PLANT
GENETICS

Genetic Diversity of Old and Local Apple (*Malus × domestica* Borkh.) Cultivars from the Collection of VIR according to AFLP Analysis

A. V. Shlyavas[a], *, A. A. Trifonova[b], L. V. Dedova[b], K. V. Boris[b], and A. M. Kudryavtsev[b]

[a]*Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg, 190121 Russia*
[b]*Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, 119991 Russia*
*e-mail: ann2668@yandex.ru

Abstract—The genetic diversity of 123 apple accessions from the collection of the research and production base Pushkin and Pavlovsk Laboratories of VIR, including landraces, Soviet cultivars, and commercial Russian and foreign cultivars, was for the first time estimated using the AFLP technique. A total of 355 fragments were obtained, 319 of which were polymorphic (89.86%), and each of the accessions had a unique set of AFLP fragments. The level of diversity of modern commercial cultivars was lower (74.11%, $H_e = 0.202$, $I = 0.302$) as compared with landraces (89.20%, $H_e = 0.238$, $I = 0.366$). A wide range of genetic differences between the studied accessions (0.99–0.63) was demonstrated. The statistical analysis of the data obtained made it possible to divide the studied samples into two groups. The first group included most of the analyzed landraces; with no clear differentiation of these landraces by origin. The second group included modern commercial Russian and foreign apple cultivars, Soviet cultivars, and some landraces.

INTRODUCTION

The apple (*Malus × domestica* Borkh.) is the most important commercial fruit crop in temperate latitudes. According to various estimates, there are from 7500 to 20000 apple cultivars in the world; of them, no more than 20 cultivars are widely cultivated in the horticulture industry [1, 2]. Such distribution of a small set of industrial cultivars leads to a displacement of local and old cultivars (and sometimes to their loss); accordingly, individual genes and gene combinations typical, for example, of the cultivars adapted to local conditions are lost, leading to a loss of genetic diversity [3].

Genetic resources of cultivated plants and their wild relatives are one of the most important components of plant biological diversity, since they have a potential value for food production and sustainable development of environmentally friendly agriculture. At present, realizing the strategic importance of plant genetic resources, most countries of the world have developed and are implementing national programs on their conservation and use [4]. National gene banks occupy a key position in solving the problems of plant genetic resources conservation strategy; the conservation of genetic resources *ex situ* is one of their main functions. In our country, the Vavilov All-Russian Institute of Plant Genetic Resources (VIR) is such an organization; at present, it has become not only

a national but also a recognized world center for the conservation and study of plant diversity [5].

VIR holds the largest collection of apple genetic resources in Russia, which includes more than 3800 cultivar and species accessions stored in the field gene banks of six branches of the institute that are located in different ecological and geographical zones of Russia. More than 500 apple accessions are preserved and studied in the conditions of the Northwest region at the research and production base Pushkin and Pavlovsk Laboratories of VIR in the collection gardens; of them, there are more than 100 accessions of Russian and European landraces: old cultivars widely cultivated in Russia and in the world and local cultivars locally grown by gardeners only in certain regions of the country. The apple landraces represented in the collection combine durability, high adaptability, and plasticity to the conditions of the northwest of Russia, as well as resistance to diseases and pests [6].

Domestic and foreign researchers and breeders developed descriptors for the study, estimation, pomological characteristics, and identification of the apple cultivars [7–9]. But the work with them is sometimes difficult because of the polygenic nature of many phenotypic traits and their dependence on environmental conditions, as well as because of a significant amount of time and labor costs required when studying large collections. Molecular markers (DNA mark-

ers) are widely used today to study the diversity of gene bank collections and to identify and certify accessions. One of the effective methods used to solve the problems of molecular characterization of species and varietal polymorphism is the multilocus AFLP (Amplified Fragment Length Polymorphism) method. This method was previously used for the identification of apple cultivars [10], creation of genetic linkage maps of two apple cultivars [11], and also, in combination with other methods, it was used for mapping apple resistance genes to aphids and scab [12−14]. AFLP analysis was successfully used to study the genetic diversity of wild species of the genus *Malus* Mill. from VIR collection [15].

At present, morphological and physiological charasteristics, biochemical composition, and field resistance to main diseases and pests are well studied in apple landraces from VIR collection; however, no molecular genetic studies of these landraces were previously carried out. Therefore, the aim of this work was to study the genetic diversity of old and local apple cultivars from the collection of the research and production base Pushkin and Pavlovsk Laboratories of VIR using AFLP analysis.

MATERIALS AND METHODS

To conduct the study, 106 apple accessions from the collection of the research and production base Pushkin and Pavlovsk Laboratories of VIR were selected: 100 landraces and six Soviet cultivars (Table 1, nos. 1−106), as well as 17 modern commercial Russian and foreign cultivars (Table 1, nos. 107−123) from the collection of VIR and the I.V. Michurin Federal Scientific Center.

DNA was isolated from fresh young leaves using the ZR Plant/Seed DNA Mini Prep (Zymo Research) kit according to the manufacturer's instructions.

AFLP analysis was conducted according to a standard method [16]. *Eco*RI and *Mse*I endonucleases (NEB) were used for restriction of genomic DNA. A combination of *Eco*RI-A and *Mse*I-C primers was used for the first round of amplification; two combinations of primers (*Eco*RI-ATG/*Mse*I-CAT and *Eco*RI-ATG/*Mse*I-CTA) were used for the second round. Polymerase chain reaction was conducted using reagent kits manufactured by Dialat-LTD in a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems). The products of amplification were separated by electrophoresis in 6% polyacrylamide gel in 1× TBE buffer in a Sequi-Gen® GT Sequencing Cell (BioRad) followed by silver nitrate staining according to the method of H. Benbouza et al. [17].

The presence or absence of amplification products was registered visually, only clearly reproduced fragments were taken into account, and the results were recorded into a binary matrix (1/0) in Microsoft Excel. The portion of polymorphic fragments was determined as the ratio of the number of polymorphic fragments to their total number, expressed as a percentage. The indicators of expected heterozygosity (H_e) and Shannon's index (I) were calculated using the GenAlEx6.41 program [18]. The calculation of the Dice genetic similarity coefficient between the samples and classical multidimensional scaling (principal coordinates analysis, PCO) were performed using the PAST 3.16 program [19]. The analysis of the genetic structure of the collection was carried out using STRUCTURE 2.3 program [20] with the following parameters: admixture model and allele frequency correlations (since all analyzed samples belong to the same species); the number of tested clusters (K) from 1 to 15, each with five repetitions; burn-in of 300000 steps; the total number of Markov chain Monte Carlo (MCMC) steps of 1000000. Selection of the optimal number of clusters into which the studied samples were divided was performed using the Structure Harvester service (http://taylor0.biology.ucla.edu/structureHarvester/) by the *deltaK* method.

RESULTS

AFLP analysis of 123 apple accessions made it possible to detect 355 fragments, 319 (89.86%) of which were polymorphic. The expected heterozygosity (H_e) was 0.243 ± 0.010, while the Shannon's index (I) was 0.373 ± 0.13. The apple landraces were characterized by a higher genetic diversity level (the portion of polymorphic fragments was 89.20%, $H_e = 0.238 \pm 0.010$, $I = 0.366 \pm 0.13$) as compared with the studied modern commercial Russian and foreign apple cultivars (the portion of polymorphic fragments was 74.11%, $H_e = 0.202 \pm 0.011$, $I = 0.302 \pm 0.15$). Each of analyzed cultivars was characterized by a unique AFLP spectrum, and sample-specific fragments were identified in the Yalkarnan Kesa and Antonovka Obyknovennaya k-711.

The Dice genetic similarity coefficients between the pairs of samples were established using the PAST 3.16 program. The maximum value of this index was 0.99 between Antonovka Obyknovennaya k-74 and k-21190 accessions. The high level of similarity (0.95) was also observed between Antonovka Monastyrskaya and Antonovka Obyknovennaya k-74. Among other accessions, Kal'vil' Belyj Letnij and Medovoe ot Verevkina (0.91), as well as Ananas Berzhanitskogo and Malinovka, Arkad Zimnij and Pudovka, and Pestrushka and Terent'evka (0.90), were the most genetically similar. The minimum level of similarity was observed between Pestrushka and Zimnee ot Berdashkevicha and Vinnoe and Antonovka Obyknovennaya k-711 (0.63) accessions, as well as Litovskoe Sakharnoe and Antonovka Obyknovennaya k-711 (0.64). The average value of the Dice similarity coefficient for the studied sample set was 0.77. The level of genetic differences in modern commercial cultivars was lower (the similarity coefficient varied from 0.72 between Golden Delicious and Bylina to 0.91 between Bylina and Blagovest, as well as Stroevskoe and Kurnakovskoe, with an average value 0.79).

Table 1. Apple accessions used in the study

No.	Accession name	VIR catalog number	Origin
		Landraces and Soviet cultivars	
1	Abrikosovoe Mazyuka	k-43438	Local cultivar of Leningrad region
2	Ananas Berzhanitskogo	k-11725	Lithuanian old cultivar
3	Anis Seryi	k-43	Old Russian cultivar
4	Anis Shatskii	k-15358	"
5	Anisovka	k-47	Local cultivar of Vologda region
6	Antonovka Krasnobochka	k-66	Old Russian cultivar
7	Antonovka Monastyrskaya	k-68	"
8	Antonovka Obyknovennaya	k-74	"
9	Antonovka Obyknovennaya	k-21190	"
10	Antonovka Obyknovennaya	k-711	"
11	Antonovka Rzhavaya	k-31709	"
12	Arkad Zimnij	k-15376	"
13	Arkad Krasnyj	k-31708	"
14	Arkad Letnij Zheltyj	k-140	"
15	Akhlebina Pozdnee	k-25247	Local cultivar of Kaluga region
16	Babushkino	k-23954	Old Russian cultivar
17	Beloe Osennee ot Ryzhego	k-23976	Local cultivar of Grodno region
18	Naliv Belyj	k-1011	Old Russian cultivar
19	Bel' Chernyshevskaya	k-25211	"
20	Berezovka ot Isakova	k-24476	Local cultivar of Smolensk region
21	Borovinka	k-276	Old Russian cultivar
22	Borovinka Akulovskaya	k-38174	Local cultivar of Volga region
23	Borovinka Ivantsovskaya	k-24781	Local cultivar of Nizhny Novgorod region
24	Borovinka Moguchaya	k-25230	Local cultivar of Kaluga region
25	Bukhovka	k-38211	Old Russian cultivar
26	Vargul'	k-350	"
27	Vinnoe	k-375	"
28	Voskovoe Prevoskhodnoe	k-20343	Local Estonian cultivar
29	Voskovoe Stepina	k-31711	Local cultivar of Kaluga region
30	Vydubetskaya Plakuchaya	k-31722	Local Ukrainian cultivar
31	Gibernal	k-23956	Old Russian cultivar
32	Golubok Kryugera	k-12247	Local Estonian cultivar
33	Gravenshteinskoe Russkoe	k-444	Western European cultivar
34	Grushovka Grebnitskogo	k-20284	Local Estonian cultivar
35	Grushovka Zimnyaya	k-462	Grushovka Moskovskaya × Kronselskoe Prozrachnoe (USSR)
36	Grushovka Moskovskaya	k-464	Old Russian cultivar
37	Grushovka Revelskaya	k-10128	Local Estonian cultivar
38	Grushovka Yudicheva	k-24484	Local cultivar of Smolensk region
39	Delyukinskoe	k-24470	"
40	Dynnoe	k-566	Old Russian cultivar
41	Zelenoe Knyazheskoe	k-25259	Local cultivar of Vologda region
42	Zimnee ot Berdashkevicha	k-25234	Local Belorussian cultivar
43	Zimnee Sikory	k-23968	Obtained from I.P. Sikora (BSSR)

Table 1. (Contd.)

No.	Accession name	VIR catalog number	Origin
44	Zimnee Uchkhozovskoe	k-23975	Local Belorussian cultivar
45	Kal'vil' Belyj Letnij	k-658	Old Russian cultivar
46	Kal'vil' Oranzhevyj	k-10134	Local cultivar of Pskov region
47	Karmazinka	k-729	Old Russian cultivar
48	Kitaika Saninskaya	k-9090	Local cultivar of Vologda region
49	Korichnoe Ananasnoe	k-29	Old Russian cultivar
50	Korichnoe Beloe	k-25257	Local cultivar of Kaluga region
51	Korichnoe Polosatoe	k-805	Old Russian cultivar
52	Korobovka	k-811	"
53	Korobovka Novaya	k-31726	Local cultivar of Kaluga region
54	Korolevskoe	k-21188	Local cultivar of Tver region
55	Lavia	k-21179	Local Finnish cultivar
56	Laizanskoe Zimnee	k-20333	Local Latvian cultivar
57	Lesnoe Otti	k-10112	Local Estonian cultivar
58	Letnee Rannee	k-25222	Local cultivar of Bryansk region
59	Letnee Sladkoe	k-23964	Obtained from I.P. Sikora (BSSR)
60	Limonnoe	k-895	Western European cultivar
61	Limonovka	k-24782	Local cultivar of Nizhny Novgorod region
62	Litovskoe Sakharnoe	k-15391	Local Lithuanian cultivar
63	Maiskoe ot Ryzhego	k-23973	Local cultivar of Grodno region
64	Malinovka	k-23971	Old Russian cultivar
65	Mamutovskoe	k-15354	"
66	Medovoe ot Verevkina	k-20275	Local cultivar of Kaliningrad region
67	Mestnoe Lezhkoe	k-20281	"
68	Naliv Dymchatyj	k-24773	Local cultivar of Nizhny Novgorod region
69	Nalivnoe Yantarnoe	k-1025	"
70	Novgorodchina	k-25217	Local cultivar of Orlov region
71	Ovechij Nosik	k-22562	Seedling of unknown large-fruited cultivar (USSR)
72	Osennee Aloe	k-21191	McIntosh × Babushkino (USSR)
73	Osennee Polosatoe	k-1083	Old Baltic cultivar
74	Osennij Kvas	k-25255	Local cultivar of Kaluga region
75	Pajdesskoe Zimnee	k-1112	Local Estonian cultivar
76	Papirovka	k-38223	Old Baltic cultivar
77	Pestrushka	k-24774	Local cultivar of Nizhny Novgorod region
78	Plodovitka Rannyaya	k-1216	Old Russian cultivar
79	Ponyavinskoe	k-1233	"
80	Pudovka	k-10140	Local cultivar of Pskov region
81	Putivka	k-25233	Old Ukrainian cultivar
82	Pyltsamaaskoe Zimnee	k-20309	Old Estonian cultivar
83	Rannyaya Pytalova	k-25254	Local cultivar of Pskov region
84	Raspisnoe	k-10126	"
85	Rozovka	k-21194	"
86	Rozovka ot Ryzhego	k-23972	"
87	Severnyj Velikan	k-1503	Local cultivar of Leningrad region
88	Seyanets Rebristogo	k-23970	Local cultivar of Grodno region

Table 1. (Contd.)

No.	Accession name	VIR catalog number	Origin
89	Seyanets Trebu	k-433	Old Baltic cultivar
90	Skryzhapel (clone)	k-15393	Old Russian cultivar
91	Sladkoe Zimnee	k-21195	Local cultivar of Pskov region
92	Slivochnoe	k-1573	Old Russian cultivar
93	Suislepskoe	k-1626	Old Baltic cultivar
94	Talve Nauding	k-38194	Akero × Streifling Herbst (ESSR)
95	Tellisaare	k-1654	Old Estonian cultivar
96	Terent'evka	k-12309	Old Russian cultivar
97	Titovka	k-1667	"
98	Titovka clone	k-21196	"
99	Tyushkinskoe Krasnoe	k-10133	Local cultivar of Pskov region
100	Fedorovskoe	k-24489	Local cultivar of Tver region
101	Chernoe Derevo	k-1766	Old Russian cultivar
102	Chulanovka	k-1774	"
103	Shelkovka	k-1809	"
104	Shtreifling Krasnyj	k-25196	Western European cultivar
105	Yablonya Dobrynicheva	k-24487	Local cultivar of Tver region
106	Yalkarnan Kesa	k-20311	Local Estonian cultivar
Commercial modern cultivars			
107	Bylina	–	Prima × Bessemyanka Michurinskaya (Russia)
108	Blagovest	–	Prima × Bessemyanka Michurinskaya (Russia)
109	Kurnakovskoe	–	814 × PA-29-1-1-63 (Russia)
110	Rozhdestvenskoe	–	Wealthy × VM41497 (Russia)
111	Svezhest'	–	Antonovka Krasnobochka × PR12T67 (Russia)
112	Skala	–	Prima × Bessemyanka Michurinskaya (Russia)
113	Stroevskoe	–	Seedling of the 814 from free pollination (Russia)
114	Uspenskoe	–	Prima × Bessemyanka Michurinskaya (Russia)
115	Flagman	–	Bogatyr' × Skala (Russia)
116	Braeburn	–	Seedling Lady Hamilton from free pollination (New Zealand)
117	Fireside	–	McIntosh × Longfield (United States)
118	Florina	k-40694	612-1 × Jonathan (France)
119	Gala	k-40702	Kidd's Orange Red × Golden Delicious (New Zealand)
120	Golden Delicious	k-40769	Unknown origin (United States)
121	Granny Smith	k-43504	Possibly a seedling of French Crab (Australia)
122	Haralson	–	Malinda × Wealthy (United States)
123	Spartan	–	McIntosh × Yellow Newtown (Canada)

On the basis of the Dice genetic similarity coefficients, principal coordinates analysis (PCO) was conducted (Fig. 1). All studied modern commercial apple cultivars are located on the right side of the graph. Soviet cultivars, as well as some local and old cultivars, are located near the group of modern cultivars: Baltic (Grushovka Gerbnitskogo, Mestnoe Lezhkoe, Seyanets Trebu, Suislepskoe, Tellisaare, Yalkarnan Kesa),

Western European cultivar Shtreifling Krasnyi, local cultivar of Grodno region Seyanets Rebristogo, and some Russian landraces (Abrikosovoe Mazyuka, Severnyi Velikan, Sladkoe Zimnee, Slivochnoe, Titovka, Titovka klon, Tyushkinskoe Krasnoe, Fedorovskoe). Other studied landraces (both Russian and foreign) are mainly located in the left and central parts of the graph, and no clear differentiation of the landra-

ces by their origin is observed. Antonovka accessions: Antonovka Monastyrskaya, Antonovka Rzhavaya, and Antonovka Obyknovennaya (k-74 and k-21190) occupy a somewhat isolated position (Fig. 1).

Subsequently, the genetic structure of the studied collection was determined using the STRUCTURE 2.3 program (Fig. 2a). Data analysis using the *deltaK* method demonstrated that the studied accessions most likely can be divided into two clusters (Fig. 2b). The first cluster includes most of the studied apple landraces, and no differentiation of Russian and Baltic landraces was detected. The second cluster includes modern and commercial apple cultivars, Soviet cultivars, and some landraces. In addition, samples that include the components of both groups according to AFLP data were detected (Fig. 2a). Such separation was also observed on the PCO graph, where these samples occupied an intermediate position between modern commercial cultivars and landraces (Fig. 1).

DISCUSSION

The conducted AFLP analysis detected a fairly high level of genetic diversity of the studied collection (89.86%, $H_e = 0.243$, $I = 0.373$); at the same time, the level of genetic diversity of modern Russian and foreign commercial cultivars was lower (74.11%, $H_e = 0.202$, $I = 0.302$) as compared with landraces (89.2%, $H_e = 0.238$, $I = 0.366$). The analysis of data obtained demonstrated that most of the studied apple accessions can be attributed with a high probability to one of two groups. The first group includes most of the analyzed landraces, while the second group includes modern Russian and foreign commercial apple cultivars. Within the first group, no clear division of the studied landraces by geographic origin was observed, which may be due to the exchange of genetic material that existed between neighboring territories of northwestern regions of Russia and Baltic countries, where most of the samples originate (Table 1). Previously, such mixing was registered when studying the genetic diversity of the collection of local Finnish and Latvian apple cultivars using microsatellite markers [21, 22].

Besides modern commercial apple cultivars, the second group included Soviet cultivars (Grushovka Zimnyaya and Osennee Aloe) created with the involvement of foreign cultivars and Estonian cultivar Talve Nauding. Zimnee Sikory and Letnee Sladkoe accessions obtained in the collection of VIR from Belorussian breeder I.P. Sikora in the 1960s are also included in this group. The parental forms of these cultivars are unknown, but Sikora often used foreign cultivars in the breeding process [1]. The second group also includes several Baltic (mainly Estonian) landraces (Grushovka Grebnitskogo, Mestnoe Lezhkoe, Seyanets Trebu, Suislepskoe, Tellisaare, Yalkarnan Kesa), Western European cultivar Shtreifling Krasnyi, local Belorussian cultivar Seyanets Rebristogo, and several Russian landraces: Abrikosovoe Mazyuka, Severnyj Velikan (Leningrad region); Sladkoe Zimnee, Tyushkinskoe Krasnoe (Pskov region); Fedor-

ovskoe (Tver region); Slivochnoe, Titovka, Titovka clone (old, once widespread cultivars), which may be associated with the use of landraces in breeding.

Modern Russian cultivars are not significantly differentiated from traditional foreign commercial cultivars; this is apparently a consequence of an increasing involvement of foreign material in domestic breeding programs and can lead to further narrowing of the genetic base of the apple cultivars in our country. However, the problem of apple genetic diversity reduction is global. At present, a relatively small number of apple cultivars are used in world production and breeding programs. Thus, 15 cultivars accounted for 90% of apple production in United States by 2008, out of which just Red Delicious cultivar took 24% [2], while only ten cultivars in Europe in 2012 accounted for 70% of the production. Thus, the Golden Delicious cultivar occupied 35% of the production area in France [23]. Such a small number of cultivars on large areas increases their vulnerability to diseases, pests, and other unfavorable environmental factors.

Wild species of the genus *Malus* are traditional sources of economically valuable traits, such as resistance to biotic and abiotic stresses [2, 24]. The study of genetic diversity of wild *Malus* species from VIR collection by AFLP analysis demonstrated high level of their polymorphism (90.2% of polymorphic loci) [15]. But in addition to valuable traits, wild species transmit undesirable traits to the offspring; in particular, the quality of fruit worsens and their size decreases. To solve this problem, re-hybridization with large-fruited best seedlings obtained from the crossing of cultivars with wild species is recommended, but this complicates and slows down the breeding work. Landraces can be a good alternative to wild species in the breeding programs, since they have passed a long selection and have fewer negative traits that are manifested during the crosses. It is necessary to note that some landraces (such as Antonovka Obyknovennaya, Borovinka, and Osennee Polosatoe) were quite widely used and continue to be used by Russian and foreign breeders as sources of early maturity, productivity, and high environmental stability. However, most of the local and old apple cultivars from the collection of Pushkin and Pavlovsk Laboratories of VIR have almost never been used in breeding. When studying these landraces in modern conditions of unstable changing climate, the genotypes with a wide norm of reaction that can be recommended for breeding as sources of valuable traits are distinguished.

Thus, the genetic diversity of the apple landraces from the collection of the research and production base Pushkin and Pavlovsk Laboratories of VIR was for the first time estimated using AFLP analysis. A high polymorphism level of the apple landraces, as well as differentiation between landraces and modern cultivars, was demonstrated. The data obtained once again confirm the importance of local and old apple cultivars preservation as a valuable source of genetic diversity.
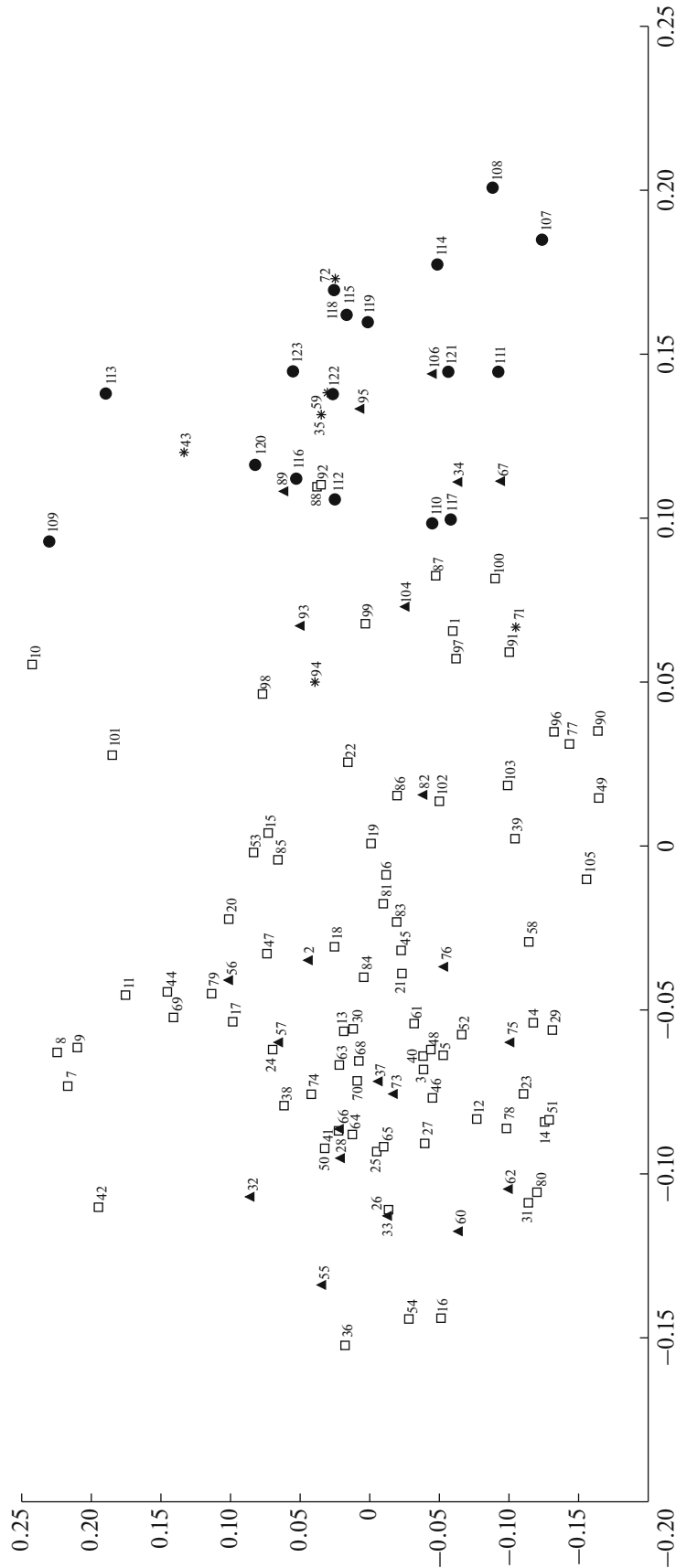
Fig. 1. Location of studied apple accessions in principal coordinates based on AFLP analysis data: (●) modern and commercial cultivars; (□) landraces of Northwest and Central Russia; (▲) landraces of Western Europe and Baltics; (*) Soviet cultivars. Sample numbers on the graph correspond to serial numbers in Table 1.
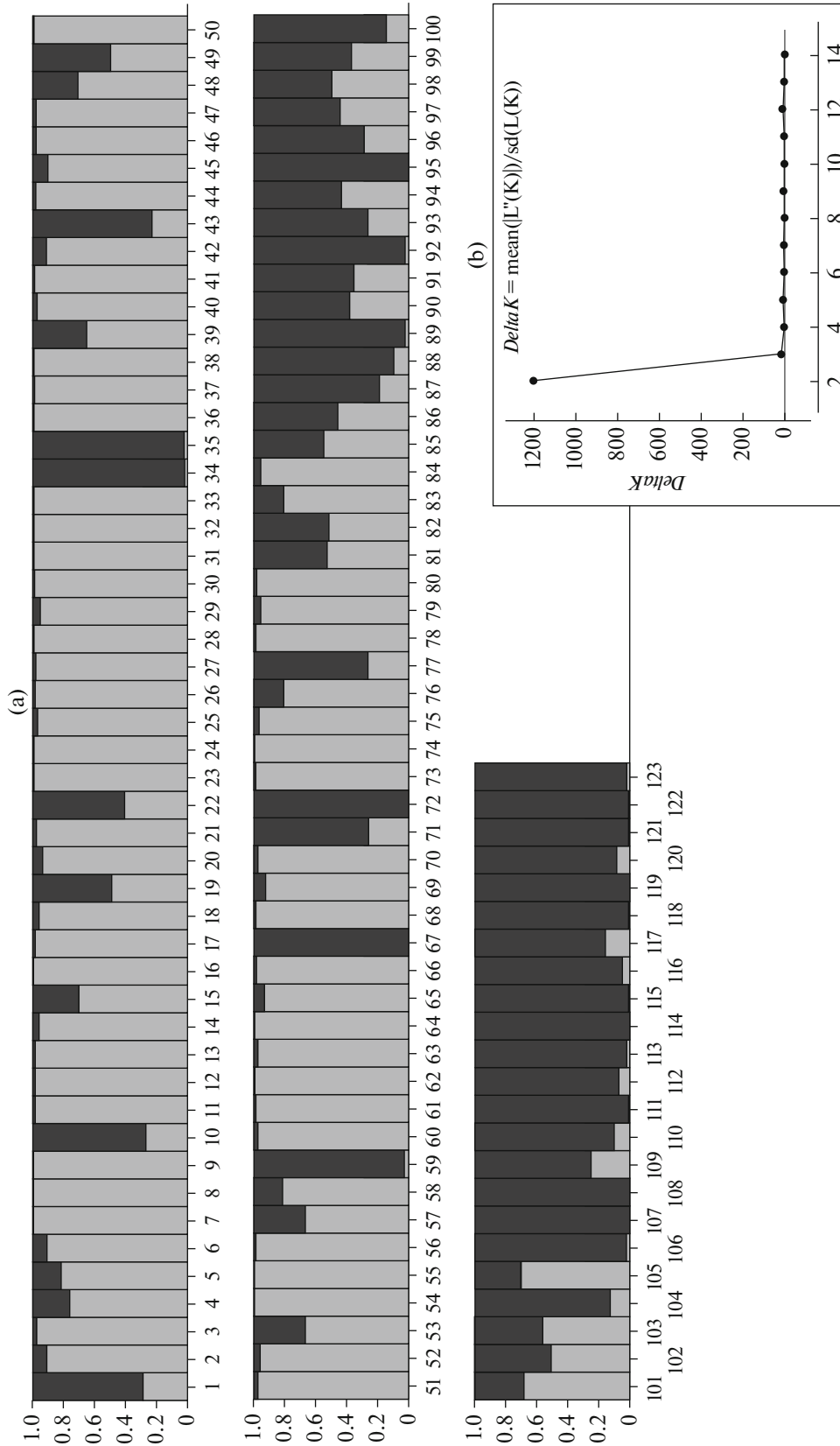
Fig. 2. (a) The probability of ascribing the studied apple accessions to one of the clusters according to the results of the analysis in the STRUCTURE 2.3 program with the number of clusters K = 2. Vertical axis, a posteriori probability of ascribing the sample to one of the clusters; horizontal axis, analyzed samples ((▢) cluster 1; (■) cluster 2); (b) Estimation of optimal number of clusters using the *deltaK* method. The sample numbers correspond to serial numbers in Table 1.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

REFERENCES

1. Kozlovskaya, Z.A., *Selektsiya yabloni v Belarusi* (Apple Tree Breeding in Belorussia), Minsk: Belaruskaya Navuka, 2015.

2. Volk, G.M., Chao, C.T., Norelli, J., et al., The vulnerability of U.S. apple (*Malus*) genetic resources, *Genet. Resour. Crop Evol.*, 2015, vol. 62, no. 5. pp. 765—794.
https://doi.org/10.1007/s10722-014-0194-2

3. http://ww.fao.org.

4. Aleksanyan, S.M., Ponomarenko, V.V., Burmistrov, L.A., et al., *Sovremennye metody i mezhdunarodnyi opyt sokhraneniya genofonda dikorastushchikh rastenii na primere dikikh plodovykh* (Modern Methods and International Experience in Preserving the Gene Pool of Wild Plants Using Wild Fruit Crops as an Example), Almaty, 2011.

5. Aleksanyan, S.M., The strategy of interaction of the world genebanks in the context of globalization, *Tr. Prikl. Bot., Genet. Sel.*, 2007, vol. 164, pp. 11—33.

6. Shlyavas, A.V., Bagmet, L.V., Trifonova, A.A., and Boris, K.V., *Katalog mirovoi kollektsii VIR: yablonya* (World Collection Catalog of Vavilov All-Russian Scientific Research Institute of Plant Genetic Resources: Apple), issue 863: *Sorta narodnoi selektsii v kollektsii geneticheskikh resursov yabloni nauchno-proizvodstvennoi bazy "Pushkinskie i Pavlovskie laboratorii VIR"* (Apple Landraces in the Genetic Resources' Collection at the Pushkin and Pavlovsk Experiment Stations of Vavilov All-Russian Institute of Plant Genetic Resources), St. Petersburg, 2018.
https://doi.org/10.30901/978-5-905954-72-6

7. Nesterov, Ya.S. and Karamysheva, V.I., *Klassifikator roda Malus Mill.* (Genus *Malus* Mill. Classifier), Leningrad: Vsesoyuz. Inst. Rastenievod., 1976.

8. Watkins, R. and Smith, R.A., *Descriptors List for Apple (Malus)*, Rome: International Board for Plant Genetic Resources Secretariat, 1982.

9. Wang, K., Liu, F.Z., and Cao, Y.F., *Descriptors and Data Standard for Apple (Malus spp. Mill.)*, Beijing: China Agriculture Press, 2005.

10. Tignon, M., Kettmann, R., and Watillon, B., AFLP: use for the identification of apple cultivars and mutants, *Acta Hortic.*, 2000, vol. 521, pp. 219—226.
https://doi.org/10.17660/ActaHortic.2000.521.24

11. Kenis, K. and Keulemans, J., Genetic linkage maps of two apple cultivars (*Malus × domestica* Borkh.) based on AFLP and microsatellite markers, *Mol. Breed.*, 2005, vol. 15, pp. 205—219.
https://doi.org/10.1007/s11032-004-5592-2

12. Cevik, V. and King, J., High-resolution genetic analysis of the Sd-1 aphid resistance locus in *Malus* spp., *Theor. Appl. Genet.*, 2002, vol. 105, pp. 346—354.
https://doi.org/10.1007/s00122-002-0904-6

13. Xu, M.L. and Korban, S.S., Saturation mapping of the apple scab resistance gene *Vf* using AFLP markers, *Theor. Appl. Genet.*, 2000, vol. 101, pp. 844—851.
https://doi.org/10.1007/s001220051551

14. Xu, M.L., Huaracha, E., and Korban, S.S., Development of sequence-characterized amplified regions (SCARs) from amplified fragment length polymorphism (AFLP) markers tightly linked to the *Vf* gene in apple, *Genome*, 2001, vol. 44, pp. 63—70.
https://doi.org/10.1139/g00-103

15. Savelyeva, E.N. and Kudryavtsev, A.M., AFLP analysis of genetic diversity in the genus *Malus* Mill. (apple), *Russ. J. Genet.*, 2015, vol. 51, no. 10, pp. 966—973.
https://doi.org/10.1134/S1022795415100154

16. Vos, P.R., Hogers, M., Bleeker, M., et al., AFLP: a new technique for DNA fingerprinting, *Nucleic Acids Res.*, 1995, vol. 23, pp. 4407—4414.

17. Benbouza, H., Jacquemin, J.M., Baudoin, J.P., and Mergeai, G., Optimization of a reliable, fast, cheap and sensitive silver staining method to detect SSR markers in polyacrylamide gels, *BASE*, 2006, vol. 10, no. 2, pp. 77—81.

18. Peakall, R. and Smouse, P.E., GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update, *Bioinformatics*, 2012, no. 28, pp. 2537—2539.
https://doi.org/10.1093/bioinformatics/bts460

19. Hammer, O., Harper, D.A.T., and Ryan, P.D., PAST: Paleontological Statistics software package for education and data analysis, *Paleontol. Electron.*, 2001, vol. 4, no. 1, pp. 1—9.

20. Pritchard, J.K., Stephens, M., and Donnelly, P., Inference of population structure using multilocus genotype data, *Genetics*, 2000, vol. 155, no. 2, pp. 945—959.

21. Lacis, G., Kota, I., Ikase, L., and Rungis, D., Molecular characterization of the Latvian apple (*Malus*) genetic resource collection based on SSR markers and scab resistance gene *Vf* analysis, *Plant Genet. Resour.: Charact. Util.*, 2011, vol. 9, no. 2, pp. 189—192.
https://doi.org/10.1017/S1479262111000384

22. Garkava-Gustavsson, L., Mujajub, C., Sehic, J., et al., Genetic diversity in Swedish and Finnish heirloom apple cultivars revealed with SSR markers, *Sci. Hortic.*, 2013, vol. 162, pp. 43—48.
https://doi.org/10.1016/j.scienta.2013.07.040

23. Lassois, L., Denance, C., Ravon, E., et al., Genetic diversity, population structure, parentage analysis, and construction of core collections in the French apple germplasm based on SSR markers, *Plant Mol. Biol. Rep.*, 2016, vol. 34, pp. 827—844.
https://doi.org/10.1007/s11105-015-0966-7

24. Pereira-Lorenzo, S., Fischer, M., Ramos-Cabrer, A.M., and Castro, I., Apple (*Malus* spp.) breeding: present and future, *Adv. Plant Breed. Strategies: Fruits*, 2018, vol. 3, pp. 3—29.
https://doi.org/10.1007/978-3-319-91944-7_1

Translated by A. Barkhash