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Characterization of kolomikta kiwi (*Actinidia kolomikta*) genetic diversity by RAPD fingerprinting

L. Èesonienë[1],

R. Daubaras[1],

B. Gelvonauskis[2]

[1] Kaunas Botanical Garden of Vytautas Magnus University Þ. E. Þilibero 6, 46324, Kaunas, Lithuania
Tel. 8 686 53684.
E-mail: bruknyte@one.lt

[2] Plant Gene Bank
Stoties 2, 58343 Akademija, Këdainiai distr., Lithuania
E-mail: b.gelvonauskis@agb.lt

Twenty-four accessions of *Actinidia kolomikta* (Maxim.) Maxim. were evaluated by the RAPD method at the Kaunas Botanical Garden collection *ex situ* for genetic diversity. Six decamer oligonucleotides generated 43 fragments, of which 30 (69.8%) were polymorphic. The UPGMA dendrogram revealed a wide range of genetic variability and a relationship between the accessions. Three fragments were detected in all genotypes. The cultivar 'Laiba' showed the highest GD_{xy} values and was selected as a genetic distinct accession in the *A. kolomikta* germplasm collection.

Key words: DNA, genetic relationship, polymorphism, RAPD

INTRODUCTION

Actinidia kolomikta (Maxim.) Maxim. is a very valuable horticultural plant because of a high level of ascorbic acid in its berries [1, 2]. This species is being cultivated and investigated in Russia. Breeding programmes for kolomikta kiwi were carried out in Russia, and the obtained cultivars were characterized by valuable agronomic traits [3]. Good results in breeding programs were obtained due to a wide range of intraspecific variations and particularly employment of rich natural genetic resources of *A. kolomikta* in the Far East of Russia. Kolomikta kiwi was introducted in Lithuania about 100 years ago [4]. Four Lithuanian cultivars, 'Paukðtës Ðakarva', 'Landë', 'Lankë' and 'Laiba', were bred under the breeding programme at the Lithuanian University of Agriculture [5, 6]. *A. kolomikta* is a very popular plant in the amateur gardens because of its ornamental as well as economically important properties. Some amateur gardeners have carried out permanent screening of kolomikta kiwi seedlings for winter hardiness, quality of berries, productivity and selected the best for further testing. The selected seedlings were named, propagated by soft- or hardwood cuttings and distributed in different regions of Lithuania.

The basis for a successful modern breeding of *A. kolomikta* is collection of genetically diverse plant germplasm. There are kolomikta kiwi cultivars of Russian origin, Lithuanian cultivars, femail and mail clones in the collection at Kaunas Botanical Garden of Vytautas Magnus University. These accessions were received from amateur gardeners and from scientific research institutes, thus we have a collection of *A. kolomikta* with a wide range of plant traits and characters. It contains unique clones selected by Dr. V. Paukðtë. The evaluation of the phenotypical diversity of *A. kolomikta* accessions at Kaunas Botanical Garden confirmed that this germplasm provides a valuable source of different traits and can be important for breeding.

The objetive of this work was to evaluate the genetic diversity of *A. kolomikta* germplasm by using RAPD fingerprints and to establish a relationship between the cultivars and clones studied.

MATERIALS AND METHODS

Twenty four cultivars and clones of *A. kolomikta* were investigated (Table 1). Each accession was represented by 3–6 plants.

Total DNA was isolated from fresh young leaf tissue using a cetyltrimethylammonium bromide (CTAB) buffer [7]. 0.2 g of leaves was finely ground in liquid nitrogen and mixed with a buffer extracted with 1 ml CTAB: 100mM TRIS-HCl, pH 8.0; 20 mM EDTA; 1.4 M NaCl, 1% PVP, 0.2% β-mercaptoethanol. The ground leaf samples were placed in Eppendorff tubes and incubated at 65 °C for 40 min. After incubation, an equal volume of chloroform/

Table 1. **The list of *Actinidia kolomikta* accessions investigated in this study**

Accession	Type of accession	Origin
'Matovaya'	Cultivar	Russia, Pavlovsk Research Station
'Krupnoplodnaya'	–"–	–"–
VIR-1	–"–	–"–
VIR-2	–"–	–"–
'Sentiabrskaya'	–"–	–"–
'Aromatnaya'	–"–	–"–
'Paukðtës Ðakarva'	–"–	Lithuanian University of Agriculture
'Landë'	–"–	–"–
'Lankë'	–"–	–"–
'Laiba'	–"–	–"–
F1	Female clone	Kaunas district, Babtai
F1M1	–"–	Elektrënai
Felë	–"–	Elektrënai
'Anykšta'	Landrace	Anykšèiai
'Dr. Szymanowski'	Cultivar	Poland
F4	Female clone	Kaunas
F2M2	–"–	Kaunas district, Ringaudai
La3	–"–	–"–
F3M3	–"–	Këdainiai district, Dotnuva-Akademija
F2	–"–	–"–
F4M4	–"–	–"–
M1	Male clone	Kaunas
M3	–"–	Kaunas district, Babtai
M6	–"–	Lithuanian University of Agriculture

Table 2. **Primers used for *A. kolomikta* DNA amplification**

Primer code	Primer synthesized by	Nucleotide sequences 5' – 3'
Akt-1	ROTH	TCGGCACGCA
Akt-2	JSC 'Fermentas'	TCCCTGTGCC
Akt-3	–"–	GAGACGTCCC
2B	–"–	CAAACGTCGG
OPA-02	–"–	TGCCGAGCTG
OPC-02	–"–	GTGAGGCBTC

isoamyl-alcohol was added and centrifuged for 10 min at 9,500 g. The supernatant was carefully transferred to a new Eppendorff tube and the same amount of cold isopropanol was added and centrifuged at 7,800 g for 5 min. DNA was washed, dryed and disolved in 0.150 ml TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA).

Six decamer oligonucleotides were used for polymerase chain reaction (PCR) amplification [8] (Table 2). DNA amplification reactions were caried out in 20 µl volumes containing 10 × PCR buffer (10 mM Tris-HCl, pH 8.0; 50 mM KCl, 3.0 mM $MgCl_2$), 200 µM of each dNTP, 0.3 µM primer, 1 unit Taq DNA polymerase and 10 ng template DNA. The tubes were placed in a thermal cycler (Eppendorf Master Gradient) programmed as follows: 5 min at 94 °C, 35 cycles of 80 s at 94 °C, 60 s at 33 °C, 90 s at 72 °C and final extension for 6 min at 94 °C. The amplified products were separated on 1% agarose gel in TAE buffer, pH 8.0 (40 mM Tris-acetate, 1 mM EDTA). All reagents used for DNA extraction and PCR were purchased from ROTH. Pairwise values of genetic distances (GD_{xy}) were calculated according to the formula [9]:

$$GD_{xy} = N_x + N_y / N_x + N_y + N_{xy},$$

where N_x is the number of fragments in line x and not in line y, N_y is the number of fragments in line y and not in line x, N_{xy} is the number of fragments shared in lines x and y.

The dendrograme was constructed by the UPGMA (unweighted pair-group method of arithmetic averages) and TREECON programme for Windows [10].

RESULTS

Six decamer oligonucleotides generated 43 fragments, of which 30 (69.8%) were polymorphic. A range of 6 to 9 amplified fragments per primer were observed, with an average of 7.2 fragments per primer (Table 3). The primers AKT-3 and OPC-02 amplified six fragments, but the primer OPA-02 amplified as many as nine fragments. The primer 2B generated the largest number of polymorphic bands (Fig. 1). The approximate size of the amplified fragments ranged from 250 to 3000 bp. Reproducible fragments

Table 3. **Number of different DNA amplified fragments**

Primer	Number of fragments observed		Percentage of polymorphic fragments
	Total	Polymorphic	
Akt-1	7	5	71.4
Akt-2	7	5	71.4
Akt-3	6	4	66.7
2B	8	6	75
OPA-02	9	5	55.6
OPC-02	6	5	83.3

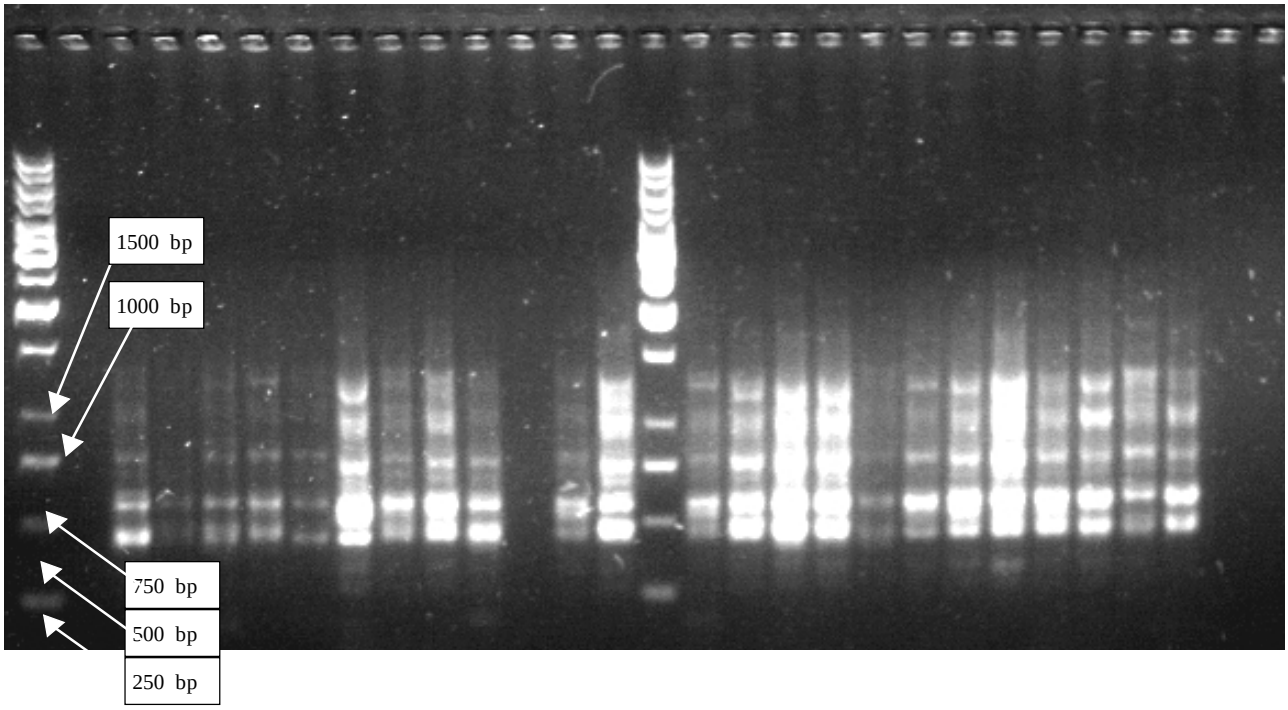

Fig. 1. Amplified polymorphic DNA profiles for *Actinidia kolomikta* generated by primer ***2B***. 1 – 'Matovaya', 2 – 'Krupnoplodnaya', 3 – 'Sentiabrskaya', 4 – 'VIR-1', 5 – 'VIR-2', 6 – 'Dr. Szimanowski', 7 – 'Lankë', 8 – 'Paukðtës Ðakarva', 9 – 'Landë', 10 – 'Laiba', 11 – 'Anykðta', 12 – 'Aromatnaya', 13 – Felë, 14 – F1, 15 – F4, 16 – F2, 17 – F1M1, 18 – F2M2, 19 – F3M3, 20 – F4M4, 21 – La, 22 – M1 ♂, 23 – M3 ♂. 24 – M6 ♂. M – DNA marker GeneRuler™ 1kb DNA Ladder Plus

with distinct bands only were scored in our evaluation.

Pairwise values of genetic distance ranged from 0.00 (for the same accession) to 0.914 (for cultivar 'Laiba' and female clone F4). The highest genetic identity and the lowest genetic distances were calculated for the female clones F2 and F4 (0.059), as well as for the male clone M1 and female clone F2M2 (0.094).

The dendrogram revealed two main clusters at a mean genetic distance of 0.55 (Fig. 2). Seventeen accessions were grouped into one cluster. The values of the genetic distance GD_{xy} in this cluster ranged from 0.059 (female clones F2 and F4) to 0.55 (male clone M6 and cultivar 'Lankë'). This cluster comprised two subclusters at a genetic distance of 0.409. One subcluster comprised related cultivars 'Matovaya' and 'Anykðta' and the other one contained two male clones M1 and M6, all female clones, except F1M1, two Lithuanian cultivars 'Paukðtës Ðakarva' and 'Lankë', Russian cultivars 'Aromatnaya', VIR-1 and the cultivar 'Dr. Szymanowski' of Polish origin. The other cluster contained one male clone M3, female clone F1M1, one Lithuanian cultivar 'Landë' and three cultivars 'Sentiabrskaya', VIR-2 and 'Krupnoplodnaya' of Russian origin. This cluster comprised two small subclusters joined at a level of genetic distance 0.512.

The highest pairwise values of GD_{xy} were calculated for the cultivar 'Laiba' from 0.615 (with VIR-2) to 0.914 (with F4).

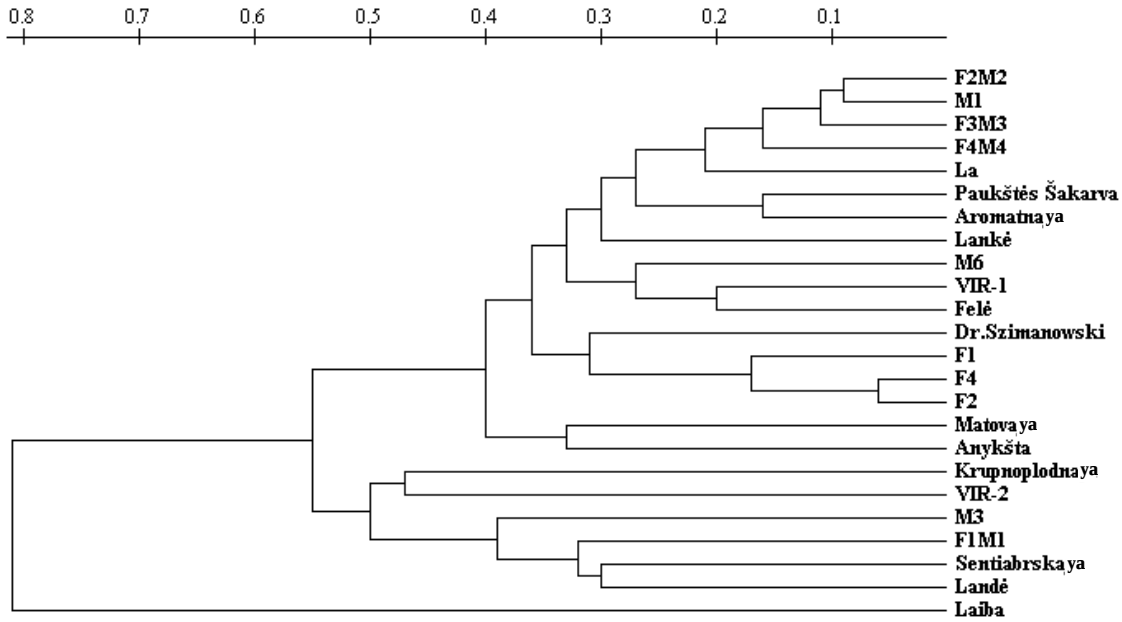

Fig. 2. Dendrogram of *Actinidia kolomikta* accessions obtained by UGMA

PCR with the primer AKT-3 generated two fragments (750 and 450 bp) and the primer OPC-2 amplified one (780 bp) fragment typical of all the accessions studied. The female clone F4M4 was distiguished by the presence of a unique polymorphic fragment, 310 bp (primer 2B), and the male clone M6 had a polymorphic fragment 550 bp (primer OPC-2).

The largest number of fragments was detected in female clones F2, F4, F1 (36, 32, 32 respectively). Six decamer primers amplified only six fragments in the cultivar 'Laiba'.

DISCUSSION

Morphological and agronomic traits were often used for characterization of *Actinidia kolomikta* cultivars and clones [1, 3, 5, 6]. At the same time it is necessary to develop molecular methods for direct investigations of the genetic diversity at the DNA level and to confirm the uniformity, stability and distinctness of each accession. Interactions between the genotype and the environment complicate the characterization [11, 12].

The results of this study demonstrate a successful fingerprinting of *A. kolomikta* cultivars using RAPD and its suitability for detection of genetic variation in kolomikta kiwi. The UPGMA dendrogram was constructed from GD_{xy} values and showed a relationship among the kolomikta kiwi cultivars and clones. The cultivars and clones of Lithuanian origin were not separated from the Russian and Polish cultivars, possibly because of the origin of the Lithuanian cultivars. The cultivars 'Landë', 'Lankë', 'Paukðtës Ðakarva' were received by selection of seedlings of the Russian cultivars 'Ananasnaya' and 'Klara Zetkin' [5]. The cultivar 'Laiba' demonstrated a genetic distinctness.

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MARGALAPIØ AKTINIDIJØ (*ACTINIDIA KOLOMIKTA*) GENETINËS ÁVAIROVËS ÁVERTINIMAS RAPD METODU

S a n t r a u k a

RAPD (atsitiktinai amplifikuotos polimorfinës DNR) metodu Kauno botanikos sodo kolekcijoje *ex situ* buvo tiriama dvidešimt keturiø *Actinidia kolomikta* (Maxim.) Maxim. pavyzdþiø genetinë ávairovë. Su 6 pradmenimis, kuriø ilgis 10 nukleotidø, amplifikuoti 43 fragmentai, ið kuriø 30 (69,8%) buvo polimorfiniai. Trys DNR fragmentai buvo bendri visiems tirtiems pavyzdþiams. Sudaryta dendrograma parodë genetiná tirtø pavyzdþiø giminingumo lygá. Veislë 'Laiba' iðsiskyrë ið tirtø veisliø ir klonø bei buvo maþiausiai jiems giminingа.