley and oat, cannot be explained only by the geographic origin or by the age of variety but by all the noted factors together. It depends on the character of each crop which factor plays the most important role in the constitution of genetic diversity. More analyses should be done on a set of genotypes where the information about the origin, pedigree, and the year of release (the age of genotype) is complete. Our intention was to provide a molecular tool to identify and characterise barley and oat accessions, estimating the genetic diversity and providing a set of genetically defined materials for experimental and breeding purposes. Microsatellites provide an optimal system with which to increase the efficiency of germplasm evaluation and to identify duplicate accessions in the barley and oat germplasm collections.

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