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GENETIC VARIATION OF EUROPEAN MAIZE GENOTYPES (*ZEA MAYS* L.) DETECTED USING SSR MARKERS

Martin Vivodík, Zdenka Gálová, Želmíra Balážová, Lenka Petrovičová

ABSTRACT

The SSR molecular markers were used to assess genetic diversity in 40 old European maize genotypes. Ten SSR primers revealed a total of 65 alleles ranging from 4 (UMC1060) to 8 (UMC2002 and UMC1155) alleles per locus with a mean value of 6.50 alleles per locus. The PIC values ranged from 0.713 (UMC1060) to 0.842 (UMC2002) with an average value of 0.810 and the DI value ranged from 0.734 (UMC1060) to 0.848 (UMC2002) with an average value of 0.819. 100% of used SSR markers had PIC and DI values higher than 0.7 that means high polymorphism of chosen markers used for analysis. Probability of identity (PI) was low ranged from 0.004 (UMC1072) to 0.022 (UMC1060) with an average of 0.008. A dendrogram was constructed from a genetic distance matrix based on profiles of the 10 maize SSR loci using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 40 diverse accessions of maize was clustered into four clusters. The first cluster contained nine genotypes of maize, while the second cluster contained the four genotypes of maize. The third cluster contained 5 maize genotypes. Cluster 4 contained five genotypes from Hungary (22.73%), two genotypes from Poland (9.10%), seven genotypes of maize from Union of Soviet Socialist Republics (31.81%), six genotypes from Czechoslovakia (27.27%), one genotype from Slovak Republic (4.55%) and one genotype of maize is from Yugoslavia (4.55%). We could not distinguish 4 maize genotypes grouped in cluster 4, (Voroneskaja and Kocovska Skora) and 2 Hungarian maize genotypes - Feheres Sarga Filleres and Mindszentpusztai Feher, which are genetically the closest.

Keywords: old maize; dendrogram; SSR markers; genetic diversity; PIC

INTRODUCTION

With the advent of the first maize hybrids, in 1933 in the US and around 1950 in Europe, maize cultivation has undergone a complete change. Numerous open-pollinated landraces adapted to specific regions were substituted by a limited number of hybrids bred from a large genetic basis **(Gay, 1984)**. Today, the main maize hybrids cultivated in the world involve a restricted number of key inbred lines. Therefore, genetic diversity of those cultivars is almost certainly limited, in comparison to the large genetic diversity available in genebanks **(Gay, 1984)**. A few years ago, the threat of genetic erosion led to a significant interest in the assessment of genetic diversity in germplasm collections and a huge number of studies on various crops **(Dubreuil and Charcosset, 1998)**.

Molecular markers based on polymerase chain reaction (PCR) methods, such as simple sequence repeats (SSRs) or microsatellites, have become important genetic markers in a wide range of crop species, including maize **(Elçi and Hançer, 2015)**. SSRs markers have many advantages over other types of molecular markers, such as co-dominance, abundant in genomes, highly polymorphisms, locus specificity, good reproducibility and random distribution throughout the genome **(Sun et al., 2011)**. These features, coupled with their ease of detection, make them ideal for identifying and distinguishing between accessions that are genetically very similar **(Saker et al, 2005)**.

For the analysis of genetic diversity of maize genotypes were used several dominant molecular markers: amplified fragment length polymorphism (AFLP) **(Roy and Kim, 2016)**, random amplified polymorphic DNA (RAPD) **(Balážová et al., 2016)**, start codon targeted (SCoT) **(Vivodík et al., 2016)**, inter-simple sequence repeat (ISSR) **(Idris et al., 2012; Žiarovská et al., 2013)** and sequence-related amplified polymorphism (SRAP) **(Abd El-Azeem et al., 2015)**. And codominant molecular markers were also used for the analysis of maize genotypes: simple sequence repeat (SSR) **(Shiri et al., 2014)**, expressed sequence tag (EST)-SSR **(Galvão et al., 2015)**, single nucleotide polymorphism (SNP) **(Sa et al., 2012)** and using protein markers (SDS-PAGE) **(Vivodík et al., 2016)**. Suitability of SSR markers for the construction of genetic maps, fingerprinting and phylogenetic studies has been proved by many authors in many crops, such as

castor **(Gálová et al., 2015)**, rye **(Balážová et al., 2015)**, wheat **(Han et al., 2015)**, barley **(Maniruzzaman et al., 2014)**, triticale **(Vyhnánek et al., 2009)**, maize **(Salami et al., 2016),** hemp **(Vyhnánek et al., 2015)** and many other crops.

The present study aimed to examine the genetic variability within and among old maize genotypes cultivated in the Europe, using SSR markers. The data collected will contribute to identification, rational exploitation and conservation of germplasms of maize genotypes.

MATERIAL AND METHODOLOGY

Maize genotypes (40) were obtained from the Gene Bank VURV Praha-Ruzine (Czech Republic) and from the Gene Bank in Piesťany, the Slovak Republic (Table 1). DNA of 40 genotypes of maize was extracted from leaves of 10 day old seedlings using the Gene JET Plant Genomic DNA Purification Mini Kit.

SSR analysis: Amplification of SSR fragments was performed according to **(Elçi and Hançer, 2015)** (Table 2). Polymerase chain reaction (PCR) were performed in 20 μL of a mixture containing 7.5 μL H_2O, 10.0 μL Master Mix (Genei, Bangalore, India), 0.75 μL of each primer (10 pmol) and 1 μL DNA (100 ng). Amplification was performed in a programmed thermocycler (Biometra, Germany) and amplification program consisted of an initial denaturing step at 94 °C for 2 min, followed by 35 cycles of amplification [95 °C (30 s), 1 min at the 55 °C, 72 °C (30 s)] and a final elongation step at 72 °C for 10 min. Amplification products were confirmed by electrophoresis in 7% denaturing polyacrylamide gels and silver stained and documented using gel documentation system Grab-It 1D for Windows.

Data analysis: For the assessment of the polymorphism between castor genotypes and usability of SSR markers in their differentiation diversity index (DI) **(Weir, 1990)**, the probability of identity (PI) **(Paetkau et al., 1995)** and polymorphic information content (PIC) **(Weber, 1990)** were used.

Table 1 List of 40 analyzed genotypes of maize.

Genotypes	Country of origin	Year of registration
1. Feheres Sarga Filleres	Hungary	1965
2. Mindszentpusztai Feher	Hungary	1964
3. Zakarpatskaja	Union of Soviet Socialist Republics	1964
4. Przebedowska Burskynowa	Poland	1964
5. Krasnodarskaja	Union of Soviet Socialist Republics	1964
6. Mesterhazy Sarga Simaszemu	Hungary	1964
7. Slovenska biela perlova	Czechoslovakia	1964
8. Zuta Brzica	Yugoslavia	1975
9. Zloty Zar	Poland	1964
10. Slovenska Florentinka	Czechoslovakia	1964
11. Juhoslavska	Yugoslavia	1964
12. Kostycevskaja	Union of Soviet Socialist Republics	1964
13. Mindszentpusztai Sarga Lofogu	Hungary	1964
14. Stodnova	Czechoslovakia	1964
15. Slovenska žltá	Slovak Republic	1964
16. Slovenska krajová velkozrná	Slovak Republic	1964
17. Partizanka	Union of Soviet Socialist Republics	1964
18. Voroneskaja	Union of Soviet Socialist Republics	1964
19. Kocovska Skora	Slovak Republic	1964
20. Milada	Czechoslovakia	1964
21. Moldavskaja	Union of Soviet Socialist Republics	1964
22. Bučiansky Konský Zub	Slovak Republic	1964
23. Hodoninský konský zub žltý	Czechoslovakia	1964
24. M Silokukurica	Hungary	1964
25. Valticka	Czechoslovakia	1964
26. Przebedowska Biala	Poland	1964
27. Toschevska	Slovak Republic	1964
28. Šamorinsky konský zub	Hungary	1964
29. Wielkopolanka	Poland	1964
30. Czechnicka	Poland	1964
31. Manalta	Czechoslovakia	1964
32. Zlota gorecka	Poland	1964
33. Celchovicka ADQ	Czechoslovakia	1964
34. Belaja mestnaja	Union of Soviet Socialist Republics	1964
35. Bučanská žltá	Slovak Republic	1964
36. Iregszemeseil 2 hetes	Hungary	1964
37. Dnepropetrovskaja	Union of Soviet Socialist Republics	1964
38. Bezuncukskaja	Union of Soviet Socialist Republics	1964
39. Mikulická	Czechoslovakia	1964
40. Aranyozon sarga lofogu	Hungary	1964

Table 2 List of SSR primers of maize **(Elçi and Hançer, 2015)**.

SSR markers	F primer	R primer
UMC1363	AAAGGCATTATGCTCACGTTGATT	TCTCCCTCCCCTGTACATGAATTA
UMC1004	CTGGGCATACAAAGCTCACA	TGCATAAACCGTTTCCACAA
UMC2002	TGACCTCAACTCAGAATGCTGTTG	CACAAAATCCTCGAGTTCTTGATTG
UMC1117	AATTCTAGTCCTGGGTCGGAACTC	CGTGGCCGTGGAGTCTACTACT
UMC1587	ATGCGTCTTTCACAAAGCATTACA	AGGTGCAGTTCATAGACTTCCTGG
UMC1060	ACAGGATTTGAGCTTCTGGACATT	GGCCTCTCCTTCATCCTATTCAA
UMC1155	TCTTTTATTGTGCCCGTTGAGATT	CCTGAGGGTGATTTGTCTGTCTCT
UMC1072	GAGGAGACCGCCTCTGGTTC	CTTCGGGTTCCTGGACCTTCT
UMC1133	ATTCGATCTAGGGTTTGGGTTCAG	GATGCAGTAGCATGCTGGATGTAG
UMC1413	CATACACCAAGAGTGCAGCAAGAG	GGAGGTCTGGAATTCTCCTCTGTT

Table 3 List of SSR primers, total number of bands and the statistical characteristics of the SSR markers used in maize.

Marker name	Number of alleles	DI	PIC	PI
UMC1363	7	0.808	0.799	0.011
UMC1004	6	0.830	0.823	0.005
UMC2002	8	0.848	0.842	0.005
UMC1117	5	0.794	0.780	0.010
UMC1587	7	0.835	0.827	0.006
UMC1060	4	0.734	0.713	0.022
UMC1155	8	0.835	0.830	0.007
UMC1072	7	0.845	0.839	0.004
UMC1133	6	0.818	0.808	0.007
UMC1413	7	0.846	0.841	0.005
Average	**6.50**	**0.819**	**0. 810**	**0.008**

Note: DI- diversity index, PIC- polymorphic information content, PI- probability of identity.

RESULTS AND DISCUSSION

Ten maize SSR primers were used for identification and estimation of the genetic relations among 40 old European maize genotypes. All 10 SSR primers generated clear banding patterns with high polymorphism (Figure 1). Ten SSR primers revealed a total of 65 alleles ranging from 4 (UMC1060) to 8 (UMC2002 and UMC1155) alleles per locus with a mean value of 6.50 alleles per locus (Table 3). Variations in DNA sequences lead to polymorphism. Greater polymorphism is indicative of greater genetic diversity. The PIC values ranged from 0.713 (UMC1060) to 0.842 (UMC2002) with an average value of 0.810 and the DI value ranged from 0.734 (UMC1060) to 0.848 (UMC2002) with an average value of 0.819 (Table 3). 100% of used SSR markers had PIC and DI values higher than 0.7 that means high polymorphism of chosen markers used for analysis. Probability of identity (PI) was low ranged from 0.004 (UMC1072) to 0.022 (UMC1060) with an average of 0.008 (Table 3).

A dendrogram was constructed from a genetic distance matrix based on profiles of the 10 maize SSR loci using the unweighted pair-group method with the arithmetic average (UPGMA). According to analysis, the collection of 40 diverse accessions of maize was clustered into four clusters (Figure 2). The first cluster contained nine genotypes of maize, while the second cluster contained the four genotypes (Šamorinsky konský zub, Wielkopolanka, Manalta and Toschevska) of maize. The third cluster contained 5 maize genotypes (Moldavskaja, Bučiansky Konský Zub, Milada, Bučanská žltá and Iregszemeseil 2 hetes) (Figure 2). Cluster 4 contained five genotypes from Hungary (22.73%), two genotypes from Poland (9.10%), seven genotypes of maize from Union of Soviet Socialist Republics (31.81%), six genotypes from Czechoslovakia (27.27%), one genotype from Slovak Republic (4.55%) and one genotype of maize is from Yugoslavia (4.55%) (Figure 2). We could not distinguish 4 maize genotypes grouped in cluster 4, (Voroneskaja and Kocovska Skora) and 2 Hungarian maize genotypes – Feheres Sarga Filleres and Mindszentpusztai Feher, which are genetically the closest.

Similar results were detected by other authors **(Krishna et al., 2012; Kanagarasu et al., 2013; Molin et al., 2013; Qu and Liu, 2013; Al-Badeiry et al., 2014; Shiri, et al., 2014; Efendi et al., 2015; Ignjatovic-Micic et al., 2015; Salami et al., 2016)** and these results presented a high level of polymorphism of old maize genotypes detected by SSR markers. In the present investigation **(Krishna et al., 2012)**, 48 microsatellite markers were used for analyzing genetic diversity among the sixty three quality protein maize lines. Polymorphic profiles for 37 simple sequence repeat (SSR) loci aided in differentiating the QPM inbred lines. Using SSR procedures, the number of alleles per locus ranged from two to six, giving a total of 151 alleles for the 37 SSR loci.

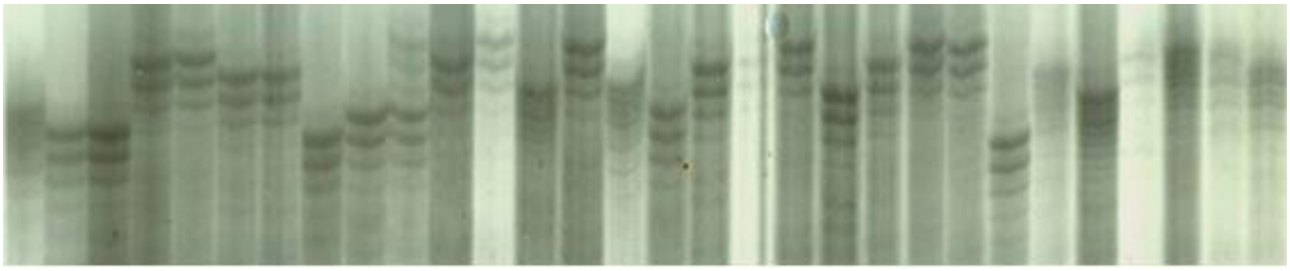

Figure 1 PCR amplification products of 30 genotypes of maize produced with SSR marker UMC1060. Lanes 1- 30 are maize genotypes (Table 1).

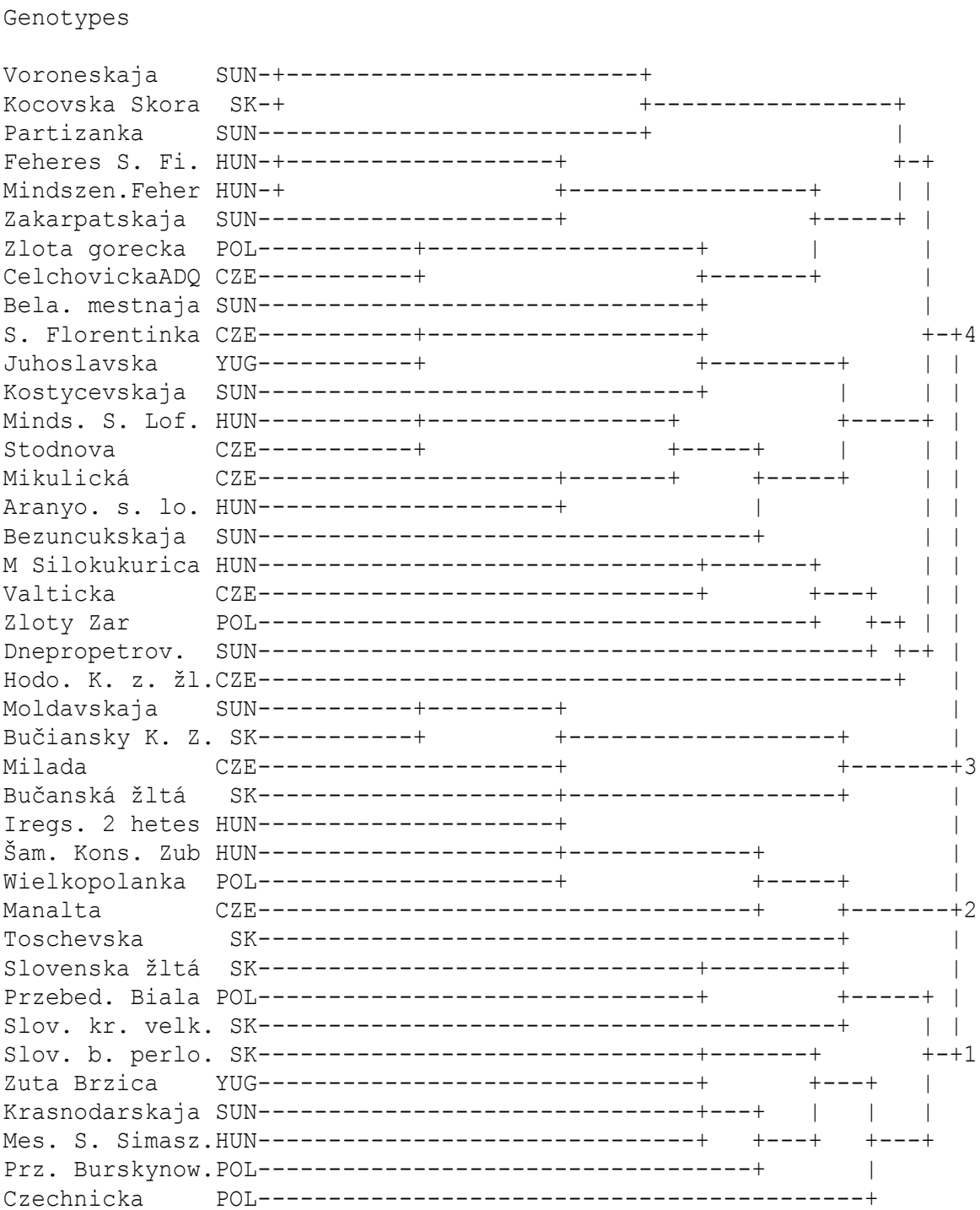

Figure 2 Dendrogram of 40 maize genotypes prepared based on SSR markers.

Kanagarasu et al. (2013) used 10 SSR molecular markers to analysis of 27 maize inbred lines. Ten SSR markers produced 23 polymorphic alleles with an average of 2.3 alleles per locus and mean polymorphic information content (PIC) of 0.45. The dendrogram generated with hierarchical unweighted pair group method with arithmetic mean (UPGMA) cluster analysis revealed five major clusters at 0.62 similarity coefficient.

The aim of **Molin et al. (2013)** was study the genetic diversity across 48 varieties of maize landraces cultivated at different locations in the States of Rio Grande do Sul and Paraná by 47 simple sequence repeat (SSR) markers. SSR analysis resulted in amplification of 105 polymorphic fragments and a polymorphic index of 78.3%. **Qu and Liu (2013)** selected SSRs with unique flanking sequences and then applied to analyze the polymorphism of next-generation sequencing data from 345 maize inbred. There were 58,946 SSRs with length information results in ten or more than ten genomes, accounting for 71.28% of SSRs with unique flanking sequences, while 55,621 SSRs had polymorphism, with an average PIC value of 0.498. **Al-Badeiry et al. (2014)** detected 41 alleles among the tested maize varieties using 10 Simple Sequence Repeat (SSR).The molecular size of bands obtained from amplification of SSR products ranged from 91 to 288 bp. Alleles ranged from one in umc1653 to ten in bnlg1189 loci. The polymorphic information content (PIC) values for the SSR loci ranged from 0.17 to 0.85, with an average of 0.44. The highest PIC values were observed in primers bnlg1017 (0.85) and umc1038 (0.79) and the lowest PIC values was observed in primer umc1946 (0.17). **Shiri, et al. (2014)** study genetic diversity of 38 maize hybrids using 12 SSR primers. The total number of PCR-amplified products was 40 bands, all of them polymorphic. Primer Phi031 generated the highest number of bands (6). Among the studied primers, UMC2359, PHI031 and UMC1862 showed the maximum polymorphism information content (PIC) and the greatest diversity. Maize hybrids were divided into three main groups based on SSR markers. The aim of **Efendi et al. (2015)** was to select homozygosity and analyze genetic diversity of 51 maize inbreds using 36 SSRs markers. The research was aimed to select among 51 maize inbreds with high homozygosity and to investigate the genetic diversity using 36 SSRs markers. The result shows that there were 30 inbreds indicating homozygosity level of more than 80%. The diversity of those inbreds was moderately high, with genetic similarity of between 0.22 and 0.87 distributed within six heterotic groups. The farthest genetic distance of 0.87 was detected on inbred pair 1044-3 vs Nei9008. **Ignjatovic-Micic et al. (2015)** analyzed nine flint and nine dent accessions from six agro-ecological groups, chosen on the basis of diverse pedigrees. Ten SSR primers revealed a total of 63 alleles. High average PIC value (0.822) also supports informativeness and utility of the markers used in this study. The aim of study **Salami et al. (2016)** was to evaluate the genetic diversity of Benin's maize accessions by SSR marker. Thus, 187 maize accessions from three areas were analyzed using three SSR markers. A total of 227 polymorphic bands were produced and showed high genetic diversity. The polymorphic information content (PIC) values for the SSR loci ranged from 0.58 to 0.81, with an average of 0.71.

CONCLUSION

In conclusion, a high level of genetic diversity exists among the old maize accessions analyzed. According to analysis, the collection of 40 diverse accessions of maize was clustered into four clusters. The first cluster contained nine genotypes of maize, while the second cluster contained the four genotypes (Šamorinsky konský zub, Wielkopolanka, Manalta and Toschevska) of maize. The third cluster contained 5 maize genotypes (Moldavskaja, Bučiansky Konský Zub, Milada, Bučanská žltá and Iregszemeseil 2 hetes). Cluster 4 contained 22 genotypes of maize. We could not distinguish 4 maize genotypes grouped in cluster 4, (Voroneskaja and Kocovska Skora) and 2 Hungarian maize genotypes - Feheres Sarga Filleres and Mindszentpusztai Feher, which are genetically the closest. A SSR marker system is a rapid and reliable method for cultivar identification that might also be used in quality control in certified seed production programs, to identify sources of seed contamination, and to maintain pure germplasm collections.

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Contact address:
Martin Vivodík, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: martin.vivodik@uniag.sk.

Zdenka Gálová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: zdenka.galova@uniag.sk.

Želmíra Balážová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: zelmira.balazova@uniag.sk.

Lenka Petrovičová, Slovak University of Agriculture, Faculty of Biotechnology and Food Sciences, Department of Biochemistry and Biotechnology, Tr. A. Hlinku 2, 949 76 Nitra, Slovakia, E-mail: lenka.petrovicova@uniag.sk.