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REGULAR ARTICLE

DIFFERENTIATION OF BARLEY GENOTYPES BASED ON DNA POLYMORPHISM

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ABSTRACT

Identification and characterization of genotypes is essential for improving the quality of cultivated varieties in breeding programs. Information about the origin of varieties can help farmers in selecting appropriate varieties to specific growing conditions or end use of crops. A set of ten microsatellite markers was used to describe genetic diversity in a sample of 30 barley (*Hordeum vulgare* L.) genotypes. A total of 55 different alleles were amplified using ten SSR markers localized on chromosomes 1H, 2H, 3H, 5H, 6H, 7H with an average number of 5.5 alleles per locus. On the basis of allele frequencies we have calculated diversity index, polymorphic information content and index of probability, which have mean values of 0.664; 0.643 and 0.126 respectively. These values indicate high differentiation ability of SSR markers. In the created dendrogram using hierarchical cluster analysis using UPGMA algorithm we were able to differentiate all 30 barley genotypes. The results show that DNA markers are suitable for the identification and differentiation of genotypes and indicated the effectiveness of microsatellite markers to describe genetic diversity.

Keywords: barley, DNA polymorphism, DNA markers, microsatellite, genetic diversity

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the oldest domesticated cereals and its origin is in the territory of the "Fertile Crescent" in the Middle East around 8000 BC. Since ancient times it was used as human food and as an ingredient in fermented beverages **(Kling *et al*., 2004).**

Biochemical studies have expanded knowledge of the molecular basis of the barley quality, which is determined by multiple genes. Using genetic mapping a number of loci affecting the quality parameters of barley were identified. These loci have been successfully used in barley breeding **(Fox *et al*., 2003)**.

DNA markers, which are characterized by frequent occurrence in the genome, the independence of the environment and high reproducibility are an important tool for genetic mapping, study of genetic variability and diversity **(Garg *et al*., 2001)**.

Microsatellites (Simple Sequence Repeats - SSR) are widely used in cereal research and their exploitation in breeding has increased the speed and efficiency of genotypes improvement **(Hayden *et al*., 2008)**. The basis of using microsatellites for multiple purposes is a methodology allowing us to identify genotypes and to obtain genetic information about large number of genes.

Macaulay *et al.* (2001) developed a ‘genotyping set’ of 48 SSR-based genetic markers for application in genetical studies of barley. These markers have specific parameters: (1) they are single locus, (2) their product quality is good under standard assay conditions, (3) they are distributed across the barley genome, and (4) they exhibit reasonably high polymorphic information content (PIC) values.

Barley microsatellite map was constructed by combining six independent genetic maps based on the mapping of populations ‘Igri x Franka’, ‘Steptoe x Morex’, ‘OWBRec x OWBDom’, ‘Lina x Canada Park’, ‘L94 x Vada’ and ‘SusPtrit x Vada’ **(Varshney *et al*., 2007)**. Resulting integrated map was created by adding more maps and this map contained 775 unique microsatellite loci. This large map provides important information for molecular breeding programs of barley (Marker Assisted Selection method) with a better choice in terms of quality of markers and higher probability of polymorphic markers in the important chromosomal segments.

The aim of our study was to identify and differentiate 30 barley genotypes on the basis of selected set of microsatellite markers.

MATERIAL AND METHODS

DNA was isolated according to the standard method for the commercial kit GeneJET Plant Genomic DNA Purification Mini Kit (Fermentas, EU).

Ten SSR primers were chosen on the basis of high level of polymorphism according to studies of **Ramsay *et al*. (2000)** and **Varshney *et al*. (2007).**

Microsatellite analysis were carried out in a volume of 25 µl, which contains of 16.8 µl ultra pure H_2O; 5 µl 5xGreen goTaq reaction buffer; 0.1 µl dNTP (200 $\mu mol.dm^{-3}$); 1 µl forward primer (0.25 $\mu mol.dm^{-3}$); 1 µl reverse primer 2 (0.25 $\mu mol.dm^{-3}$); 0.1 µl Taq-DNA polymerase (5 $U.mm^{-3}$); 30 ng DNA

The PCR conditions were: initial denaturation at 93 °C for 2 minutes, 30 cycles consisting of denaturation at 93 °C for 1 minute, annealing at 54 °C for 2 minutes and polymerization at 72 °C for 2 minutes and final cool down to 10 °C.

Amplified fragments were separated in 6 % polyacrylamid gels denaturated with urea. Gel solution contains 18.5 ml 40 % acrylamid/bis (19:1), 5 ml 10xTBE (107.8 g Tris, 7.44 g EDTA and 55 g boric acid in 1 liter of distilled H_2O, adjust to pH 8,3), 20 ml redistilled H_2O, 56,5 ml hot urea solution. After cooling this gel solution, 180 µl of TEMED and 500 µl were added.

For electrophoretic separation of amplified fragments 5 µl of sample were used. Separation was carried out at maximum voltage of 90 mA in 1xTBE buffer for 3 to 4 hours according to expected size of the fragments. After separation, gels were stained according to **Bassam *et al*. (1991).**

The evaluation of DNA polymorphism and usability of SSR markers for differentiation of barley genotypes was based on values of diversity index (DI), the probability of identity (PI) and polymorphic information content (PIC), which were calculated on the basis of frequencies of identified alleles. Using hierarchical cluster analysis the dendrogram was constructed.

RESULTS AND DISCUSSION

The number of alleles per locus is a significant indicator of genetic diversity. We have identified a total of 55 alleles at 10 microsatellite loci, which are located on chromosomes 1H, 2H, 3H, 5H, 6H a 7H. In the individual loci we have detected from 3 to 9 alleles with an average of 5.5 alleles per locus. The most polymorphic marker was *Bmag0222* (9 alleles)

(figure 1) and the least polymorphic marker was *Bmag 0013*, which amplified only 3 alleles. Frequencies for all identified alleles varied from 2.63 % to 61.29 %.

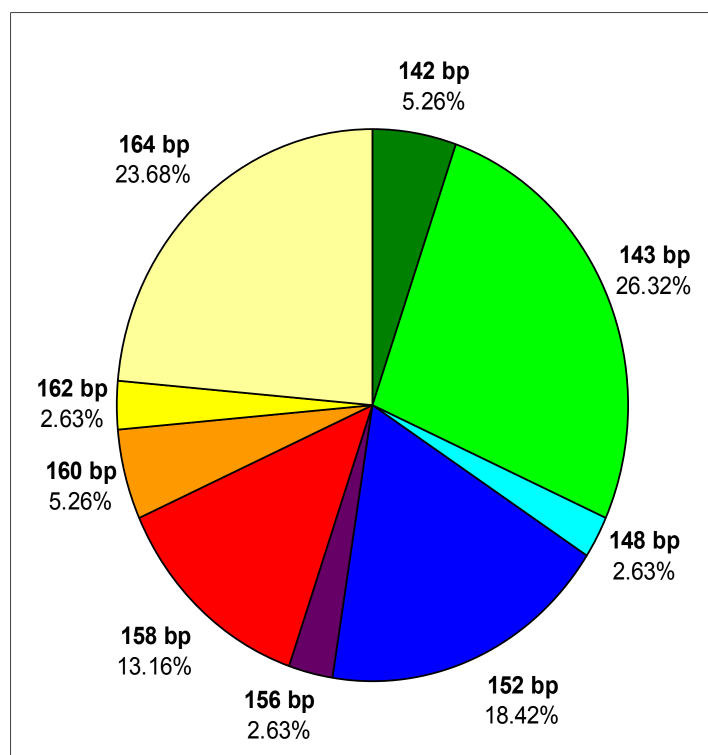

Figure 1 Percentage of identified alleles at locus *Bmag 0222*

Compared with our results **Wang *et al.* (2010)** identified from 1 to 5 alleles with average number of alleles per locus 2.4 in the collection of 40 barley genotypes from 8 different countries. On the other hand **Jilal *et al.* (2008)** detected a total of 403 alleles with average 20.2 alleles per locus. This high value of alleles per locus was caused by larger collection of analyzed barley genotypes (304 varieties).

On the basis of allele frequencies three indexes were calculated (table 1). Diversity index ranged from 0.506 (*Bmac 0134*) to 0.820 (*Bmac 0040*) with an average value of 0.664. Similar values were detected for polymorphic information content which varied from 0.501 (*Bmac 0134*) to 0.815 (*Bmac 0040* and *Bmag 0222*) with the average value of 0.643. Probability of identity values ranged from 0.011 (*Bmag 0222*) to 0.233 (*Bmac 0134*) with the average value of 0.126, what indicates that analyzed barley genotypes are genetically very distant.

Table 1 Parameter overview of individual microsatellite markers

Marker	Number of alleles	Allele size	DI	PIC	PI
Bmac 0040	8	208 – 222 bp	0,820	0,815	0,020
Bmac 0067	5	172 – 188 bp	0,696	0,665	0,112
Bmac 0093	4	148 – 154 bp	0,556	0,541	0,174
Bmac 0134	6	134 – 168 bp	0,506	0,501	0,233
Bmac 0156	4	162 – 168 bp	0,611	0,603	0,122
Bmag 0013	3	154 – 158 bp	0,571	0,502	0,250
Bmag 0125	6	132 – 142 bp	0,714	0,696	0,081
Bmag 0173	4	148 – 154 bp	0,578	0,536	0,215
Bmag 0211	6	144 – 166 bp	0,771	0,760	0,043
Bmag 0222	9	142 – 164 bp	0,816	0,815	0,011
Average	**5,5**	**-**	**0,664**	**0,643**	**0,126**

Legend: DI – Diversity index, PIC – Polymorphic information content, PI – Probability index

Baek *et al.* (2003) detected lower average diversity index (0.512) for SSR markers based on frequency of alleles in the Jordanian barley genotypes. Comparable value for this index has been calculated by **Kolodinska Brantestam *et al.* (2007)** (0,623). **Ganjkhanloo *et al.* (2012)** have detected higher values of diversity index which varied from 0.53 up to 0.93.

Based on our results dendrogram was created (figure 2), which is useful tool for studying genetic relations among analyzed collection of barley genotypes. In this dendrogram, genotypes were separated in to two clusters. First cluster contains 3 genotypes and the second contains 27 genotypes. In this major cluster, older genotypes were grouped into one subcluster. Using dendrogram we were able to differentiate all 30 genotypes and also we can see, that genotypes SK 5835 and SK 5407 are close genetic related.

Wang *et al.* (2010) have differentiated Tibetan and Middle-east barley genotypes in their created dendrogram. **Pillen *et al.* (2000)** analyzed German barleys and using dendrogram, they were able to differentiate spring and winter barleys.

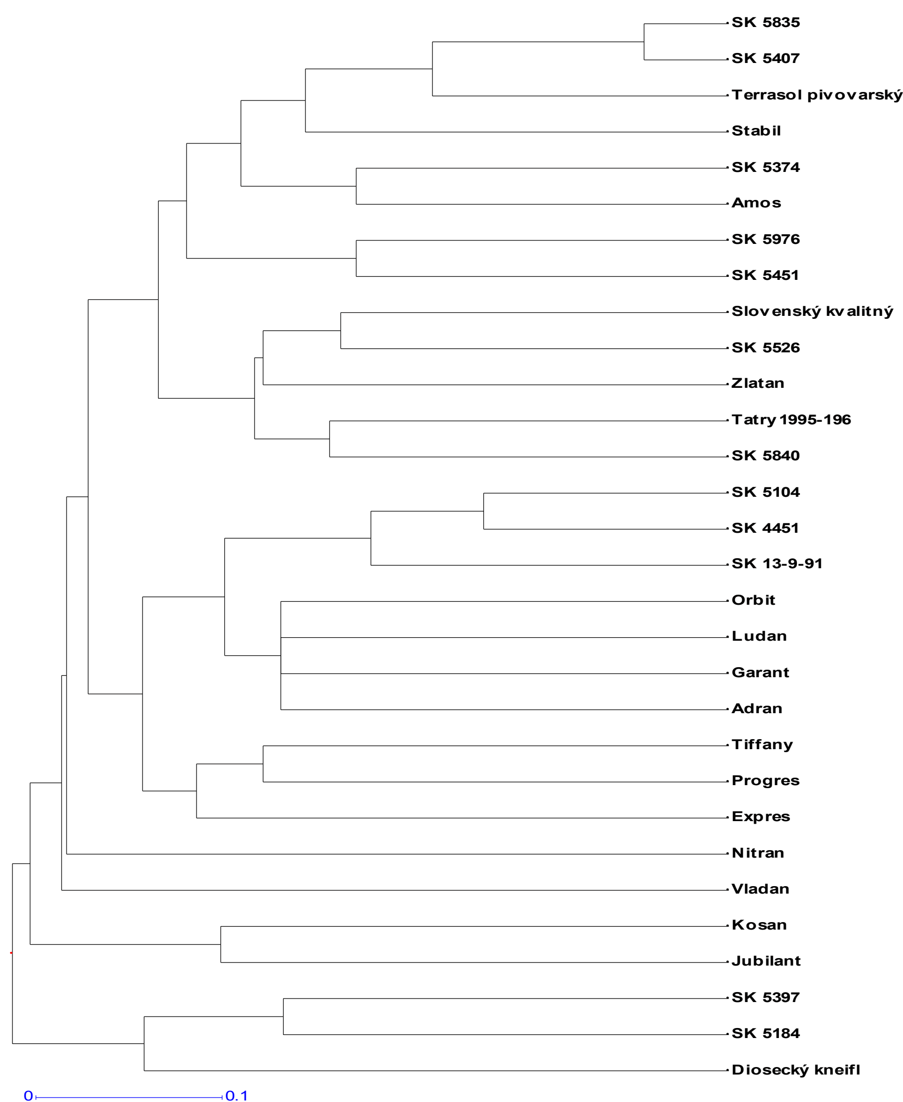

Figure 2 Dendrogram of analyzed barley genotypes

CONCLUSION

Our results confirmed that microsatellite markers are important tool for characterization, identification and differentiation of different plant species. Compared with different author's results, we have detected lower level of polymorphism in microsatellite loci, but we were able to differentiate all 30 barley genotypes nonetheless. Using five microsatellite markers we could detect heterozygotes, what indicate that SSR markers are suitable for studying intravarietal polymorphism. Microsatellite marker *Bmag 0173*, which is in close relation with

genes for resistance to barley blotch disease, can be used in Marker Assisted Selection for the development of new barley varieties.

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