EVALUATION OF GENETIC DIVERSITY AND GENETIC RELATIONSHIPS AMONG FEMALE LITHUANIAN ACCESSIONS OF KOLOMIKTA KIWI

Laima Česonienė[1], Remigijus Daubaras[1] and Bronislovas Gelvonauskis[2]

[1]Kaunas Botanical Garden of Vytautas Magnus University, Ž.E. Žilibero 6, Kaunas LT–46324, LITHUANIA
[2]Plant Gene Bank, Stoties 2, Akademija, Kėdainiai distr., LITHUANIA
Corresponding author: l.cesoniene@bs.vdu.lt, fax.+370 37 390133

(Received July 26, 2007/Accepted September 13, 2007)

A B S T R A C T

The estimation of genetic heterogeneity of female *Actinidia kolomikta* accessions from the pomological collection of Kaunas Botanical Garden of Vytautas Magnus University was accomplished. DNA analysis by RAPD (Randomly Amplified Polymorhic DNA) technique showed significant genetic diversity of Lithuanian accessions and allowed to estimate their relationship. The percentage of polymorphism ranged from 55.6 to 80.0%. Two specific markers were identified with the primers OPC–02 and 2B for the cultivar 'Laiba' and female clone F4M4. The dendrogram grouping the accessions by UPGMA method revealed two main clusters. 'Laiba' was the most divergent cultivar and was separated from other accessions by 0.824 genetic distance.

Key words: cluster, dendrogram, genetic distance, kolomikta kiwi

INTRODUCTION

The non-traditional fruit plants which are valuable economically and/or because of their health-promoting properties are common objects of evaluation in recent decades. The berries of the genus *Actinidia* Lindl. contain significant amounts of biologically active compounds with antioxidative properties (Ferguson and MacRae, 1992; Moskvina et al., 1998; Chesoniene et al., 2004).

Kolomikta kiwi, *Actinidia kolomikta* (Maxim.) Maxim., is a widely spread species in Lithuania. It is a perennial, dioecious climbing deciduous plant. (Ferguson, 1984). Male kolomikta kiwi plants are more ornamental because of their intensive leaf variegation while female clones produce valuable berries with intense flavour. Introduction of kolomikta kiwi occurred in Lithuania by introducing gradually this plant into culture and assessing its ability to adapt to the new climatic conditions. The selection work resulted in breeding four Lithuanian cultivars of *A. kolomikta* at the Lithuanian University of Agriculture during 1972-1996 (Pranckietis, 1998; Pranckietis and Pranckietienė, 2000).

Morphological description was accomplished and successfully used for identification of clones and cultivars in kolomikta kiwi collection (Chesoniene, 2000). In addition, the molecular characterization should enable to evaluate genetic relationships and promote the breeding of new cultivars with valuable agronomic properties. As several authors noted, unusually wide genetic diversity of this species and rich genetic resources provide tremendous potential for selection of superior genotypes (Ferguson, 1984; Osipova, 1989; Plekhanova, 1990; Huang et al., 2003).

Biochemical markers, such as isozymes, were used in assessing genetic diversity in kiwifruit. However, their usefulness is limited. (Messina et al., 1991).Recently techniques based on DNA markers are used more often to detect variation at DNA level. Effectiveness of RAPD in distinguishing between closely related genotypes was proved in the last several years (Williams et al., 1990; Shirkot et al., 2002, Xiao et al., 2003). PCR techniques ensure identification of kolomikta kiwi cultivars or clones which are morphologically similar. Another advantage of molecular methods is that they are not affected by environmental factors.

The aim of this study was to assess the genetic diversity of Lithuanian female cultivars and clones of kolomikta kiwi and deter-mine their genetic relationships by RAPD technique.

MATERIAL AND METHODS

The biological material was obtained from the collection of *A. kolomikta* at Kaunas Botanical Garden of Vytautas Magnus University and was represented by 14 female accessions (Tab. 1). The cultivars and clones for this collection were gathered in consideration of fundamental principles of selection of genetically diverse plant material (Brown and Marshall, 1995).

Genomic DNA was isolated from leaf tissue by the cetyltrimethylammonium (CTAB) method (Doyle and Doyle, 1990). 0.2g of young leaves was quickly frozen in liquid nitrogen, pulverized and mixed in Eppendorf tubes with 1 ml CTAB buffer: 100 mM TRIS-HCL, pH 8.0; 20 mM EDTA; 1.4 M NaCl; 1% PVP; 0.2% ß–mercapthoetanol.

Table 1. The list of female *Actinidia kolomikta* accessions investigated

Accession	Type of accession	Place of origin
'Paukštės Šakarva'	female cultivar	Lithuanian University of Agriculture
'Landė'	– „ –	– „ –
'Lankė'	– „ –	– „ –
'Laiba'	– „ –	– „ –
F1	female clone	Kaunas distr., Babtai
F1M1	– „ –	Elektrėnai
'Anykšta'	female cultivar	Anykščiai
Felė	female clone	Elektrėnai
F2	– „ –	Kėdainiai distr., Dotnuva–Akademija
F4	– „ –	Kaunas
F2M2	– „ –	Kaunas distr., Ringaudai
F3M3	– „ –	Kėdainiai distr., Dotnuva–Akademija
F4M4	– „ –	Kėdainiai distr., Dotnuva–Akademija
La3	– „ –	Kaunas distr., Ringaudai

The mixture was incubated at 65°C for 40 min. Then, an equal volume of chloroform/isoamyl alcohol was added and, after vortexing, centrifuged for 10 min at 9,500 g. The supernatant was transferred to a new Eppendorf tube, the equal amount of isopropanol was added and the mixture was centrifuged at 7,800 g for 5 min. The supernatant was discarded and precipitated DNA was dissolved in 0.150 ml TE buffer (10 mM Tris-HCl, pH 8.0; 1 mM EDTA). The quality of such prepared DNA was checked by electrophoresis in 1% agarose gel.

The primers used for RAPD reactions were established empirically by their ability to initiate amplification of distinct fragments of DNA from kolomikta kiwi accessions. In effect, six decamer oligonucleotides were selected for priming polymerase chain reaction (PCR): Akt–1, Akt–2, Akt–3, 2B, OPA–02, OPC–02. DNA amplification was carried out in 20 µl volume of PCR buffer (10 mMTris-HCl, pH 8.0; 50 mM KCl; 3.0 mM $MgCl_2$, 20 µM of each dNTP) with addition of 0.3 µM primer, 1 unit of Taq polymerase and 10 ng of template DNA. The tubes were placed in a thermocycler (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. The final step consisted of 6 min incubation at 94°C. The amplified products were separated in 1% agarose gel in TAE buffer, pH 8.0 (40 mM Tris-acetate, 1 mM EDTA).

Of all amplified RAPD fragments, reproducible and clear bands only were taken into consideration.

The presence or absence of fragments was recorded as 1 or 0, respectively. Pair-wise comparison was accomplished according to the formula (Link et al., 1995):

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

where N_x is the number of fragments in line x but not in line y; N_y is the number of fragments in line y but not in line x; N_{xy} is the number of fragments shared in lines x and y. The data were presented as a dendrogram of genetic distances using UPGMA (unweighted pair-group method of arithmetic averages) and TREECON programme for Windows. The bootstrap method was employed to evaluate the reliability of tree topology. Numbers of dendrogram branches indicate bootstrap values (%) generated after 1000 permutations.

RESULTS

Amplification products of 14 accessions of *A. kolomikta* with 6 decamer primers yielded a total of 42 distinct fragments, 29 of which were polymorphic. The size of the ampli-fication products ranged from 250 to 3000 bp (Tab. 2).

From 5 to 9 fragments per primer was amplified, with an average 7 fragments. The largest number of fragments (9) was observed with the primer OPA–02, whereas the primer 2B generated the largest number of polymorphic fragments (6). The lowest number of fragments was obtained with the primer OPC–02 (5); however, 4 of them were polymorphic. The percentage of polymorphism ranged from 55.6% (primer OPA–02) to 80.0% (primer OPC–02). The female clone F2 was distinguished by the largest total number of RAPD products (36) as well as for the number of polymorphic fragments (21). The primers Akt–1, Akt–2, 2B and OPA–02 did not generate any fragments for the cultivar 'Laiba', while the primer Akt–3 and OPC–02 generated 6 fragments for this cultivar. Cultivar 'Laiba' was characterized exclusively by the 620 bp polymorphic fragment generated with primer OPC–02. The clone F4M4 was distinguished by the 320 bp fragment generated with the primer 2B. The 750 bp and 450 bp fragments (primer Akt–3) and 780 bp fragment (primer OPC–02) were typical for all accessions investigated.

The accessions were grouped on the basis of genetic distances by UPGMA method (unweighted pair–group method using arithmetic average) (Fig. 1). The genetic distance matrix was compiled by accomplishing of 91 pair-wise comparisons.

The dendrogram revealed two main clusters at a level of 0.500 genetic distance. The first cluster consisted of two accessions (cultivar 'Landė' and female clone F1M1) joined at a level of 0.333 genetic distance with the high bootstrap value of 84%. The second cluster consisted of two subclusters at the 0.350 genetic distance. The first subcluster contained four clones (F2, F4, F1 and Felė). The second cluster consisted of six other accessions, namely the clones La3, F4M4, F3M3

Table 2. The results of amplification of *Actinidia kolomikta* accessions

Primer code	Sequences, 5'–3'	Fragment size (bp)	Number of fragments observed		Percentage of polymorphic fragments
			total	poly-morphic	
Akt–1*	TCGGCACGCA	300–1500	7	5	71.4
Akt–2 •	TCCCTGTGCC	250–1500	7	5	71.4
Akt–3 •	GAGACGTCCC	450–1160	6	4	66.7
2B •	CAAACGTCGG	320–1350	8	6	75.0
OPA–02 •	TGCCGAGCTG	600–3000	9	5	55.6
OPC–02 •	GTGAGGCBTC	250–780	5	4	80.0

* – primer synthesized by ROTH, Germany
• – primer synthesized by JSC 'Fermentas', Lithuania

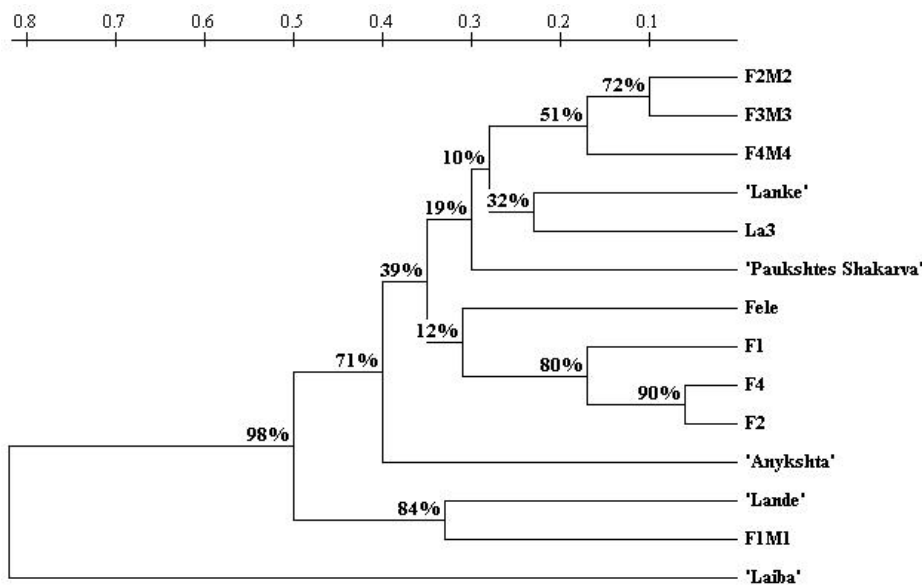

Figure 1. UPGMA dendrogram of female Lithuanian accessions of *A. kolomikta*

and F2M2 as well as the cultivars 'Lankė' and 'Paukštės Šakarva'. The cultivar 'Anykšta' joined this subcluster at a level of 0.400 genetic distance with the high bootstrap value of 71%. The lowest genetic distances were calculated for the clones F2 and F4 (0.059) as well as for the clones F2M2 and F3M3 (0.097). These clones appeared to be closely related. The cultivar 'Laiba' was characterized the highest genetic distances from other accessions of Lithuanian origin: from 0.700 with the cultivar 'Landė' to 0.914 with the clone F4.

The bootstrap values for this dendrogram ranged from 10 to 98%. The bootstrap values below 50% suggest that the positions of these genotypes may change if other primers were used or other genotypes are involved in the analysis.

DISCUSSION

Heterogeneous genetic material of *A. kolomikta* collected at Kaunas Botanical Garden was a main precondition for the comprehensive investigations of morphological and genetic peculiarities. The previous investigations of phenotypic diversity of cultivars and clones provided the main criteria for purposive collection of *A. kolomikta* genetic resources (Chesoniene, 2000; Daubaras et al., 2002). Data on morphological characteristics could be useful for describing new accessions and for comparing clones or cultivars grown in the same agroclimatic conditions. However, morphological markers are not reliable when precise identification of an accession is desired (Nuel et al., 2001). Moreover, interaction between genotype and environment complicate the evaluation of clone and cultivars.

The results of this study corroborated significant genetic diversity of Lithuanian accessions and defined the level of their relationships. In order to characterize further the structure and grouping of 14 kolomikta kiwi accessions of Lithuanian origin the dendrogram was designed on the basis of genetic distances determination by the UPGMA method. Previous investigations by RAPD markers revealed phylogenetic relationships in genus *Actinidia* (Cipriani et al., 1997; Huang et al., 2002; Marsh et al., 2003). RAPD analysis was successfully used to examine interspecific *A. arguta* and *A. deliciosa* hybrids as well (Kokudo et al., 2003). Molecular markers increase reliability of decisions in breeding programmes and save experimental materials and time (Novy and Vorsa, 1995; Hodkin et al., 2001).

Traditional methods, including morphological descriptions and isozyme pattern analysis can not provide sufficient information on the genetic diversity. The techniques based on molecular markers (i.e. RFLP, RAPD, ISSR-PCR) may provide a more efficient screening method. Genetic markers should help avoiding false identification of clones and cultivars of *A. kolomikta* as well as allow eliminating duplication in germplasm collection.

RAPD markers identified in this investigation could be used to assist breeding programme by establishing of relationships among kolomikta kiwi cultivars and clones. Clone-specific fragments were identified in this study with the primers OPC–02 and 2B. The cultivar 'Laiba' and the female clone F4M4 could be distinguished from other Lithuanian accessions by the presence of unique fragments 620 bp and 320 bp, respectively.

Acknowledgements: This study was supported by the Lithuanian Ministry of Education and Science programme 'Genefund'.

REFERENCES

Brown A.H.D., Marshall D.R. 1995. A basic sampling strategy: theory and practice. In: Guarino L., Ramantha Rao V. and Reid R. (eds). Collecting Plant Genetic Diversity. Cambridge, pp. 75-91.

Chesoniene L. 2000. Comparison of some biological features and fruiting potential of *Actinidia kolomikta* cultivars. ACTA HORT. 538: 769-774.

Chesoniene L., Daubaras R., Viskelis P. 2004. Biochemical composition of berries of some kolomikta kiwi (*Actinidia kolomikta*) cultivars and detection of harvest maturity. ACTA HORT. 663: 305-308.

Cipriani G., Di Bella R., Testolin R. 1997. Typing kiwifruit cultivars by means of RAPD markers. ACTA HORT. 444: 103-108.

Daubaras R., Česonienė L., Ašmonienė V. 2002. Biodiversity and conservation prospects of local and introduced berry plants in Lithuania. BIOLOGIJA 4 (supplement): 47-50.

Doyle J.J., Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. FOCUS 12: 13-15.

Ferguson A.R. 1984. Kiwifruit. A botanical review. HORT. REV. 6: 1-64.

Ferguson A.R., MacRae E.A. 1992. Vitamin C in *Actinidia*. ACTA HORT. 297: 481-488.

Hodkin T., Roviglioni R., De Vicente M.C., Dudnik N. 2001. Molecular methods in the conservation and use of plant genetic resources. ACTA HORT. 546: 107-118.

Huang H., Li Z., Li J., Kubisiak T.L., Layne D.R. 2002. Phylogenetic relationships in *Actinidia* as revealed by RAPD analysis. HORTSCIENCE 127(5): 759-766.

Huang H., Wang S., Jiang Z., Zhang Z., Gong J. 2003. Exploration of *Actinidia* genetic resources and development of kiwifruit industry in China. ACTA HORT. 610: 29-43.

Kokudo K., Beppu K., Kataoka I., Fukuda T., Mabuchi S., Suezawa K. 2003. Phylogenetic classification of introduced and indigenous *Actinidia* in Japan and identification of interspecific hybrids using RAPD analysis. ACTA HORT. 610: 351-356.

Link W., Dixhens C., Singh M., Scwall M., Melchinger A.E. 1995. Genetic diversity in European and Mediterranean faba bean germplasm revealed by RAPD markers. THEORET. APPLIED GENET. 90: 27-32.

Marsh H.D., Paterson T., Seal A.G., McNeilage M.A. 2003. Heritability estimates in kiwifruit. ACTA HORT. 622: 221-229.

Messina R., Testolin R., Morgante M. 1991. Isozymes for cultivar identification in kiwifruit. HORTSCIENCE 26: 899-902.

Moskvina O.A., Plekhanova M.N., Kolbasina E.I. 1998. *A. kolomikta* – a promising crop per new food technologies. FORESTRY STUDIES XXX: 113-115.

Novy R.G., Vorsa N. 1995. Identifi-cation of intracultivar genetic heterogeneity in cranberry using silver-stained RAPDs. HORTSCIENCE 30: 600-604.

Nuel G., Baril C., Robin S. 2001. Varietal distinctness assisted by molecular markers: a methodological approach. ACTA HORT. 546: 65-71.

Osipova N.V. 1989. Lianas. Moskva (in Russian), 182 p.

Plekhanova M.N. 1990. Actinidia, schizandra, honeysuckle. Leningrad (in Russian), 111 p.

Pranckietis V. 1998. New cultivars of *Actinidia kolomikta*, created in Lithuania. HORT. VEGET. GROWING 4: 33-43.

Pranckietis V., Pranckietienė I. 2000. Breeding and crop quality of *Actinidia kolomikta* in Lithuania. FRUIT SCI. REP. 207: 231-235.

Shirkot P., Sharma D.R., Mohapatra T., Shirkot C.K. 2002. Partial molecular characterization of some kiwifruit cultivars by RAPD markers. INDIAN J. EXPERIM. BIOLOGY 40: 233-236.

Williams G.K., Kubelik A.R., Livek J., Rafalski J.A., Tingey S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. NUCLEIC ACIDS RES. 18: 6531-6535.

Xiao X., Zhang L., Li S., Testolin R., Cipriani G. 2003. Molecular markers for early sex determination in *Actinidia*. ACTA HORT. 610: 533-538.

OCENA ZRÓŻNICOWANIA I POWINOWACTWA GENETYCZNEGO ŻEŃSKICH GENOTYPÓW AKTINIDII PSTROLISTNEJ

Laima Česonienė, Remigijus Daubaras i Bronislovas Gelvonauskis

S T R E S Z C Z E N I E

Dokonano oceny zróżnicowania genetycznego żeńskich genotypów *Actinidia kolomikta* z kolekcji sadowniczej Ogrodu Botanicznego Uniwersytetu Witolda Wielkiego w Kownie. Analiza DNA za pomocą RAPD (Randomly Amplified Polymorphic DNA – losowa amplifikacja polimorficznych fragmentów DNA) wykazała znaczące zróżnicowanie genotypów z kolekcji litewskiej i pozwoliła na określenie ich powinowactwa. Procent polimorfizmu wynosił od 55,6 to 80,0%. Zidentyfikowano dwa specyficzne markery dla identyfikacji odmiany 'Laiba' i klonu F4M4, amplifikowane przy użyciu starterów OPC–02 i 2B. Dendrogram grupujący rośliny z kolekcji, sporządzony przy użyciu metody UPGMA, wykazał istnienie dwóch głównych gron (klastrów). Odmiana 'Laiba' była genetycznie najbardziej oddalona, oddzielona od reszty genotypów z kolekcji na 0.824 dystansu genetycznego.

Słowa kluczowe: grono, dendrogram, dystans genetyczny, aktinidia pstra