The Application of Microsatellite Analysis in Barley Malting Quality Breeding Programmes

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Abstract

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A set of 43 microsatellite loci was used to characterise the malting barley varieties and breeding lines used for the Protected Geographical Indication (PGI) "České pivo" ("Czech beer") and for other beer types. Genotype data were compared with technological malting quality parameters. The analysis of variance showed significant differences between varieties used for the given PGI and varieties used for other beer types in six variables. Three clusters of varieties specific to the PGI and three clusters specific to malting barley genotypes for other beer types were identified using a Bayesian approach. The remaining four clusters expressed effects of parent lines. The discrimination analysis based on malting quality and molecular variables identified only one barley breeding line suitable for the PGI "České pivo"".

Keywords: "České pivo"; discriminant analysis; diversity; markers; SSR

Barley (*Hordeum vulgare* L.) is one of the most adapted plant species grown around the world. In the Czech Republic, barley takes up the fourth largest production area of all agricultural plants and is the fourth most important agricultural plant, mainly due to its use as the raw material for malt and subsequent beer production. Beer has hundreds of years of history and the tradition of brewing has been passed from generation to generation. In the 19th century, the type of beer designated as Czech or Pilsen was well established (Kosař *et al.* 2004). In 2008, the name "České pivo" was added to the list of products from the Czech Republic entered in the Register of Protected Designations of Origin and Protected Geographical Indications (Official Journal 2008). According to the Official Journal of the European Union, the distinctiveness of the protected geographical indication (PGI) "České pivo" is based on many factors, including raw material (malt and hop) and special brewing procedure. Pale malt, also called "Pilsen malt", made from two-row spring barley, is used for brewing České pivo (Psota 2008).

Quality requirements for malting barley are directly related to the processing efficiency and product quality in the malting and brewing industries. The current requirements for the quality of malting barley dictate that only varieties possessing high enzymatic activity, high content of extract and high values of final attenuation may be used. Barley varieties intended for the PGI "České pivo" production are characterised by a lower level of proteolysis, cytological changes leading to cleavages and attenuation, resulting in the presence of residual extract in the final product (Kosař *et al.* 2004; Psota 2008).

Malting quality is a complex character, controlled by multiple genes with strong interactions with the environment (Molina-Cano *et al.* 1997). Quantitative trait locus (QTL) methods represent a step forward in applied genetics, allowing the assessment of the localisation of numerous genes involved in phenotypic traits. The first systematic QTL mapping of malting quality traits in barley was reported by Hayes *et al.* (1993), in which 62 QTLs of 8 malting quality traits were mapped. Since then, several QTL

analyses of malting quality have been reported for different germplasm combinations (Ayoub *et al.* 2002; Emebiri *et al.* 2004; Walker *et al.* 2013; and many others). Microsatellites very often used in QTL mapping are simple sequence repeats (SSR) interspersed ubiquitously in eukaryotic genomes. The main advantages of SSR are the following: high levels of polymorphisms and information content; unambiguous designation of alleles; selectively neutral markers; high reproducibility; codominance; and simple assaying of genotypes. SSR have been used extensively for genome mapping and cultivar identification (Goldstein & Schlötterer 1999; Pillen *et al.* 2000).

Gianinetti *et al.* (2005) proposed a system of malting quality evaluation based on only a few traits, depending on environmental conditions as little as possible. This system was intended for breeders for line testing in early generations of the selection process. The same method of discriminant analysis was used for discrimination between barley germplasms suitable for PGI "České pivo" (group C) and for other conventional beers (group E). Several hypotheses were tested in this study: (a) whether there is any difference in the allelic composition of microsatellites between the two groups of barley varieties (C, E); (b) if yes, whether it is possible to use microsatellite analysis to choose breeding lines during the selection process. To address these hypotheses, fifty breeding lines were analysed.

MATERIAL AND METHODS

Plant material and DNA extraction. Twenty barley varieties and 50 breeding lines were chosen for this study (Table 1). Four barley varieties previously classified into group C (PGI "České pivo"), four varieties belonging to group E (other malting barley) and 12 varieties used only for genotyping studies were selected as standards of SSR alleles. Barley varieties classified into group C and E were grown in three localities (Table 1). Harvested seed samples were sent to the Research Institute of Brewing and Malting , Prague (RIBM) for the determination of technological parameters and to the Crop Research Institute in Prague (RICP), where genotyping was performed. Breeding lines and allele standards were classified as group O.

In RICP, plants were grown in greenhouse conditions and approximately 30 plants per accession were pooled and frozen at –80°C. Genomic DNA was extracted using CTAB detergent (Saghai-Maroof *et al.* 1984). The quality and concentration of DNA were verified using agarose gel electrophoresis. A λ HindIII (Fermentas, Lithuania) marker was used as a size and concentration standard.

Micromalting protocol and technological parameter determination. Technological parameters were determined in malt and sweet wort according to the EBC (2009) and MEBAK (2011) methods. The malting schedule consisted of an initial wash for 15 min. to remove surface dust from the barley kernel followed by a 5-hour steep on the first day, 4-hour steep on the second day, and a third day of spraying or steeping to reach a grain water content of 45.5%. Water and air temperature during the air interval was held at 14.5°C. The barley then underwent germination for 144 h at a temperature of 14.5°C with constant air passing through revolving drums. Kilning was performed in a one-floor electrically heated kiln for 22 h. Pre-kilning was carried out at 55°C and kilning at a temperature of 80°C for 4 h. Technological parameters, namely the protein content of the barley grain (Pb, in %), extract of malt (E, in %), Kolbach index (KI, in %), diastatic power (DP, in WK), apparent final attenuation (AFA, in %), friability (F, in %), percent partly unmodified grains (PUG, in %), β-glucan content in wort (BGw, in mg/dm^3), colour of malt (Cw, in EBC units) and viscosity of wort (Vw, in mPa·s) were measured according to EBC (2009) and the relative extract at 45°C (VZ 45, in %) according to MEBAK (2011) and bulk density of barley (Bdb in g/l).

Microsatellite analysis. A set of 43 microsatellite markers was chosen from several publications (Becker & Heun 1995; Liu *et al.* 1996; Russel *et al.* 1997; Ramsay *et al.* 2000) to allow at least four microsatellite loci per chromosome. PCR reactions with fluorescently labelled primers (6-fam, vic, ned and pet) were performed in a reaction volume of 15 µl consisting of 1U of *Tth* polymerase (Biotools, Madrid, Spain), 1× PCR buffer, 2mM of $MgCl_2$, 0.25mM of each dNTP, and 0.33µM of each primer. Reactions were performed in a Labcycler (SensoQuest, Goettingen, Germany). Amplification products were analysed using capillary electrophoresis on an ABI PRISM 3130 genetic analyser (Applied Biosystems, Foster City, USA). A multiplexed configuration of four reactions was used in one analysis. LIZ500 (Applied Biosystems) was used as a size standard. Electrophoretograms were processed by GeneMapper software (Applied Biosystems).

Data evaluation. Cluster analysis was performed to study the relationships among genotypes. On the basis of the presence or absence of an amplification

Table 1. Barley varieties and lines used in the study

DNA samples	Group	Name of variety	Localities of growing			K	DNA samples	Group	Name of variety	Localities of growing	K
1, 2	C	Aksamit (ST)	ST	PO	HE	8	57		ST_804/12	ST	1+4+8+10
3, 4, 5	C	Blaník (ST)	ST	PO	HE	2	58		ST_810/12	ST	7+8+9
6, 7, 8	C	Bojos (ST)	ST	PO	HE	2	59		ST_815/12	ST	1+10
9, 10, 11	C	Malz (ST)	ST	PO	HE	6	60		ST_816/12	ST	4
12, 13, 14	E	Kangoo (ST)	ST	PO	HE	1+10	61		ST_818/12	ST	4
15, 16, 17	E	Sebastian (ST)	ST	PO	HE	1	62		ST_819/12	ST	4
18, 19, 20	E	Xanadu (ST)	ST	PO	HE	1	63		ST_832/12	ST	10
21, 22, 23	E	Zeppelin (ST)	ST	PO	HE	9	64		ST_857/12	ST	1+5
24		HE_2488/2.6			HE	2+7+9	65		ST_858/12	ST	1+5
25		HE_2490/2.11			HE	2+3+7	66		ST_860/12	ST	1+4+10
26		HE_2499/2.1			HE	1	67		ST_863/12	ST	8
27		HE_2499/2.4			HE	1	68		ST_884/12	ST	8+10
28		HE_2500/2.3			HE	1+2+7	69		ST_886/12	ST	1+3+7
29		HE_2501/2.10			HE	7+10	70		ST_888/12	ST	4+8
30		HE_2508/2.4			HE	2+7+10	71		ST_890/12	ST	1+6+8
31		HE_2523/2.6			HE	1+10	72		ST_903/12	ST	8
32		HE_2549/2.5			HE	1+10	73		ST_912/12	ST	1+8+10
33		HE_2583/2.5			HE	2+10	74		Beate	SSR allele standard	3+8
34		HE_2642/2.13			HE	7					
35		HE_2645/2.1			HE	7+10	75		CI 739	SSR allele standard	3
36		HE_2669/2.4			HE	2+9+10					
37		HE_2713/2.15			HE	1+2	76		Diplom	SSR allele standard	2+8
38		HE_2716/2.13			HE	5+10					
39		HE_2716/2.5			HE	1+10	77		Forum	SSR allele standard	3+7+8
40		HE_2722/2.14			HE	7+8+10					
41		HE_2722/2.2			HE	7+8+10	78		Heris	SSR allele standard	5
42		HE_2729/2.2			HE	2+6+7					
43		HE_2730/2.1			HE	2+10	79		Jersey	SSR allele standard	3+7
44		HE_2745/2.3			HE	2+3					
45		HE_2751/2.8			HE	2+10	80		Kompakt	SSR allele standard	3
46		HE_2755/2.10B			HE	2+10					
47		HE_2768/2.2			HE	2+7	81		PI 31900	SSR allele standard	3
48		HE_2819/2.2			HE	5+8					
49		ST_704/12	ST			1+2+8+10	82		Prestig	SSR allele standard	7+8
50		ST_705/12	ST			8+10					
51		ST_722/12	ST			8+10	83		Radegast	SSR allele standard	2+5+8
52		ST_723/12	ST			1+8+10					
53		ST_747/12	ST			1+2	84		Tifang	SSR allele standard	3
54		ST_793/12	ST			1+4+8					
55		ST_798/12	ST			1+4	85		Tolar	SSR allele standard	3+8
56		ST_800/12	ST			1+5					

C – protected geographical indications (PGI) "České pivo"; E – other types of beer; ST – Stupice, PO – Pohořelice; HE – Hrubčice; KR – Krukanice; K – classification of the barley genotypes into ten inferred clusters K with the probability $P > 0.10$

product, binary data matrices were built. A dissimilarity matrix was computed with DARwin software using the Jaccard coefficient (Perrier *et al*. 2003; Perrier & Jacquemoud-Collet 2006). A dendrogram was constructed using an unweighted neighbour joining method. Bootstrap analysis with 2000 replicates was performed to estimate the robustness of a tree. The degree of the subdivision of the two groups of barley genotypes was measured by Wright's fixation index (F_{st}). An exact test for population differentiation was calculated using the Tools for Population Genetic Analyses (TFPGA; version 1.3; Miller 1997) with 10 000 permutation steps.

Another approach to studying genetic diversity is based on Bayesian statistics. The Structure version 2.3.4 software (Pritchard *et al.* 2000) was used to determine the genetic architecture of barley genotypes. Ten independent runs of 1–20 groups (K = 1–20) were performed using 10 000 Markov chain iterations after a burn-in period of 10 000 iterations. The number (K) of clusters into which the sample data (X) were fitted with posterior probability Pr (X|K) was estimated using a model with admixture and correlated allele frequency (Falush *et al.* 2003). The optimal value of K was estimated based on ln(K) and on the ΔK calculation, which considers the rate of change in the lnP(D) values among successive K runs to account for patterns of dispersal that are not homogeneous among populations (Evanno *et al.* 2005).

ANOVA and discriminant analysis were performed using Statistica software (StatSoft, Prague, Czech Republic). Before analysis, arcsin transformation of the data given in percentages was performed. The discriminant power of the proposed model was then tested by Wilks' criterion λ. The classification of the samples into groups was performed on the basis of their Mahalanobis distance and a posteriori probabilities.

RESULTS AND DISCUSSION

Several quality parameters have been used for the evaluation of malting barley. In this study, malting technological parameters and microsatellite analysis were followed by 58 barley accessions.

Genotyping analysis was performed on four barley varieties from group C and four varieties from group E grown in three localities, together with 50 breeding lines and 12 microsatellite allele standards (Table 1). In total, 86 samples were analysed. Microsatellite analysis was carried out with 43 microsatellite loci. At least three microsatellite markers were chosen per chromosome. The total number of alleles per locus ranged from 1 to 11, with an average of 5.5 alleles per locus. This level of barley collection genetic variability is rather low. It is even lower than in a previous study in which an average of 12.6 alleles per locus was found (Leišová *et al.* 2007). This indicates that the breeding process pointed only to selected features, which leads to a decrease in the genetic diversity, as previously mentioned (Vellvé 1993).

While each of the barley varieties, Bojos, Malz, Blanik, Sebastian, Kangoo, Xanadu and Zeppelin, always showed the same microsatellite allele composition regardless of the growing locality, Aksamit grown in Stupice differed a lot in allele composition. As this was most likely the result of sample confusion, this item was omitted from the subsequent analyses.

Cluster analyses based on the microsatellite data of barley genotypes is given in Figure 1. There are five main clusters with low bootstrap support (< 50%). While the main clusters are not statistically significant, each of the barley varieties declared as group C or E, and several other barley genotypes, form clusters with high bootstrap support (Figure 1). Varieties Bojos and Blaník occur in one cluster and Xanadu, Sebastian and Kangoo are involved in another cluster. On the other hand, Aksamit occurs in a cluster together with Zeppelin and with those of allele standards showing a good resistance to several fungal pathogens.

Exact tests of population differentiation were carried out to test the null hypothesis that there is no difference in genetic variability between barley varieties classified into groups C and E. Only eight barley varieties grown in three localities were taken into account in this analysis. The analysis was performed three times with 1 000, 10 000 and 20 000 permutations, and *P*-values were significant (0.0006, 0.0004, 0.0003, respectively) in all three analyses. Therefore, the null hypothesis could be rejected. The differences between the barley varieties recommended for the production of "České pivo" and the varieties recommended for other types of beer were found at the level of the technological parameters, although they were also influenced by external factors (Psota 2008). In this study, the analysis of variance of the technological parameters found significant differences between C and E in the malting quality index ($P = 0.048$), relative extract at 45°C ($P = 0.003$), Kolbach index ($P = 0.011$), β-glucan in wort ($P = 0.013$) and colour of malt ($P = 0.032$) (Table 2). The most significant differences were found between C and E

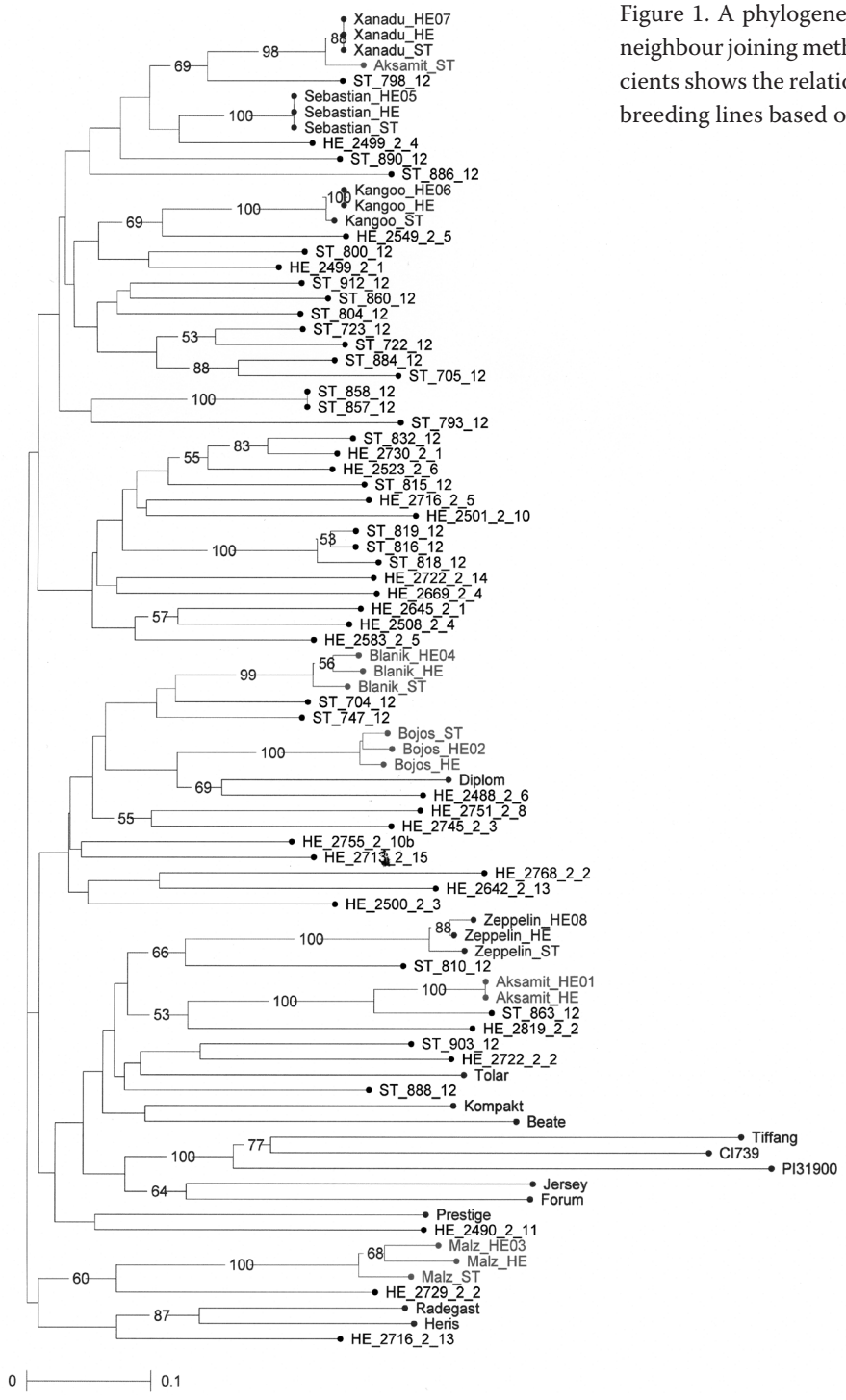

Figure 1. A phylogenetic tree formed by the unweighted neighbour joining method with Jaccard dissimilarity coefficients shows the relationship between barley varieties and breeding lines based on microsatellite analysis

in the relative extract at 45°C and the β-glucan in wort that are the least affected by locality and year (Dráb *et al.* 2013). The relative extract at 45°C indicates the level of proteolytic modification and it is an important feature especially in Central Europe (Psota & Kosař 2002).

The genetic structure of barley genotypes was also evaluated using a Bayesian approach as implemented by the Structure software to find the number of diverged groups hidden in the data. According to the ΔK value, ten clusters (K = 10) were identified within the studied set of barley genotypes. The mean value of α was 0.059, indicating that most of the barley genotypes are homogeneous. In other words they belong genetically to only one cluster (Falush *et al.* 2003). Based on the proportion of the membership of each population in each of the 10 clusters, clusters 2, 6 and 8 consisted of barley varieties C (Aksamit,

Table 2. Differences in average values of malting quality technological parameters of group C and E of barley varieties

Technological parameters (abbreviation, unit)	Mean ± SD		ANOVA	
	C	E	*F*	*P*
Protein content of the barley grain (Pb, %)	13.33 ± 2.23	12.86 ± 2.37	0.218	0.654
Extract of malt (E, %)	80.95 ± 1.52	81.83 ± 1.42	1.790	0.195
Relative extract at 45°C (VZ45, %)	40.85 ± 3.44	46.63 ± 4.42	11.167	0.003
Kolbach index (KI, %)	39.31 ± 3.75	43.11 ± 2.43	7.682	0.011
Diastatic power (DP, WK)	387.64 ± 88.60	391.67 ± 123.65	0.007	0.933
Apparent final attenuation (AFA, %)	79.92 ± 1.05	80.75 ± 0.87	3.940	0.060
Friability (F, %)	81.82 ± 8.26	84.5 ± 8.36	0.604	0.446
β-glucan content in wort (BGw, mg/l)	164.28 ± 55.42	103.08 ± 47.67	7.392	0.013
Percent partly unmodified grains (PUG, %)	2.6 ± 2.26	1.67 ± 1.11	0.304	0.587
Viscosity of wort (Vw, mPa·s)	1.48 ± 0.03	1.47 ± 0.02	0.680	0.420
Colour of malt (Cw, EBS u)	3.31 ± 0.43	3.80 ± 0.53	5.291	0.032
Bulk density of barley (Bdb, g/l)	69.67 ± 1.51	69.20 ± 0.96	0.750	0.340

SD – standared deviation

Blanik, Bojos and Malz) with an incidence of more than 0.10 (Table 3). Clusters 1, 9 and 10 included barley varieties from group E (Kangoo, Sebastian, Xanadu and Zeppelin). Cluster 3 is represented by barley material used as a source of resistances (CI739, PI31900 and Tiffang). In our experiment, these were used as standards of alleles. Genetic sources presented in clusters 4, 5 and 7 remain unknown. While the barley varieties appeared to be genetically uniform in this experimental configuration, barley breeding material appears to be a mixture of mostly parental genotypes (Figure 2). Breeding programs usually involve more than one intention, including breeding for quality, for yield, for different resistance to diseases, and other factors. That is why breeding lines can be a mixture of parental genotypes other than those classified into group C or E. A similar hidden genetic structure was found, for example, in the *Chrysoporthe cubensis* population (Van der Merwe *et al.* 2010).

The expected heterozygosity between individuals within the same cluster ranged from 0.01 to 0.25, with an average of 0.074 (Table 3). Figure 2 represents the cluster analysis of all individuals in the three populations (C, E and O) with K = 10. This analysis shows a limited admixture among barley varieties in groups C and E and a higher level of admixture among breeding lines and other varieties used as standards of alleles (Figure 2). Table 3 also shows F_{ST} values of each inferred cluster. Except for cluster 3 (F_{ST} = 0.01), all the clusters showed very high differentiation, which indicates the presence of genetically diverged groups in the data.

Table 3. The proportion of membership of each predefined population in each of the 10 clusters computed using a Bayesian approach to the results of the barley microsatellite analysis

Population		No. of individuals	Clusters									
			1	2	3	4	5	6	7	8	9	10
Varieties	C	11	0.01	0.53	0.00	0.00	0.00	0.27	0.00	0.18	0.00	0.01
	E	12	0.63	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.25	0.10
Breeding lines		62	0.17	0.11	0.12	0.07	0.07	0.02	0.08	0.15	0.03	0.18
F_{ST}		85	0.79	0.74	0.01	0.92	0.89	0.90	0.55	0.66	0.96	0.91
h		85	0.05	0.06	0.25	0.02	0.06	0.03	0.14	0.09	0.01	0.03

Mean value of alpha = 0.059; C – protected geographical indications (PGI)"České pivo"; E – other types of beer; F_{ST} – Fixation indices; h – expected heterozygosity between individuals within the same cluster

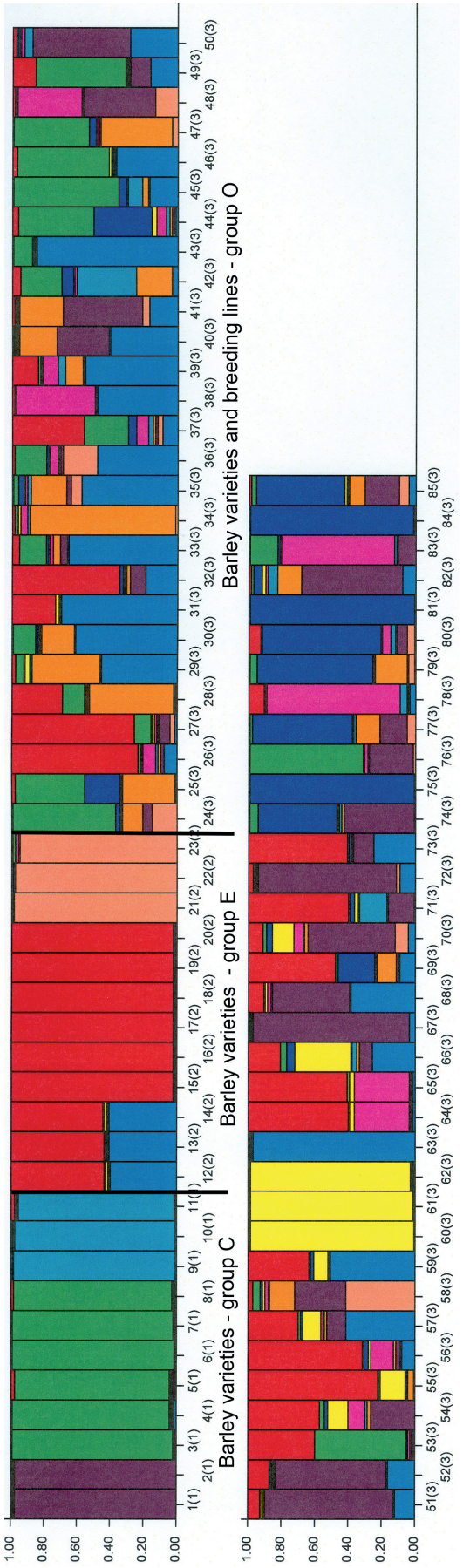

Figure 2. A cluster analysis of barley varieties, breeding lines and allele standards based on a Bayesian approach applied to the microsatellite analysis results; each genotype is represented by a bar, divided into K colours, where K is the number of clusters assumed: 1 – red; 2 – green; 3 – dark blue; 4 – yellow; 5 – violet; 6 – emerald; 7 – ochre; 8 – brown; 9 – apricot; 10 – light blue.; individuals are sorted according to the group they belong to: C – PGI "České pivo", E – other types of beer, O – breeding lines

Discriminant analysis is a classification method used to identify to which of a set of categories a new observation belongs. The purpose of the analysis in our study was to learn how breeding material could be distinguished in the three groups of barley genotypes (C, E and O) based on the technological parameters and microsatellite analysis data.

First, a model with only eight barley varieties with known membership in the two groups C and E and with only technological parameters was built. Highly correlated variables ($r \geq 0.9$) were left out. Out of the original 28 malting quality parameters, 18 were used in the next step of the analysis. Using a forward stepwise method, the following seven variables were added to the model: relative extract at 45°C, protein content in barley grain, final attenuation, β-glucan content in wort, bulk density of barley, viscosity of wort and partly unmodified grains. Wilks' λ value of 0.036 indicates the good discriminatory power of the model. The least value of partial Wilks' λ indicated that the variable relative extract at 45°C contributes most to the overall discrimination.

The proposed model was used in a second analysis where all varieties of all breeding lines were included in the evaluation. Given that the impact of non-malting genotypes was largely found in breeding lines, they were pre-classified as group O. First, the results of only the technological parameters were used. After the application of a forward stepwise method, the six variables mentioned above, except partly unmodified grains, remained in the model (VZ 45, Pb, AFA, BGw, Bdb, Vw). Wilks' λ increased to a value of 0.206. Discriminant functions (roots) computed by canonical analysis were considered statistically significant (root 1: $\chi^2 = 106.75$; $P < 0.001$; root 2: $\chi^2 = 14.34$; $P = 0.014$) but root 2 had much less discriminatory power. These results were visualised as a scatterplot of canonical scores (Figure 3a). The first discriminant function (root) discriminates between the varieties and breeding lines, and the second function provides discrimination between the barley genotypes of group C and E, but with less discriminatory power. These results were confirmed by further classification based on the Mahalanobis distances and posterior probabilities (Table 4). Despite breeding lines that were correctly classified into group O (100%), the percentage of well-classified barley varieties in class E was as low as 58%.

In the next analysis, the posterior probabilities data from the individual categorisation into 10 clusters computed by the Structure software were added. This approach has already been used by Gutierrez *et al.*

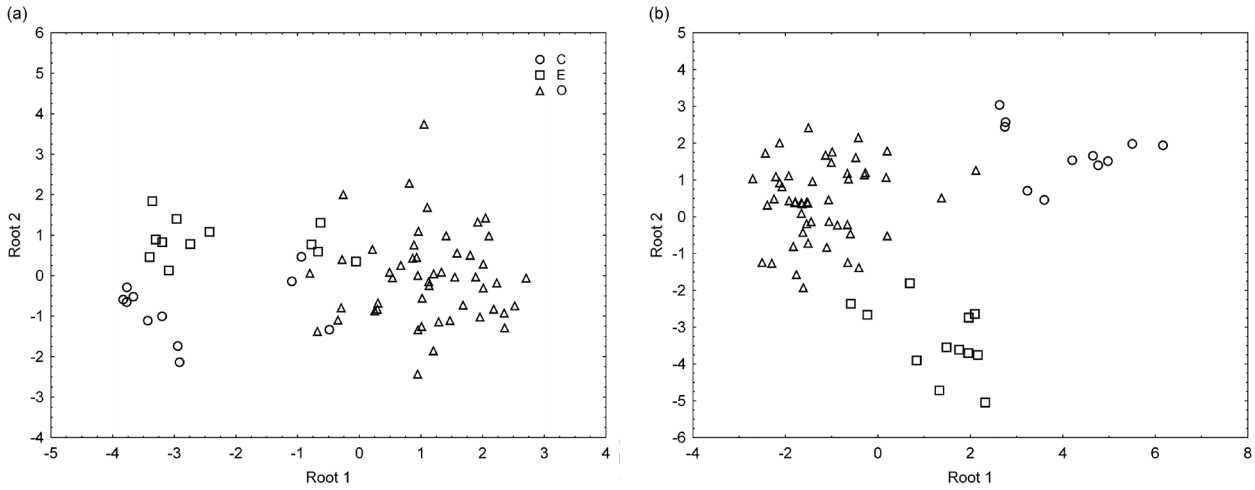

Figure 3. A scatterplot of canonical scores; C – group of barley genotypes for protected geographical indications (PGI) "České pivo"; E – other types of beer; O – group of breeding lines

(2011), who compared the results of technological parameters with SNP analysis to perform association mapping of barley malting quality. The discriminant function obtained by the forward stepwise method contains the following 11 variables: Pb, VZ45, BGw, Vw, PUG and clusters K1, K2, K5, K6, K8, and K9. Wilks' λ increased to a value of 0.056. Both discriminant functions (roots) were considered statistically significant (root 1: $\chi^2 = 187.41$; $P < 0.001$; root 2: $\chi^2 = 82.57$; $P < 0.001$). Figure 3b shows a scatterplot of two canonical scores. The three clusters correspond well to the three input groups. Using both roots, it is possible to discriminate between the barley genotypes of group C and E. Further classification based on the Mahalanobis distances and posterior probabilities (Table 4) proved the original classification of the barley varieties (Table 4). Only one misclassification was found in the breeding line ST704-12, which was classified by the model into group C, and thus, as suitable for PGI "České pivo". Based on the a posteriori probabilities, except for ST704-12 (91.1%), only one breeding line ST747-12 is closer (6.7%) to group C. The three breeding lines HE2499-2.1, ST886-12 and ST912-12 come close to being classified into group E with probabilities of 1.9%, 1.6%, and 4.8%, respectively.

Breeding for malting quality is difficult work, as many parameters must be tested. Many of them are highly correlated and give redundant information (Nielsen & Munck 2003). Moreover, they are often influenced by environmental factors and by technological treatment (Fox *et al.* 2003). Discriminant analysis can be a method for selecting the most suitable set of variables for distinguishing between selected features (Gianinetti *et al.* 2005). In our study, discriminant analysis was employed to distinguish between barley genotypes suitable or unsuitable for PGI "České pivo".

In conclusion, discriminant functions distinguishing between groups C, E and non-malting barleys were found. The model places weight on traits with low levels of environmental variability, including molecular markers that are not impacted by environmental factors. The structure analysis of genotyping data provided information about the genetic composition

Table 4. Classification matrices; a classification matrix based on the malting technological parameters only (a) and a classification matrix based on the malting technological parameters and molecular data (b)

Group	Percent correct	C $P = 0.151$	E $P = 0.164$	O $P = 0.685$
Malting technological parameters only				
C	72.73	8	0	3
E	58.33	1	7	4
O	100.00	0	0	50
Total	89.04	9	7	57
Malting technological parameters and molecular data				
C	100.00	11	0	0
E	100.00	0	12	0
O	98.00	1	0	49
Total	98.63	12	12	49

Rows contain the observed classification; columns include the predicted classification; C – protected geographical indications (PGI) "České pivo"; E – other types of beer; O – breeding lines

of the barley genotypes studied. Although only one promising breeding line was found, the model is ready for use in future barley breeding programmes.

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