**FOLIA**
**HORTICULTURAE**
Ann. 16/2, 2004, 87-94

# Analysis of genetic similarity of apple tree cultivars

*Miłosz Smolik [1], Danuta Rzepka-Plevneš [1], Izabela Stankiewicz [1], Piotr Chełpiński [2], Katarzyna Kowalczys[1]*

[1] Department of Horticultural Plant Breeding
[2] Pomology Department
Agricultural University in Szczecin
Janosika 8, 71-422 Szczecin
e-mail: smolik@ns.rektor.ar.szczecin.pl

Key words: *Malus* × *domestica*, DNA, polymorphism, ISSR-PCR

ABSTRACT

Eight phenotypically differing apple tree cultivars from the collection of the Agricultural University of Szczecin were analyzed using the ISSR anchored polymerase chain reaction. Out of 30 primers, 11 were chosen for the final study. Those amplified a total of 414 bands out of which 342 (83%) were polymorphic. Specific ISSR products were detected for each apple cultivar. A dendrogram was constructed using the UPGMA method which revealed three distinct clusters: I – 'Jonagold' and 'Jerseymac', II – 'Katja' and 'Lired', III – 'Gloster' and 'Oliwka Żółta'. Similarity within each group was 67.2%, 71.5%, and 63.5%, respectively. 'Gala' and 'Freedom' constituted a separate group, differing from other cultivars.

Abbreviations:

ISSR – inter-simple sequence repeat
PCR – polymerase chain reaction
UPGMA – unweighted pair-group method with arithmetic mean
TBE – tris-borate EDTA
AFLP – amplified fragment length polymorphisms
RAPD – random amplified polymorphic DNA

## INTRODUCTION

Apple (*Malus* × *domestica* Borkh.) is economically the most important fruit tree crop and a high number of commercial cultivars are available, in result of open-pollinated seedlings, controlled crosses, and somatic mutations induced in adapted cultivars. A genetic characterization of the apple cultivars is essential to breeding programs, patent protection and nursery control (Goulão and Oliveira 2001). Inter simple sequence repeat (ISSR) analysis is a simple, quick and efficient technique based on the PCR method, which involves amplification and analysis of DNA located between two microsatellite repeat regions. The technique uses primers, usually 18-25 bp long, in a single primer PCR reaction targeting loci to amplify mainly the inter simple sequence repeat of different size. The utility of the technique has been demonstrated in a wide range of applications in molecular biology (Nagaoka and Ogihara 1996, Guilford et al. 1997).

The aim of the present study was to determine the genetic variability of several phenotypically differing apple trees, based on DNA polymorphism analysis in regions between the microsatellite sequences.

## MATERIAL AND METHODS

The material comprised 8 apple cultivars (‘Gloster’, ‘Gala’, ‘Katja’, ‘Jonagold’, ‘Oliwka Żółta’, ‘Lired’, ‘Freedom’, and ‘Jerseymac’) from the Fruit Growing Research Station in Rajkowo. The DNA was extracted from young leaves of four-year old apple trees using the A&A Biotechnology “Genomic DNA Prep Plus” Kit. Thirty ISSR primers used are listed in Table 1. Reaction mixture (25 µl) contained 10x PCR buffer with $(NH_4)_2SO_4$ (750 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween 20), 0.2 mM of dNTPs, 2 mM $MgCl_2$, 0.25 µM of primer, 1u of *Taq* DNA polymerase enzyme (Fermentas) and 45 ng of template DNA. Samples were overlaid with 15 µl of mineral oil and reactions were carried out on a HotShot-12 thermal cycler (DNA Gdańsk II), using the following reaction conditions: the

initial denaturation at 94°C for 7 min, each cycle comprised 30 s denaturation at 94°C, 50 s annealing at 50°C, 2 min extension at 72°C with a final extension for 7 min at 72°C at the end of 40 cycles. The annealing temperature was usually adjusted to fit the Tm of the primer being used in the reaction. Amplified products were mixed with 6x Orange Loading Dye Solution and were analyzed by electrophoresis on a 2% agarose gel using 1x TBE buffer at a room temperature. Gels were photographed (Polaroid DS-34). Each fragment that was amplified using ISSR primers, was coded in a binary form by 0 or 1, indicating its absence or presence in each cultivar, respectively. The 0/1 matrix was used to calculate similarity/genetic distance (Jaccard coefficient) and later was employed to construct an UPGMA – dendrogram using software packages Diversity one 1.3 (Pharmacia LKB). Molecular weights of each band were calculated using the same software package.

## RESULTS AND DISCUSSION

To estimate the genetic diversity among 8 apple cultivars 30 anchored primers were tested, which were homologous to microsatellite repeats and contained anchor nucleotides. Most of them consisted of dinucleotide repeats with one to six 3'-anchor nucleotides. In addition, two trinucleotide, one tetranucleotide, and one pentanucleotide repeats were analyzed (Table 1). From 30 primers that gave amplification products in the initial screening, 11 primers (810, 811, 818, 825, 827, 834, 841, 842, 847, 851, 873) were selected (Table 1). Most of the ISSR primers, which were selected for analyzing the apple genome, had already been successfully used to reveal polymorphisms in other plants (Prevost and Wilkinson 1999, Joshi et al. 2000, Xue-Jun et al. 2003).

Table 1. ISSR-PCR primers used in the present study

| Primer number | Sequence (5` - 3`) | Primer number | Sequence (5` - 3`) | Primer number | Sequence (5` - 3`) |
|---|---|---|---|---|---|
| 808 | $(AG)_8C$ | 826 | $(AC)_8C$ | 848 | $(CA)_8GC$ |
| 810 | $(GA)_8T$ | 827 | $(AC)_8GG$ | 849 | $(GT)_7GAA$ |
| 811 | $CAC(CA)_6AT$ | 830 | $(TG)_8G$ | 851 | $(GT)_8CG$ |
| 815 | $(CT)_8GT$ | 834 | $(AG)_8GC$ | 856 | $(AC)_8G$ |
| 818 | $(CA)_8G$ | 840 | $(GA)_8GT$ | 857 | $(AC)_8GGTC$ |
| 819 | $(GT)_8A$ | 841 | $(GA)_8AT$ | 860 | $(TG)_8CA$ |
| 822 | $(TC)_7TAC$ | 842 | $(GA)_6CCCGGG$ | 864 | $(ATG)_5TG$ |
| 823 | $(TC)_8C$ | 845 | $(CT)_8GT$ | 865 | $(ATG)_5TCC$ |
| 824 | $(TC)_8G$ | 847 | $(CA)_8GC$ | 873 | $(GACA)_4$ |
| 825 | $(AC)_8T$ | 826 | $(AC)_8C$ | 880 | $(GGAGA)_3GT$ |

ISSR-PCR with genomic DNA of apple cultivars yielded DNA fragments ranging from 147 bp (primer 873) to 3997 bp (primer 811) and their number varied from 2 to 7 fragments per primer. On average, 5 fragments per primer were amplified. In total, 414 ISSR fragments of the apple genome were obtained. Of these, 342 (83%) were polymorphic, and only 9 fragments were monomorphic, occurring in patterns of all representatives of the genus (Table 2). The level of polymorphism of the ISSR markers observed was higher than reported for AFLP markers reported by Goulão and Oliveira (2001), and that of RAPD markers, described by Zhou and Li (2000) and Forte et al. (2002). The minimum and maximum numbers of polymorphic fragments were obtained with primer 842 (15) and 847 (53) respectively. Most of the 11 primers used were dinucleotide repeats varying in size and the 3'-anchor sequence. The sequences of repeats and anchored nucleotides are randomly selected. Each of these primers allowed amplification and revealed polymorphism of the apple genome. Generally, the number of fragments in an ISSR pattern and the polymorphism observed were linked (related) to the primer sequence (Table 1). As shown in Fig. 1, amplification with primers 810, 811, 827, and 842 yielded highly informative patterns. Specific ISSR markers, those that were either present or absent in one apple cultivar exclusively, were detected. Specifically, the apple cultivar ‘Oliwka Żółta’ was distinguished by the presence of the 1215, 1044, 1726, and 1093 bp fragments (per primers 818, 841, and 842, respectively) which were absent in other cultivars. Cultivar-specific ISSR fragments were found in the patterns of all apple cultivars (Table 3).

Table 2. Polymorphic and non-polymorphic amplification products generated with ISSR primers in apple cultivars

| Primer | Amplified products (loci) | | | |
|---|---|---|---|---|
| | polymorphic | | monomorphic | |
| | number | length (bp) | number | length (bp) |
| 810 | 13 | [2832, 2210, 1756, 1319, 1140, 883, 780, 700, 482, 445, 420, 411, 347] | 3 | [570, 288, 245] |
| 811 | 14 | [3997, 3400, 2893, 2550, 2210, 1951, 1765, 1661, 1478, 970, 832, 418, 371, 250] | 2 | [750, 585] |
| 818 | 15 | [1354, 1115, 1044, 1000, 877, 750, 671, 629, 581, 546, 474, 440, 400, 375, 325] | | |
| 825 | 9 | [1288, 1220, 1117, 1081, 980, 873, 789, 755, 673] | | |
| 827 | 15 | [1321, 1027, 889, 820, 700, 621, 574, 500, 488, 465, 455, 442, 414, 385, 378] | | |
| 834 | 10 | [1130, 982, 813, 744, 553, 371, 312, 273, 141, 128] | 1 | [205] |
| 841 | 7 | [1726, 1220, 980, 789, 768, 567, 506] | | |
| 842 | 9 | [1623, 1355, 1120, 1093, 1020, 920, 740, 452, 392] | | |
| 847 | 10 | [1456, 1320, 1219, 1061, 911, 708, 647, 591, 401, 337] | 1 | [359] |
| 851 | 9 | [1840, 1647, 1356, 1283, 1148, 956, 800, 705, 608] | | |
| 873 | 9 | [528, 492, 467, 385, 347, 281, 188, 169, 147] | 2 | [318, 224] |
| Total | 120 | | 9 | |

Table 3. Cultivar-specific bands revealed through ISSR fingerprinting

| Cultivars | Primers and length of amplification products (bp) |
|---|---|
| 'Oliwka Żółta' | 818 [1215, 1044], 841 [1726], 842 [1093] |
| 'Katja' | 825 [1288], 842 ]1623, 392], 851 [1356] |
| 'Freedom' | 818 [440], 827 [1321], 834 [1130], 847 [1219, 1061] |
| 'Lired' | 818 [546], 825 [673], 873 [492] |
| 'Gala' | 847 [1456, 401] |
| 'Gloster' | 827 [1021, 621] |
| 'Jonagold' | 818 [1354], 842 [920], 873 [467, 147] |
| 'Jerseymac' | 810 [2832], 811 [2210], 818 [375], 825 [1220], 842 [1355], 851 [1840] |

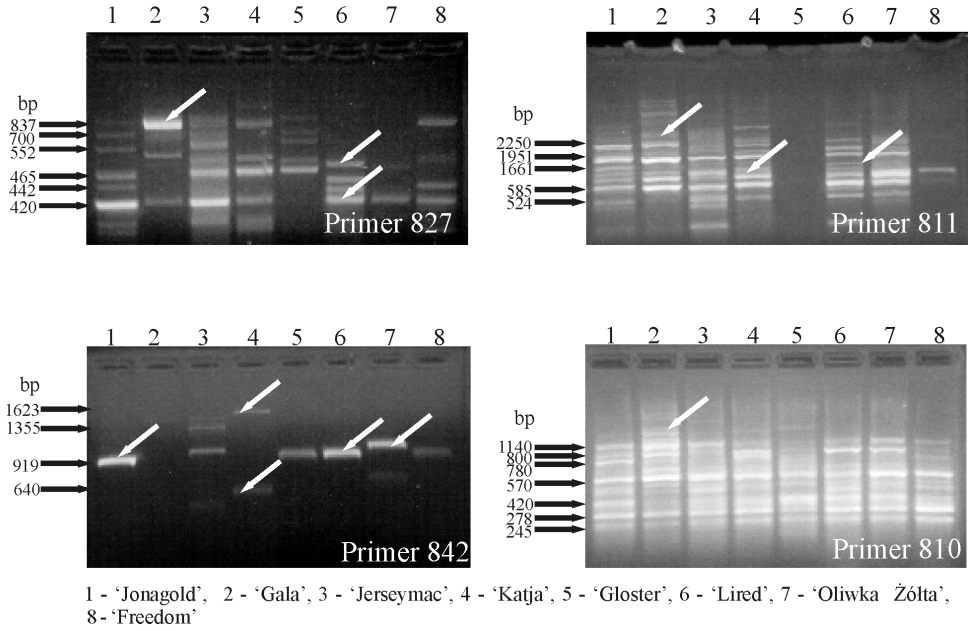


Figure 1. Fingerprints of the eight apple cultivars using four ISSR primers

Basing on the results of ISSR analysis, pairwise similarity coefficients were computed, from which an ISSR dendrogram was constructed with the use of hierarchic cluster analysis (UPGMA – Diversity one 1.3, Pharmacia LKB). The genetic similarity for the analyzed apple cultivars is presented in Fig. 2. Only two cultivars, 'Gala' and 'Freedom', were clearly separated from the other cultivars. The other apple cultivars formed three clusters (Fig. 2): the first one included 'Jonagold' and 'Jerseymac' (67.2% similarity), the second one – 'Katja' and 'Lired' (71.5% similarity), and the third one – 'Gloster' and 'Oliwka Żółta' (63.5% similarity).

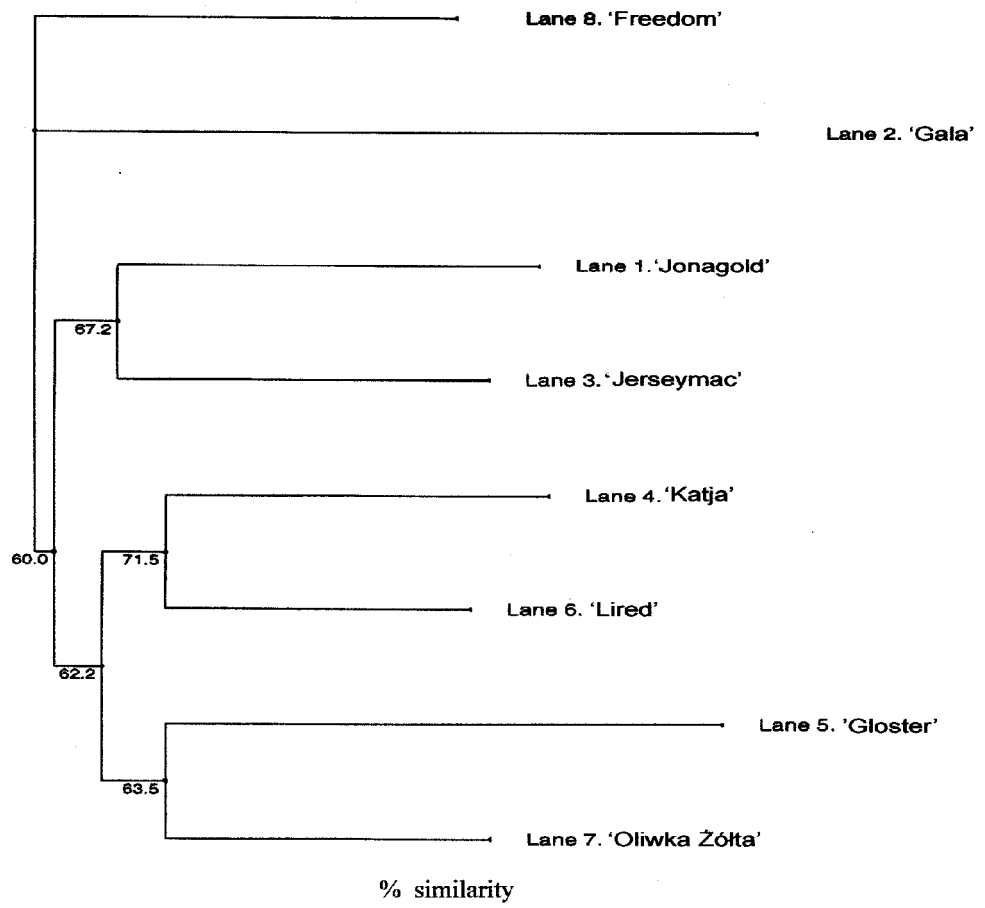


Figure 2. UPGMA dendrograms, representing genetic relationships among the eight apple cultivars, analysed by ISSR markers

In summary, the present results demonstrated a high level of the intercultivar ISSR polymorphism in the apple trees which were analyzed. Therefore, for assessing genetic relationships between apple cultivars, ISSR-PCR could be effectively used in apple breeding programs for designing crosses according to the origin of the apple form.

## CONCLUSIONS

1. The ISSR-PCR technique used in the present study is a suitable method for the evaluation of genetic similarity within the apple cultivars being analyzed.

2. Among 30 ISSR primers representing di-, tri-, tetra-, and penta- repeats, 11 primers revealed polymorphic patterns. Using those 11 primers, 414 bands in total were obtained, out of which 342 were polymorphic and scored giving an average of 5 bands amplified per primer. The used primers produced a specific patterns for each apple cultivar.
3. The analysis of the tree of genetic similarity showed that the apple cultivars used in the experiment were similar to each other in a range of 63.5-71.5%.

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ANALIZA PODOBIEŃSTWA GENETYCZNEGO ODMIAN JABŁONI

Streszczenie: Przy zastosowaniu techniki ISSR-PCR, określono podobieństwo genetyczne ośmiu, różniących się fenotypowo, odmian jabłoni, pochodzących z kolekcji Akademii Rolniczej w Szczecinie. Spośród 30 wybranych, w doświadczeniu zastosowano 11 starterów. W wyniku przeprowadzonych amplifikacji DNA ogółem otrzymano 414 prążków, z których 342 (83%) były polimorficzne. Dla każdej odmiany jabłoni wykryto specyficzne produkty ISSR. Dendrogram utworzony dla jabłoni w oparciu o algorytm – UPGMA, zawierał trzy odrębne skupienia, do których należały: I – 'Jonagold' i 'Jerseymac', II – 'Katja' i 'Lired', III – 'Gloster' i 'Oliwka Żółta'. Podobieństwo genetyczne między nimi wynosiło odpowiednio: 67,2%, 71,5% i 63,5%. Odmiany 'Gala' i 'Freedom' stanowiły oddzielne grupy podobieństw.

Received February 26, 2004; accepted October 26, 2004