

POTENTIAL OF SELECTED SSR MARKERS FOR IDENTIFICATION OF MALTING BARLEY GENOTYPES

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ABSTRACT

Cereals are main part of human nutrition and strategic resources. Thousands of barley varieties are used in food industry and especially for the production of malt in the brewing industry. In the present study, we were focused on utilization of SSR markers for differentiation and characterization of different barley genotypes. Barley collection was analyzed by 4 pure and 1 compound markers. Using STMS method we have revealed significant polymorphism. A total of 27 alleles were detected among 24 varieties with an average of 5.4 alleles per locus. Average values for index of diversity (DI), polymorphic information content (PIC) were 0.767, 0.756 respectively. The highest level of polymorphism was detected with SSR marker *Bmag 0222* (7 alleles) which also revealed one heterozygous variety. Dendrogram was created by hierarchic cluster analysis using UPGMA algorithm on the basis of detected alleles. Analyzed genotypes were divided into three clusters so it can be used to study genetic relations among collection of analyzed barley varieties.

Keywords: Barley, Genetic diversity, Simple sequence repeat (SSR), DNA marker

INTRODUCTION

Cereals are grown across the globe for several thousand years, and after all these years have been subjected to various exogenous and endogenous factors which have formed their respective genomes. Barley (*Hordeum vulgare*, L.) is economically important crops mainly for food production and malting, but the value is increased due to use as a functional food with a variety of health benefits **Hua et al. (2015)**. In order to increase the nutritional quality of grain, barley crop passed through the targeted selection and hybridization. The long process of barley breeding has resulted in increasing diversity of barley varieties **Ivandic et al. (2003)**. Growers can select the best variety according to specific climate and end use of this crop such as human consumption, malt in brewing and distilling industry or animal feeding **Ferreira et al. (2016)**. At present approximately hundred varieties of barley genotypes are registered in Slovakia, of which 20% have domestic origin. Wild barleys (*Hordeum spontaneum*) can be used as donors of important genes that could be used for improvement of different barley parameters **Shakhatreh et al. (2016)**. Molecular markers such as SSR, SNP, STS **Kojima et al. (2007)**, **Thormann et al. (2016)** or DArT **Lamaraet al. (2013)** are often used for genotype identification and characterization and thus may provide more accurate genome assessment **Yadav et al. (2015)**. Association mapping is a powerful tool in improving barley breeding via precise identification of markers significantly associated with important traits, which is vitally important for marker-assisted breeding **Abou-Elwafa (2016)**, **Elakhdar et al. (2016)**. Molecular markers are very useful for mapping and tagging the loci affecting malting quality **Han et al. (1997)** and largely fulfill most of the user requirements **Groven and Sharma (2016)**. Using molecular markers to select for specific

chromosome regions with potential positive yield contributions enhanced the breeding success for high yield while maintaining traditional malting quality **Schmierer et al. (2005)**.

The aim of this study was to analyze genetic diversity among the set of 24 barley genotypes using 5 SSR markers and to evaluate their ability to identify malting barley genotypes.

MATERIAL AND METHODS

Collection of 24 barley genotypes contained 8 winter form (Tiffany, Monaco, Cedeco, Premuda, Graciosa, Barcelona, Metaxa, Heidi) and 16 spring form (Malz, Kangoo, Overture, Signora, Nadir, Laudis 550, Karmel, Antigone, Troon, Prodeum, Valis, Exalis, Kumran, Kompakt, Novum and Madonna) barleys. Some of these genotypes are commonly used in brewing industry. DNA was isolated from dry whole grain according to the methodology for a commercial kit GeneJET Genomic DNA Purification Plant Mini Kit from the Thermo Fisher Company.

Microsatellite analyses were carried out in 25 µl volume using Biorad C 1000 Thermocycler. As primers we used specific oligonucleotide sequences 18-23 bp, described by **Ramsay et al. (2000)**. The amplification conditions for each microsatellite marker were set according to **Ramsay et al. (2000)** and **Varshney et al. (2007)**. Primers (Table 1) were selected on the basis of our previous research **Tomka et al. (2013)**, on the basis of best values for DI and PIC due to ability of differentiation.

Table 1 List of used SSR markers

Markers	Chromosome	Primer sequence (5'-3')	Repetition
<i>Bmac 0040</i>	6H	AGCCCGATCAGATTTACG TTCTCCCTTTGGTCCTTG	(AC) ₂₀
<i>Bmac 0134</i>	2H	CCAAGTGTGTCGATCTCG CTTCGTTGCTTCTCTACCTT	(AC) ₂₈
<i>Bmag 0125</i>	2H	AATTAGCGAGAACAAAATCAC AGATAACGATGCACCACC	(AG) ₁₉
<i>Bmag 0211</i>	1H	ATTCATCGATCTTGTATTAGTCC ACATCATGTCGATCAAAGC	(CT) ₁₆
<i>Bmag 0222</i>	5H	ATGCTACTCTGGAGTGGAGTA GACCTCAACTTTGCCTTATA	(AC) ₉ (AG) ₁₇

Amplified alleles were separated in the 6 % polyacrylamide gels denatured with urea. Preparation of the gel solution to a total volume of 100 ml: 18.5 ml 40 % acrylamid / bisacrylamid in ratio 19:1; 5 ml 10 x TBE (107.8 g Tris-base, 7.44 g EDTA and 55 g H₃BO₃ in 1L solution, pH 8.3); 20 ml redistilled water; 55.82 ml 66.3 % urea; 180 µl TEMED and 500 µl 10 % APS solution.

The separation of amplified alleles was conducted at OWLTM electrophoresis unit at maximum current 90 mA in a buffer system 1 x TBE for 3-4 hours, depending on the expected size of fragments. After electrophoretic separation of DNA, bands were visualized by silver staining followed **Benbouza et al. (2006)**: 5 minutes in fixative solution (10 % ethanol; 0.5 % acetic acid), 6-7 minutes in a dye solution (1.5 g AgNO₃, 1.5 ml 37 % H₂CO, H₂O to 1000 ml).

1 second washing in ultrapure water,

3-5 minutes in develop solution (15 g NaOH, 2 ml 37 % H₂CO, H₂O to 1000 ml), 2 minutes in fixative solution (10 % ethanol; 0.5 % acetic acid).

The PCR products separated in polyacrylamide gels were scanned using ChemiDoc Imaging System followed by evaluation using the GelAnalyzer 2010 software (**Lazar 2010**). On the basis of allele frequency diversity index (DI) (**Weir, 1990**) and polymorphic information content (PIC) (**Weber, 1990**) were calculated.

RESULTS AND DISCUSSION

Number of alleles per locus is an important indicator of genetic diversity. For some authors it is the only and main parameter for study of genetic relations. **Kalinowsky (2002)** statistically confirmed that it is not required to examine

highly polymorphic loci or large numbers of loci. The only requirement is that a sufficient number of alleles have been detected. Overall, we have identified 27 alleles at five loci which were located on chromosomes 1H, 2H, 5H and 6H. Number of allele per locus varied from 4 (*Bmag 0211*) to 7 (*Bmag 0222*) with average value of 5.4. Frequencies of detected alleles ranged from 4.0 % to 50.0 % (figure 1). **Shakhatreh et al. (2016)** analyzed comparable collection of cultivated barleys (27 genotypes) but have used 11 markers and therefore identified more alleles (95) with an average 8.6 allele per locus. **Yadav et al. (2015)** used even more markers (47 SSR's) but his collection of barley contained just 10 barley cultivars. Number of alleles per locus varied from 2 to 7 with an average 3.52 alleles per locus. Using markers *Bmac 0040* and *Bmag 0222* they have identified 5 and 6 alleles respectively, which is very close to our results. Similar results obtained **Nandh and Singh (2014)** who analyzed 27 wild and 20 cultivated barley accessions and identified 5 alleles at loci *Bmag 0125* and also *Bmag 0211* in cultivated barley collection. **Khodayari et al. (2014)** analyzed 40 wild barley genotypes and have identified from 1 to 17 alleles but SSR marker *Bmag 0125* amplified only 4 alleles. **Hua et al. (2015)** identified 204 alleles with mean value of 5.83 in collection of 277 colored barley varieties. Due to very large collection of genotypes, number of alleles detected in the same loci were much higher *Bmac 0040* (42 alleles), *Bmac 0134* (35 alleles), *Bmag 0222* (19 alleles). **Ferreira et al. (2016)** analyzed 64 Brazilian barley genotypes and in 34 different loci they have identified from 1 up to 18 alleles. Using marker *Bmag 0211* they have identified 6 alleles but in the case of marker *Bmag 0125* number of detected alleles (10) was double compared to our results.

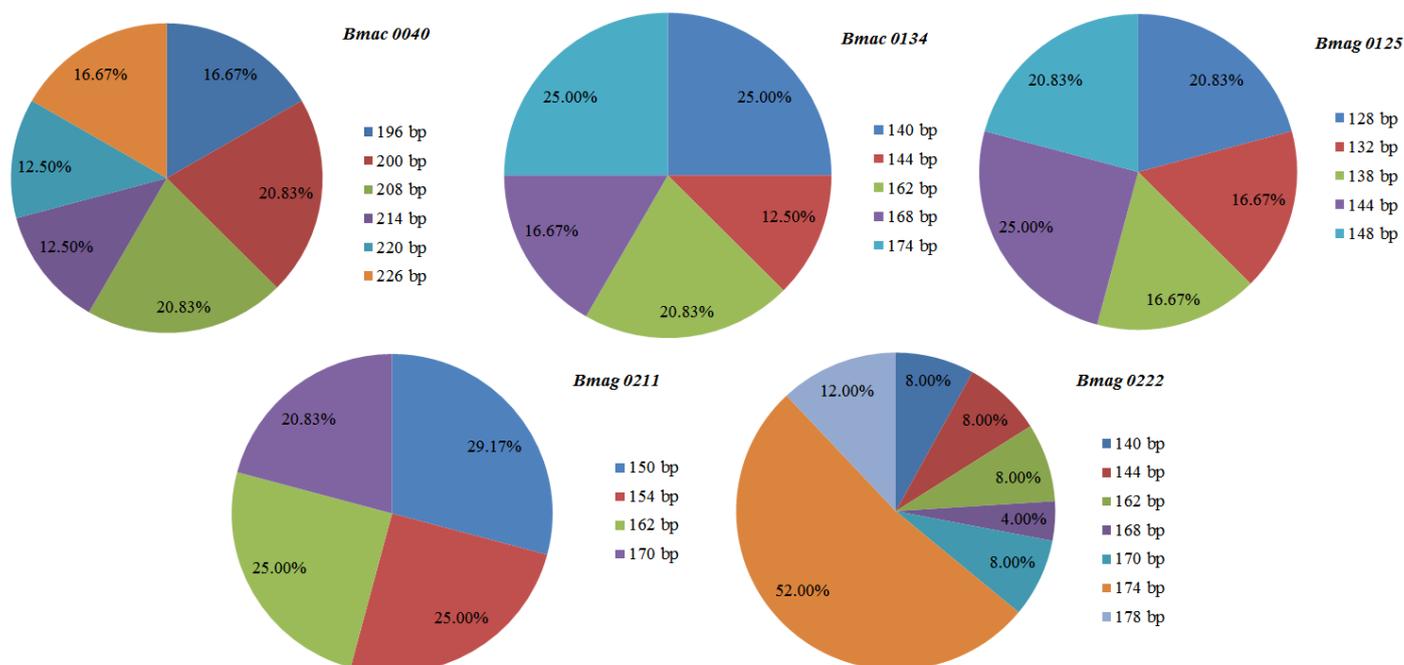


Figure 1 Preview of detected alleles in 5 SSR loci and their frequencies

Calculated indexes of DI, PIC are used to evaluate the level of polymorphism in analyzed collections of different species. The higher value for these indexes is calculated the better identification they provide (table 2). PIC values are used as a measure of a marker's usefulness for linkage analysis. Values of index diversity for our SSR markers were calculated and varied from 0.688 to 0.826 with a mean value of 0.767. For the next index (PIC) values ranged from 0.677 to 0.817 with an average value of 0.756. These values indicate that these markers are able to

detect high level of polymorphism because markers with DI and PIC values over 0.6 are considered as useful tool for genotype differentiation. **Nandh and Singh (2014)** in comparable collection of 20 cultivated barleys estimated average value of diversity index of 0.729, what is very close to our result. Polymorphic information content indexes for SSR markers *Bmac 0134* (0.835), *Bmag 0125* (0.685) and *Bmag 0211* (0.760) were comparable to our values. **Khodayari et al. (2014)** computed values for PIC index from 0.304 to 0.913 with mean value of

0.711. Also Lamara et al. (2013) calculated similar average PIC index (0.690). Other authors Yadav et al. (2015) and Hua et al. (2015) acquired lower mean values of PIC 0.551 and 0.549 but this was affected with some SSR markers with low level of polymorphism and thus lower value of PIC 0.286 and 0.407.

Table 2 Characteristics of individual SSR markers

Markers	Number of alleles	Allele size	DI	PIC
Bmac 0040	6	196 - 226 bp	0.826	0.817
Bmac 0134	5	140 - 174 bp	0.788	0.779
Bmag 0125	5	128 - 148 bp	0.795	0.782
Bmag 0211	4	150 - 170 bp	0.747	0.723
Bmag 0222	7	140 - 178 bp	0.688	0.677

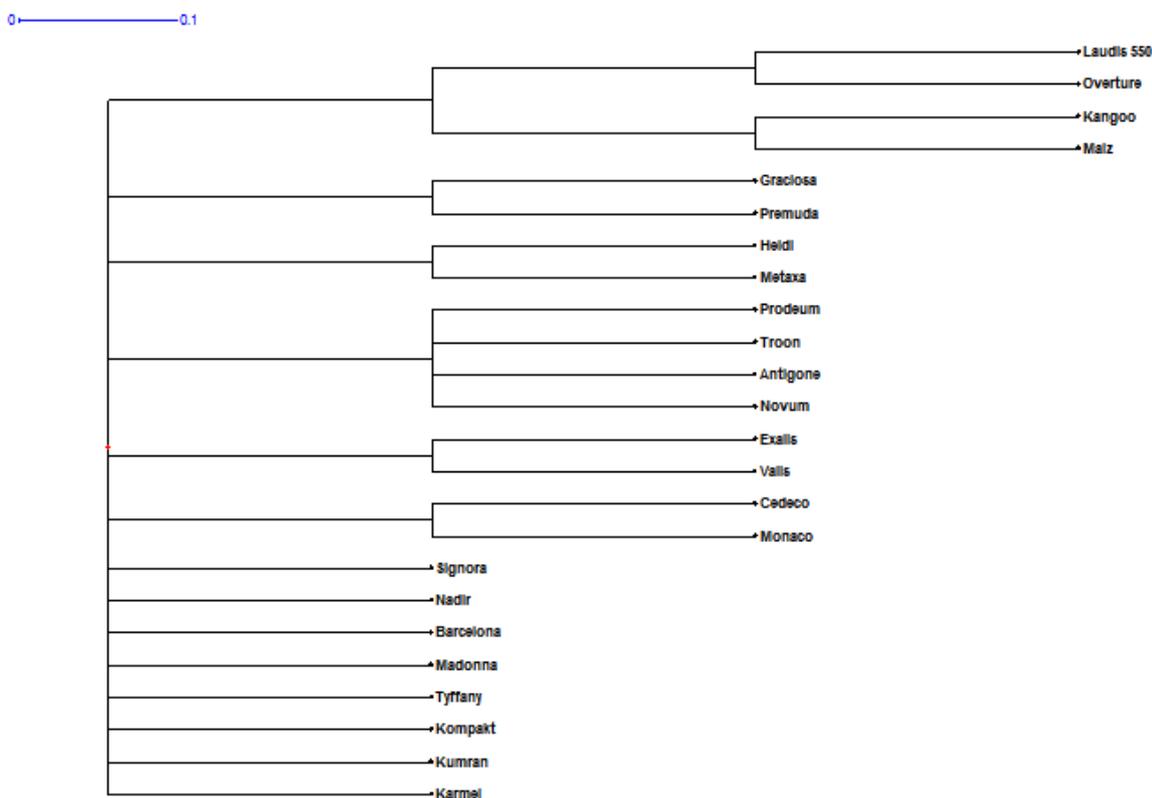


Figure 2 Dendrogram of 24 barley genotypes on the basis of 5 SRR markers

CONCLUSION

Molecular markers have been used many years for different applications mainly genotype and gene identification and genome mapping. According to obtained results, they are still very popular tool which helps growers and breeders to select the elite genotypes for their needs. Our results demonstrate the effectiveness of used SSR marker collection for differentiation of barley varieties and proved that SSR markers from our collection are still used worldwide for different applications. Comparable results with other authors confirmed potential of analyzed SSR markers for barley genotypes identification and characterization. Created dendrogram revealed relations between barley varieties and showed that this collection of SSR markers have some potential to differentiate malting barley cultivars.

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Average	5.4	-	0.767	0.756
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Legend: DI- diversity index, PIC- polymorphic information content,

DNA fragments amplified by 5 SSR markers were evaluated and on the basis of their presence or absence binary matrix was created. In the next step on the basis of this matrix dendrogram (figure 2) was created which revealed genetic relations between barley genotypes. We have successfully differentiated all 24 genotypes but we have failed to separate spring and winter forms as Pillen et al. (2000) in their study but they have used almost 40 markers. We can get better results by choosing different collection of SSR markers or by using more markers. On the other hand our set of markers showed some potential to differentiate malting barleys when 4 of them created own cluster, moreover genotypes Laudis 550, Overture and Kangoo are considered as varieties with premium malting quality, what is claimed by producers.

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