SHORT COMMUNICATION

EVALUATION OF OLD AND LOCAL APPLE (*MALUS* × *DOMESTICA* BORKH.) VARIETIES FROM GENETIC RESOURCES BY MOLECULAR GENETIC SSR ANALYSIS

HODNOCENÍ GENOFONDU STARÝCH A LOKÁLNÍCH ODRŮD JABLONÍ (*MALUS* × *DOMESTICA* BORKH.) MOLEKULÁRNĚ GENETICKOU SSR ANALÝZOU

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PATZAK, J. – HENYCHOVÁ, A. – PAPRŠTEIN, F.: Evaluation of old and local apple (*Malus* × *domestica* Borkh.) varieties from genetic resources by molecular genetic SSR analysis. Agriculture (Poľnohospodárstvo), vol. 55, 2009, N. 1, pp. 55–57.

The worldwide apple genetic resources (1087 varieties) are maintained in the Research and Breeding Institute of Pomology Holovousy Ltd. At present, the utilisation of DNA molecular genetic methods is the best suitable method for the evaluation of individual genotypes, the elimination of duplications and characterisation of genetic relationships. In our preliminary study, we used 102 old and local apple varieties for DNA molecular genetic evaluation. SSR molecular genetic analysis of six loci was carried out on these samples. We found a high amplified polymorphism of SSR markers among local apple varieties, as all amplified products (85) were polymorphic. A cluster analysis was used for the evaluation of genetic relationships of individual genotypes of local apple varieties. No duplication was found in the set of genotypes and wide molecular genetic biodiversity of local apple varieties was mapped.

Key words: apple, genetic resources, SSR molecular analysis

Apples belong to the main fruit species and they are the most important fruit in Europe. Apple cultivars are classified to *Malus* × *domestica* Borkh. species. Only a few varieties are used in the intensive orchard production for the world market. However, the maintenance of wide biodiversity is necessary for the utilisation of the whole crop potential and prevention of genetic losses. The objective evaluation of variability and genotype characterisation is necessary for the preparation of effective apple core-collection. The utilisation of DNA molecular genetic methods is the best suitable method for the evaluation of individual genotypes, so that eliminated duplications and characterised genetic relationships. In the last ten years, the development of DNA technology has provided a large number of SSR markers available in apples (Gianfranceschi et al. 1998; Goulao and Oliviera 2001; Guilford et al. 1997; Hemmat et al. 2003;

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Hofer et al. 2002; Hokanson et al. 1998; Liebhard et al. 2002; 2003; Pedryc et al. 2002; Silfverberg-Dilworth et al. 2006). These markers proved to be extremely useful for genotyping, mapping, marker assistant selection, etc. In our preliminary study, we aimed to use SSR markers for molecular genetic analysis of variability and evaluation of biodiversity, of regional and local apple varieties in Czech genetic resources.

In our experiment, we used 102 genotypes (Fig. 1) of regional and local apples varieties from apple (1087 cultivars) genetic resources of the Research and Breeding Institute of Pomology in Holovousy (CR). DNA was isolated from young leaves according to Goulao et al. (2001). For molecular analyses, we used six SSR loci (Hemmat et al. 2003; Liebhard et al. 2002; 2003) in a typical PCR reaction (*Taq* PCR master mix kit, Qiagen, FRG) and amplification conditions: 2 min at 94°C, 35 cycles/ (30 s at 94°C; 60 s at 54°C, 90 s at 72°C); 10 min at 72°C, in TGradient thermocycler (Biometra, FRG). Sequencing vertical polyacrylamide gel (5% gel, 8M urea) electrophoresis was used for separating SSR products at 45W (Patzak et al. 2001). Polyacrylamide gels were stained according to Promega (USA) silver staining protocol. Stained and dried gels were duplicated to opaque daylight film (Promega, USA).

We found a high polymorphism of SSR markers, when 85 different microsatellite alleles were amplified by six SSR loci. Likewise, Goulao and Oliviera (2001) found 85 different microsatellite alleles, by thirteen SSR loci for 41 apple cultivars. Hokanson et al. (1998) used for SSR analysis a broader core collection of 66 apple cultivars and they found 97 different microsatellite alleles by eight SSR loci. It was suggested that increasing the number of analysed genotypes encountered more allelic diversity.

A cluster analysis was used for the statistical analysis of genetic relationships of individual genotypes of local apple varieties. Genetic similarity was estimated using Jaccard's similarity coefficient (Jaccard 1908), which ranges from 0 (all products between evaluated cultivars were different) to 1 (all products between evaluated cultivars were identical). The dendrogram was generated using the unweighted pair group method with arithmetic mean (UPGMA) clustering procedure (NTSYS-pc v. 2.11V for WINDOWS, Exeter Software, USA). The resulting dendrogram (Fig 1.) shows the wide molecular genetic biodiversity of local apple varieties. Duplication was not found in this set of genotypes. Pairwise values of Jaccard's coefficients ranged from 0 to 0.687 for varieties Blenheimská reneta and Vilémovo. Hvězdnatá reneta was the most molecular genetically distant variety of all. The dendrogram was not divided into distinctively noticeable groups.

In this study, we didn't evaluate the correlation of molecular markers to morphological traits, pedigree or geographical origins of old and local apple varieties. We proved that SSR

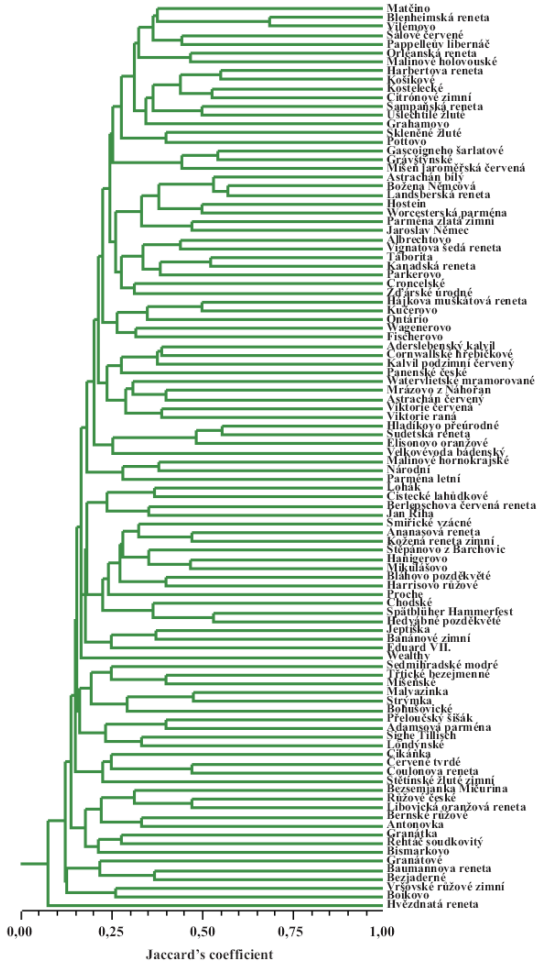

Fig. 1. Cluster analysis of old and local apple varieties based on 85 SSR polymorphic markers revealed by NTSYS-pc v.2.01 (Exeter software, USA)

markers could be successfully used for the analysis of genetic diversity in the Czech genetic resources of regional and local apple varieties. In the future, we will use more SSR loci and other commercial world apple varieties for a better evaluation of genetic biodiversity with a comparison to morphological traits, pedigree and geographical origins.

Acknowledgements. This work was supported by NAAR of the Ministry of Agriculture of CR in project QH72163.

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SOUHRN

Na pracovišti Výzkumného a šlechtitelského ústavu ovocinářského Holovousy s.r.o. je udržován rozsáhlý světový genofond jabloní (1087 odrůd). Využití molekulárně genetických metod studia DNA je v současnosti nejvhodnější metodou hodnocení jednotlivých genotypů, eliminace duplikací a charakterizace genetické příbuznosti. V naší předběžné studii bylo použito 102 starých a lokálních odrůd jabloní pro DNA molekulárně-genetické hodnocení. Na těchto vzorcích byla provedena molekulárně genetická analýza šesti SSR lokusů. Zjištěný amplifikovaný polymorfismus SSR markerů mezi krajovými odrůdami jabloní byl vysoký, když všechny amplifikované produkty (85) byly polymorfní. Shluková analýza byla použita pro hodnocení genetické příbuznosti jednotlivých genotypů krajových odrůd jabloní. V souboru genotypů nebyla nalezena žádná duplikace a byla zmapována široká molekulárně genetická biodiversita krajových odrůd jabloní.

Klúčová slova: jabloň, genetické zdroje, SSR molekulární analýza