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Study of genetic diversity in wild raspberry (*Rubus idaeus* L.) germplasm collection using morphological characters and RAPD markers

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Information about patterns of genetic diversity in wild relatives of crop species can be used for the improvement of genetic properties of new cultivars. Forty-nine wild raspberry (*Rubus idaeus* L.) clonal accessions collected from different locations in Lithuania and currently maintained in the field collection of Vilnius University Botanical Garden were examined by comparing the variation of morphological traits, RAPD markers and geographical distribution of the original locations. Thirteen morphological and 48 polymorphic RAPD markers were assessed in this study. Six preselected oligonucleotide primers produced highly polymorphic and reproducible RAPD patterns. By means of RAPD fingerprints generated using at least two primers, all the plants studied were genotyped. The UPGMA dendrogram demonstrated a high level of genetic variation among accessions. The Euclidean and molecular genetic distance matrices were calculated using morphological and RAPD data, respectively. A correlation between morphological and molecular variation was assessed. A weak but significant correlation between matrices created on the basis of molecular (RAPD) and morphological data was observed ($r = 0{,}073$; $p = 0.012$). A slight but significant correlation was established between Nei and Li's genetic distance matrix and the Euclidean geographical distance matrix ($r = 0.123$; $p = 0.001$). Results of the study are intended for the management and utilization of genetic resources of wild raspberry.

Key words: *Rubus idaeus*, raspberry, RAPD, morphological variation, genetic resources, collection

INTRODUCTION

Wild raspberry (*Rubus idaeus* L.) is widely spread in temperate regions of the Northern hemisphere and exhibits a high level of morphological, phenological, genetic and habitat variation [1–4]. The species is a wild relative to the multitude of commercial raspberry cultivars. Wild relatives of contemporary crops often possess many traits, gene alleles and genetic structures not found in domesticated representatives of the species. The process of domestication is usually associated with the loss of genetic diversity because of predefined breeding targets and a limited number of genotypes used in breeding programs. Thus, cultivated forms are more genetically homogeneous if compared to their wild progenitors [5–7]. Raspberry is just the case. Modern cultivars of red raspberry are morphologically and genetically similar and have a narrow genetic base [1, 8–10]. Therefore, the maintenance and study of the natural germplasm of raspberry as a potential gene donor is important for the conservation and utilization of raspberry genetic resources.

The characterization of wild raspberry germplasm has been carried out using morphological, biochemical and DNA markers [1–4, 11, 12]. The morphological traits have been used in breeding programs for a very long time. Unfortunately, the genetic determinants of these traits alone represent only a small part of the plant genome. Besides, they are strongly influenced by environmental conditions and heavily depend on the developmental stage [13]. In this situation, the molecular markers can compensate some disadvantages of morphological markers and have rather a good perspective in plant breeding process [14, 15]. The exploitation of both types of markers is more valuable in studies of plant genetic resources [16].

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In our work, we used morphological and molecular (RAPD) markers for the evaluation of genetic diversity in the clonal germplasm collection of wild raspberry at the Vilnius University Botanical Garden. The main objectives of our study were: 1) the phenotypic (morphological) evaluation of wild raspberry accessions; 2) the genotyping of these accessions and the assessment of genetic diversity in the collection using RAPD markers; 3) determination of correlation between morphological variation, DNA polymorphism and geographical origin of the genotypes.

MATERIALS AND METHODS

Plant material. Forty-nine wild raspberry samples from the field collection of the Vilnius University Botanical Garden were studied (Table 1). The collection was established in 2001 and consists of wild raspberry samples collected from different locations of Lithuania [17] and presently maintained in an experimental plot under the same field conditions.

Morphological characterization. The morphological characterization of raspberry accessions employing 13 quantitative morphological characters was being performed for 2 to 5 years during the period of 2003–2007 (Table 2).

RAPD analysis. Genomic DNA was extracted from samples of young leaves (0.1 g) taken from the upper part of floricanes (usually third-fifth leaves) as described previously [18]. DNA extraction was carried out in 2002–2003. DNA concentration was established using a biophotometer (Eppendorf). DNA samples were diluted to ca. 50 ng/µl. The preliminary selection of suitable oligonucleotide primers was performed on 20 genotypes to select informative and suitable primers for the RAPD-PCR analysis [18]. Six primers were chosen to analyse 49 wild raspberry genotypes for RAPD variation. RAPD-PCR was performed as described earlier [19]. Each reaction was repeated at least two times.

Data analysis. Using the geographical coordinates (Table 1) of the original collecting sites, the geographical Euclidean distance matrix was calculated. Normality test for morphological data was performed using the SAS procedure UNIVARIATE. The variation coefficients of morphological traits were calculated using the SAS procedure MEANS. To assess the morphological diversity among raspberry accessions, the quantitative morphological data were standardized and the Euclidean distance matrix was calculated (PopTools 2.7.5) [20]. This phenotypic distance matrix was evaluated by estimating differences between 1176 possible pairs of 49 accessions for 13 traits.

Table 1. **Accession numbers, original locations and collecting coordinates of the field accessions of Rubus idaeus**

Access. number	Original location	Collecting coordinates		Access. number	Original location	Collecting coordinates	
		Latit., N	Long., E			Latit., N	Long., E
JL01	Lazdijai, Dusia	54°17′	23°40′	JL34	Marijampolė, Bukta	54°26′	23°25′
JL02	Alytus, Pocelonys	54°21′	24°13′	JL35	Molėtai, Mindūnai	55°12′	25°35′
JL03	Varėna, Valkininkai	54°20′	24°45′	JL36	Raseiniai, Steponkaimis	55°25′	23°24′
JL04	Akmenė, Venta	56°06′	22°51′	JL37	Kaunas, Babtai	55°02′	23°47′
JL05	Kėdainiai, Krakės	55°24′	23°47′	JL39	Šiauliai, Kuršėnai	55°59′	23°03′
JL06	Trakai, Spindis	54°33′	24°42′	JL40	Radviliškis, Arimaičiai	55°46′	23°40′
JL07	Vilnius, Kairėnai	54°43′	25°24′	JL41	Pakruojis, Žvirbloniai	55°51′	23°57′
JL08	Ukmergė, Užulienis	55°24′	24°32′	JL42	Panevėžys, Alantė	55°31′	24°34′
JL09	Panevėžys, Ustronė	55°37′	24°05′	JL43	Šakiai, Baltrušiai	54°44′	23°12′
JL10	Prienai, Vėžionys	54°32′	24°09′	JL44	Vilkaviškis, Gurbšilis	54°37′	23°12′
JL11	Vilnius, Verkiai	54°45′	25°18′	JL45	Lazdijai, Trako m.	54°13′	23°46′
JL12	Kaišiadorys, Žiežmariai	54°48′	24°28′	JL47	Švenčionys, Mociškė	55°12′	26°40′
JL13	Vilnius, Pavilniai	54°41′	25°21′	JL52	Vilnius, Gailiūnai	54°43′	25°29′
JL14	Šalčininkai, B. Vokė	54°28′	25°08′	JL54	Jonava, Upininkai	55°03′	24°33′
JL15	Vilnius, Bezdonys	54°47′	25°27′	JL56	Širvintos, Pakalniškiai	54°54′	24°50′
JL16	Vilnius, Melkys	54°51′	25°12′	JL61	Varėna, Dargužiai	54°24′	24°51′
JL17	Neringa, Nida	55°17′	20°58′	JL64	Kelmė, Raudgiris	55°32′	22°38′
JL18	Neringa, Juodkrantė	55°35′	21°07′	JL65	Šilalė, Pagramantis	55°24′	22°13′
JL19	Jurbarkas, Lenkčiai	55°18′	22°44′	JL72	Biržai, Latveliai	56°20′	24°49′
JL20	Kaunas, Girionys	54°51′	24°02′	JL76	Anykščiai, Pelyša	55°39′	25°05′
JL22	Vilnius, Turgeliai	54°17′	23°40′	LB01	Prienai, Mauručiai	54°46′	23°45′
JL23	Vilnius, M. Kuosinė	54°34′	25°41′	LB02	Kupiškis, Šepeta	55°48′	25°02′
JL25	Vilnius, Mickūnai	54°43′	25°27′	IŽ01	Zarasai, Salakas	55°36′	26°07′
JL32	Tauragė, Liaudginai	55°19′	22°33′	SS01	Zarasai, Puščia	55°41′	26°05′
JL33	Klaipėda, Girininkai	55°39′	21°31′				

Table 2. **Means and standard deviations of 13 morphological characters of *R. idaeus* accessions**

Accession	Leaf length, cm	Leaf width, cm	Length of terminal leaflet in compound leaf, cm	Width of terminal leaflet in compound leaf, cm	Length of petiole, cm	Length of rachis, cm	Number of drupelets in aggregate fruit	Fruit weight, g	Fruit length, mm	Fruit diameter, mm	Floricane height, m	Floricane diameter at the height of 30 cm	Flower diameter, cm
JL01	12.07 ± 2.46	10.03 ± 1.94	6.13 ± 1.11	5.02 ± 1.21	1.84 ± 0.54	4.04 ± 0.95	39.00 ± 8.34	0.43 ± 0.03	9.20 ± 0.28	10.50 ± 0.28	1.18 ± 0.03	0.85 ± 0.03	1.91 ± 0.04
JL02	9.73 ± 0.73	8.80 ± 0.49	5.10 ± 0.20	3.75 ± 0.34	1.27 ± 0.26	3.23 ± 0.53	34.65 ± 1.48	0.39 ± 0.01	9.70 ± 0.57	10.45 ± 0.21	1.13 ± 0.22	0.80 ± 0.04	2.17 ± 0.13
JL03	12.10 ± 2.55	10.49 ± 2.39	6.27 ± 1.38	3.94 ± 0.84	1.59 ± 0.40	4.15 ± 0.95	36.75 ± 6.01	0.46 ± 0.01	9.50 ± 0.85	9.90 ± 0.71	0.94 ± 0.19	0.59 ± 0.29	1.75 ± 0.13
JL04	11.44 ± 0.41	10.70 ± 0.97	6.23 ± 0.58	3.70 ± 0.27	1.69 ± 0.23	3.55 ± 0.28	32.20 ± 5.66	0.41 ± 0.02	8.20 ± 0.99	9.95 ± 0.49	1.44 ± 0.03	0.63 ± 0.03	1.87 ± 0.16
JL05	12.31 ± 2.20	11.25 ± 2.04	6.77 ± 1.54	4.32 ± 0.71	1.61 ± 0.38	3.93 ± 0.48	41.15 ± 10.82	0.55 ± 0.10	11.80 ± 0.00	11.40 ± 0.00	1.29 ± 0.11	0.68 ± 0.05	1.49 ± 0.27
JL06	12.41 ± 2.34	10.29 ± 2.51	5.92 ± 1.24	4.30 ± 1.30	2.13 ± 0.66	4.52 ± 0.60	42.29 ± 5.11	0.46 ± 0.05	9.37 ± 1.46	10.12 ± 1.11	1.18 ± 0.08	0.87 ± 0.06	2.10 ± 0.00
JL07	10.64 ± 1.43	9.90 ± 0.74	6.01 ± 0.82	4.12 ± 0.44	1.46 ± 0.22	2.96 ± 0.40	38.70 ± 5.23	0.72 ± 0.27	11.45 ± 2.33	12.00 ± 1.41	1.20 ± 0.21	0.63 ± 0.12	1.92 ± 0.11
JL08	13.77 ± 2.39	13.81 ± 2.17	8.19 ± 1.75	4.69 ± 1.05	1.78 ± 0.41	3.63 ± 0.48	38.40 ± 3.11	0.69 ± 0.12	10.80 ± 0.28	12.00 ± 0.00	1.50 ± 0.10	0.70 ± 0.17	2.01 ± 0.04
JL09	11.87 ± 1.69	10.76 ± 1.61	6.28 ± 1.05	4.56 ± 0.64	1.56 ± 0.45	3.91 ± 0.36	42.90 ± 5.80	0.75 ± 0.05	12.30 ± 0.42	12.75 ± 0.07	1.56 ± 0.11	0.70 ± 0.13	2.18 ± 0.00
JL10	13.26 ± 1.31	12.12 ± 1.63	6.85 ± 1.11	4.54 ± 0.55	2.00 ± 0.36	4.31 ± 0.59	46.60 ± 4.38	0.59 ± 0.02	11.10 ± 0.28	11.75 ± 0.21	1.54 ± 0.08	0.68 ± 0.04	1.78 ± 0.00
JL11	11.33 ± 2.16	10.02 ± 2.01	6.12 ± 1.39	4.53 ± 0.73	1.69 ± 0.40	3.44 ± 0.64	33.40 ± 3.11	0.58 ± 0.04	9.85 ± 0.35	11.75 ± 0.07	1.24 ± 0.10	0.64 ± 0.05	1.58 ± 0.06
JL12	11.58 ± 2.50	9.99 ± 1.85	5.99 ± 1.45	4.35 ± 0.97	1.75 ± 0.41	3.83 ± 0.79	36.75 ± 1.20	0.55 ± 0.08	10.60 ± 0.42	11.05 ± 0.21	1.51 ± 0.04	0.65 ± 0.00	1.66 ± 0.06
JL13	9.04 ± 0.82	9.03 ± 0.69	5.11 ± 0.56	3.27 ± 0.10	1.01 ± 0.18	2.84 ± 0.33	47.65 ± 16.33	0.46 ± 0.13	10.05 ± 1.20	10.25 ± 0.78	0.97 ± 0.19	0.61 ± 0.15	1.73 ± 0.04
JL14	11.11 ± 2.81	10.18 ± 2.66	5.55 ± 1.63	3.73 ± 1.01	1.85 ± 0.56	3.47 ± 0.53	28.31 ± 9.06	0.32 ± 0.05	8.31 ± 0.58	9.70 ± 0.42	1.48 ± 0.14	0.75 ± 0.03	1.57 ± 0.09
JL15	10.23 ± 1.25	9.62 ± 1.15	5.59 ± 0.75	4.07 ± 0.57	1.28 ± 0.25	3.19 ± 0.45	38.70 ± 4.67	0.55 ± 0.13	9.70 ± 1.41	11.25 ± 0.64	1.09 ± 0.14	0.62 ± 0.13	1.58 ± 0.25
JL16	12.46 ± 1.57	12.08 ± 2.01	6.92 ± 1.25	4.99 ± 0.84	1.77 ± 0.42	3.69 ± 0.28	56.50 ± 5.37	0.86 ± 0.08	13.10 ± 1.41	12.95 ± 0.49	1.39 ± 0.12	0.69 ± 0.07	2.14 ± 0.08
JL17	10.48 ± 1.31	10.19 ± 1.05	6.08 ± 0.91	3.86 ± 0.33	1.36 ± 0.26	2.91 ± 0.30	35.30 ± 17.96	0.49 ± 0.08	10.35 ± 0.92	10.75 ± 0.07	1.35 ± 0.09	0.68 ± 0.14	1.67 ± 0.07
JL18	11.66 ± 2.01	10.53 ± 2.17	6.36 ± 1.36	4.94 ± 0.72	1.59 ± 0.45	3.47 ± 0.45	37.51 ± 0.16	0.56 ± 0.10	9.43 ± 0.25	11.88 ± 0.18	1.46 ± 0.05	0.74 ± 0.05	1.54 ± 0.06
JL19	9.41 ± 0.76	9.05 ± 0.48	5.36 ± 0.25	3.39 ± 0.33	1.39 ± 0.27	2.46 ± 0.27	43.45 ± 0.64	0.50 ± 0.20	9.80 ± 0.99	10.55 ± 1.06	1.51 ± 0.25	0.69 ± 0.09	1.62 ± 0.22
JL20	11.57 ± 1.44	10.96 ± 0.89	6.64 ± 0.92	4.36 ± 0.72	1.64 ± 0.27	3.26 ± 0.50	37.27 ± 1.32	0.47 ± 0.04	10.12 ± 0.31	10.88 ± 0.40	1.27 ± 0.14	0.61 ± 0.08	2.15 ± 0.58
JL22	12.01 ± 2.39	11.38 ± 3.00	6.58 ± 1.80	4.62 ± 1.81	1.72 ± 0.61	3.65 ± 0.51	39.75 ± 1.06	0.66 ± 0.10	10.73 ± 1.66	12.38 ± 1.16	1.40 ± 0.02	0.70 ± 0.16	2.08 ± 0.12
JL23	10.55 ± 2.13	9.09 ± 1.48	5.41 ± 0.88	3.85 ± 0.44	1.15 ± 0.41	4.00 ± 1.02	51.00 ± 0.57	0.85 ± 0.09	12.45 ± 0.21	13.15 ± 0.21	1.16 ± 0.44	0.63 ± 0.20	1.83 ± 0.38
JL25	11.29 ± 0.62	9.71 ± 0.51	5.52 ± 0.51	4.14 ± 0.30	1.90 ± 0.18	3.72 ± 0.18	40.65 ± 15.06	0.45 ± 0.04	9.80 ± 0.71	10.50 ± 0.00	1.44 ± 0.19	0.74 ± 0.03	1.63 ± 0.15
JL32	12.14 ± 2.47	11.02 ± 1.99	6.66 ± 1.23	5.16 ± 0.89	1.42 ± 0.38	3.95 ± 0.80	38.10 ± 5.23	0.93 ± 0.06	11.75 ± 0.35	14.05 ± 0.6	1.40 ± 0.20	0.72 ± 0.04	1.82 ± 0.02
JL33	10.65 ± 1.67	10.29 ± 1.02	6.18 ± 0.76	3.88 ± 0.64	1.38 ± 0.27	2.96 ± 0.56	47.15 ± 2.19	0.71 ± 0.17	10.80 ± 0.57	12.15 ± 0.78	1.66 ± 0.15	0.88 ± 0.01	2.08 ± 0.00
JL34	10.73 ± 2.17	9.88 ± 2.41	5.78 ± 1.34	4.06 ± 0.56	1.73 ± 0.49	3.02 ± 0.47	46.85 ± 4.45	0.71 ± 0.21	11.50 ± 1.56	12.30 ± 1.13	1.42 ± 0.24	0.69 ± 0.08	1.76 ± 0.00

Table 2. **Continued**

Accession	Leaf length, cm	Leaf width, cm	Length of terminal leaflet in compound leaf, cm	Width of terminal leaflet in compound leaf, cm	Length of petiole, cm	Length of rachis, cm	Number of drupelets in aggregate fruit	Fruit weight, g	Fruit length, mm	Fruit diameter, mm	Floricane height, m	Floricane diameter at the height of 30 cm	Flower diameter, cm
JL35	11.13 ± 0.72	11.11 ± 1.16	6.58 ± 0.51	4.57 ± 0.54	1.48 ± 0.17	2.92 ± 0.13	41.05 ± 0.21	0.92 ± 0.09	11.90 ± 0.28	12.85 ± 0.64	1.60 ± 0.09	0.92 ± 0.15	2.24 ± 0.03
JL36	14.64 ± 2.22	13.23 ± 2.11	7.90 ± 1.90	5.49 ± 0.81	2.22 ± 0.56	4.51 ± 0.47	40.35 ± 5.30	0.70 ± 0.03	11.70 ± 0.28	11.70 ± 0.72	1.68 ± 0.10	0.73 ± 0.05	2.06 ± 0.29
JL37	12.30 ± 1.72	11.74 ± 1.68	6.73 ± 1.05	5.27 ± 1.11	1.92 ± 0.60	3.54 ± 0.34	32.50 ± 5.23	0.60 ± 0.04	10.20 ± 1.70	11.30 ± 0.71	1.22 ± 0.03	0.78 ± 0.07	1.95 ± 0.04
JL39	9.23 ± 1.04	8.94 ± 0.77	5.41 ± 0.60	3.50 ± 0.60	1.18 ± 0.38	2.65 ± 0.53	35.95 ± 0.07	0.47 ± 0.16	10.39 ± 1.18	10.56 ± 2.20	1.21 ± 0.08	0.60 ± 0.11	1.88 ± 0.20
JL40	10.68 ± 1.00	9.78 ± 0.62	5.77 ± 0.55	4.09 ± 0.14	1.62 ± 0.15	3.16 ± 0.23	45.50 ± 10.47	0.62 ± 0.04	10.35 ± 1.06	11.35 ± 0.07	1.22 ± 0.21	0.78 ± 0.07	1.93 ± 0.00
JL41	11.62 ± 2.21	11.31 ± 2.42	6.56 ± 1.39	4.35 ± 0.97	1.45 ± 0.49	3.34 ± 0.20	24.00 ± 0.99	0.35 ± 0.02	8.35 ± 0.07	10.00 ± 0.28	1.37 ± 0.15	0.75 ± 0.13	2.17 ± 0.00
JL42	11.24 ± 3.45	10.73 ± 2.68	5.86 ± 1.19	4.09 ± 0.90	1.51 ± 0.64	3.66 ± 1.41	47.27 ± 14.62	0.49 ± 0.20	9.99 ± 2.13	9.75 ± 1.91	1.39 ± 0.07	0.68 ± 0.11	1.65 ± 0.00
JL43	10.94 ± 3.11	10.52 ± 2.57	5.97 ± 1.77	3.59 ± 0.67	1.22 ± 0.49	3.56 ± 0.91	29.05 ± 8.98	0.47 ± 0.02	9.35 ± 0.07	10.95 ± 0.78	1.56 ± 0.11	0.82 ± 0.21	1.88 ± 0.03
JL44	11.24 ± 1.15	10.79 ± 0.83	6.18 ± 0.78	3.68 ± 0.44	1.62 ± 0.33	3.30 ± 0.30	39.35 ± 7.71	0.77 ± 0.06	11.95 ± 0.64	12.25 ± 0.07	1.52 ± 0.07	0.78 ± 0.02	1.88 ± 0.17
JL45	10.61 ± 1.01	9.91 ± 0.91	6.23 ± 0.57	4.57 ± 0.70	1.14 ± 0.21	3.00 ± 0.33	44.30 ± 3.39	0.88 ± 0.31	12.40 ± 2.26	12.45 ± 1.48	1.55 ± 0.02	0.77 ± 0.16	2.04 ± 0.01
JL47	10.67 ± 0.93	10.83 ± 1.07	6.43 ± 0.74	3.65 ± 0.59	1.16 ± 0.08	2.91 ± 0.15	37.40 ± 4.24	0.56 ± 0.05	9.40 ± 0.00	11.30 ± 0.00	1.38 ± 0.05	0.73 ± 0.08	1.65 ± 0.02
JL52	11.50 ± 4.62	9.68 ± 3.25	5.81 ± 2.14	3.86 ± 1.14	1.59 ± 0.88	4.09 ± 1.71	41.74 ± 14.08	0.44 ± 0.10	9.53 ± 0.19	10.11 ± 0.16	1.36 ± 0.10	0.65 ± 0.04	1.64 ± 0.15
JL54	9.11 ± 0.60	8.85 ± 0.59	4.73 ± 0.24	3.60 ± 0.18	1.20 ± 0.14	3.01 ± 0.37	32.98 ± 0.82	0.65 ± 0.14	10.03 ± 0.45	11.46 ± 0.93	1.24 ± 0.08	0.67 ± 0.17	1.61 ± 0.04
JL56	11.29 ± 1.26	10.44 ± 1.01	5.71 ± 0.49	4.32 ± 0.71	1.54 ± 0.43	3.96 ± 0.44	35.50 ± 2.55	0.50 ± 0.07	10.05 ± 1.48	10.30 ± 0.14	1.31 ± 0.24	0.79 ± 0.19	2.35 ± 0.12
JL61	11.85 ± 1.00	10.51 ± 0.65	6.49 ± 0.67	4.05 ± 0.11	1.29 ± 0.22	3.97 ± 0.23	37.80 ± 2.12	0.46 ± 0.11	9.60 ± 0.14	10.30 ± 0.85	1.30 ± 0.03	0.66 ± 0.02	1.65 ± 0.12
JL64	11.50 ± 1.17	10.71 ± 1.09	5.78 ± 0.72	5.07 ± 0.48	1.95 ± 0.30	3.70 ± 0.45	48.30 ± 14,14	0.64 ± 0.07	11.65 ± 1.91	12.45 ± 2.05	1.51 ± 0.14	0.83 ± 0.14	1.94 ± 0.00
JL65	12.43 ± 3.39	12.20 ± 3.23	7.07 ± 1.91	3.46 ± 0.80	1.57 ± 0.49	3.58 ± 0.93	40.90 ± 4.67	0.53 ± 0.06	9.55 ± 0.07	10.60 ± 0.71	1.23 ± 0.39	0.69 ± 0.22	1.92 ± 0.00
JL72	13.33 ± 1.55	12.10 ± 0.61	7.58 ± 1.35	4.00 ± 0.57	2.05 ± 0.19	3.76 ± 0.57	56.05 ± 6.01	0.65 ± 0.12	10.95 ± 0.07	11.40 ± 1.13	1.55 ± 0.18	0.72 ± 0.10	1.65 ± 0.00
JL76	11.67 ± 0.69	11.36 ± 0.22	6.66 ± 0.27	4.10 ± 0.19	1.41 ± 0.03	3.48 ± 0.46	47.61 ± 12.00	0.63 ± 0.12	11.15 ± 0.92	11.45 ± 0.78	1.48 ± 0.13	0.85 ± 0.16	1.86 ± 0.00
LB01	11.08 ± 0.73	9.98 ± 0.74	5.93 ± 0.60	4.13 ± 0.38	1.60 ± 0.06	3.54 ± 0.35	41.70 ± 0.71	0.74 ± 0.05	11.20 ± 0.14	11.75 ± 0.07	0.84 ± 0.06	0.60 ± 0.16	1.72 ± 1.01
LB02	12.00 ± 0.51	11.25 ± 0.45	6.28 ± 0.44	4.77 ± 0.22	2.07 ± 0.07	3.53 ± 0.14	50.52 ± 8.74	0.73 ± 0.24	10.95 ± 1.34	11.74 ± 1.36	1.49 ± 0.08	0.71 ± 0.17	2.04 ± 0.32
IŽ01	10.34 ± 0.75	9.53 ± 0.69	5.63 ± 0.32	4.37 ± 0.11	1.83 ± 0.15	2.81 ± 0.56	27.06 ± 2.13	0.40 ± 0.09	8.34 ± 0.48	9.13 ± 0.35	1.12 ± 0.14	0.70 ± 0.16	2.03 ± 0.60
SS01	12.69 ± 2.67	12.27 ± 1.91	7.13 ± 1.28	4.86 ± 0.80	1.50 ± 0.40	3.86 ± 1.04	57.75 ± 7.57	1.01 ± 0.00	12.90 ± 0.57	13.65 ± 0.07	1.43 ± 0.21	0.81 ± 0.19	1.73 ± 0.00
Average	11.41 ± 1.70	10.59 ± 1.49	6.20 ± 1.00	4.24 ± 0.64	1.58 ± 0.35	3.51 ± 0.58	40.43 ± 7.14	0.60 ± 0.16	10.48 ± 1.21	11.33 ± 1.10	1.35 ± 0.19	0.72 ± 0.08	1.86 ± 0.22
Data of	5 years						2 years				3 years		2 years

RAPD data were scored in the presence (1) or absence (0) of a given amplification product in each genotype. The resulting binary matrix was used to calculate the genetic distance coefficients: Nei and Li's – GD_{NL} [21]; Link – GD_{L} [22]; simple matching – GD_{SM} [23] and to estimate the genetic diversity among the genotypes. The genetic distance matrices were calculated from polymorphic RAPD loci (bands). A locus was considered polymorphic if the most common "allele" was detected at a frequency less than 95% among the wild raspberry accessions. Correlations between these matrices were estimated using STATISTICA 7 [24].

The correlation between the morphological Euclidean and molecular GD_{NL} matrices, morphological Euclidean and geographical Euclidean matrices, the correlation between the GD_{NL} matrix and the geographical Euclidean distance matrix were calculated and tested by the Mantel test using PopTools 2.7.5. Pearson's r-value was used to measure a linear correlation between two matrices. The p-value was calculated using the distribution of r(AB) estimated from 10000 permutations. The SAS procedure GENMOD (generalized linear models) with model options of the link function 'logit' and the binomial distribution variance function were used for estimating of the effect of presence or absence of a locus band on geographic or morphologic traits [25]. The UPGMA (i. e. unweighted pair-group method using an arithmetical mean) dendrogram based on morphological data was generated using STATISTICA 7 [24]. The UPGMA dendrogram based on molecular data (GD_{NL}) was constructed using the TREECON computer program [26].

RESULTS

The mean values and standard deviations of the morphological characters studied (Table 2) show a rather high morphological variation among the accessions. Checking the normality of data distribution revealed that only data of three characters (floricane height, floricane diameter and flower diameter) showed a departure from normality. The Mantel test showed that the correlation coefficient between the morphological Euclidean distance matrix and the Euclidean geographical distance matrix was very small, r = 0.070 (p = 0.017).

The UPGMA dendrogram based on the average Euclidean distances estimated from morphological data is shown in Fig. 1. The dendrogram consists of two clusters. These clusters include groups of accessions demonstrating certain values of morphological characters. Each cluster is composed of two smaller groups (subclusters). For example, the first cluster joins genotypes with characters that are strongly expressed. Among them, six accessions (JL32, SS01, JL16, JL45, JL35, JL33) have larger and heavier fruits and five genotypes (subcluster) have larger leaves (JL65, JL72, JL10, JL37, JL08, JL36). The second cluster includes groups of individuals with smaller leaves (JL19, JL54, JL39, JL13, JL02) and fruits (JL41, JL20, IŽ01, JL14, JL04). Based on the morphological Euclidean distance matrix, the most similar genotypes were JL17 and JL47, followed by JL52 and JL42, while the most different were JL13 and JL36.

RAPD-PCRs were performed with 49 *R. idaeus* genotypes (Table 1). The amplification products of wild raspberry genomic DNA were highly polymorphic. An example of RAPD phenotypes of *R. idaeus* is shown in Fig. 2. A total of 6 primers, summarized in Table 3, generated 63 RAPD bands in the range of 390–2900 bp; 76.2% of amplification products were polymorphic. The average percentage of polymorphic DNA fragments amplified per primer was 77.83%, ranging between 64% (Roth 270–6) and 89% (MP4). On the basis on 48 polymorphic DNA fragments, genetic distances were calculated among the genotypes. Three types of genetic distance matrices were generated (GD_{NL}, GD_{L} and GD_{SM}). Correlations among these matrices were very high (GD_{SM}–GD_{L} r = 0.96; GD_{NL}–GD_{SM} r = 0.95; GD_{NL}–GD_{L} r = 0.998). The correlation between Nei and Li's genetic distance matrix and the Euclidean geographical distance matrix (r = 0.123; p = 0.001) was slight but significant. The interdependence of these two variables is illustrated in Fig. 3.

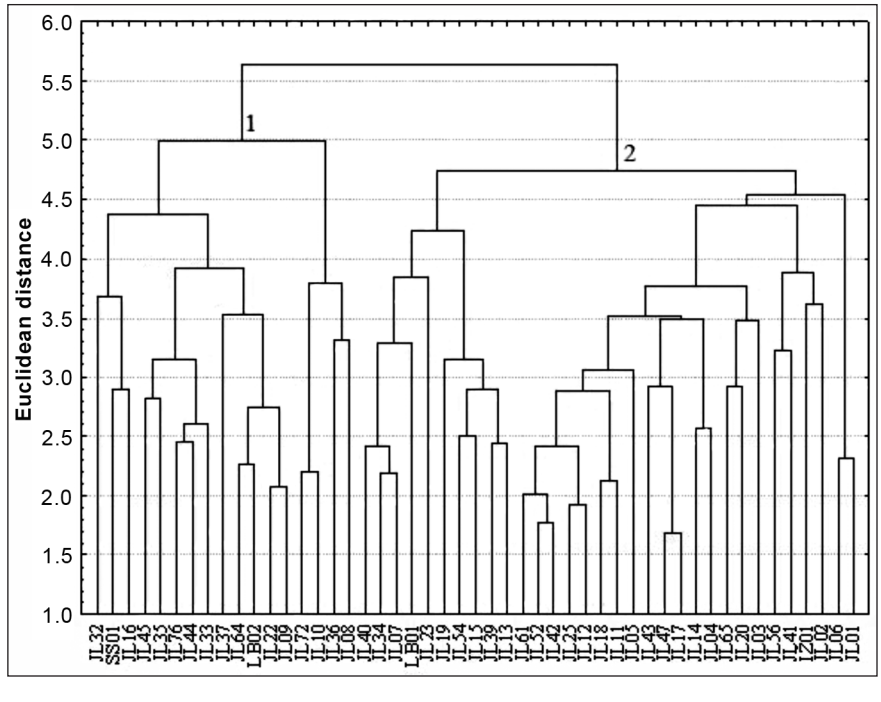

Fig. 1. UPGMA dendrogram showing relationships between 49 accessions of *Rubus idaeus* based on morphological Euclidean distance matrix

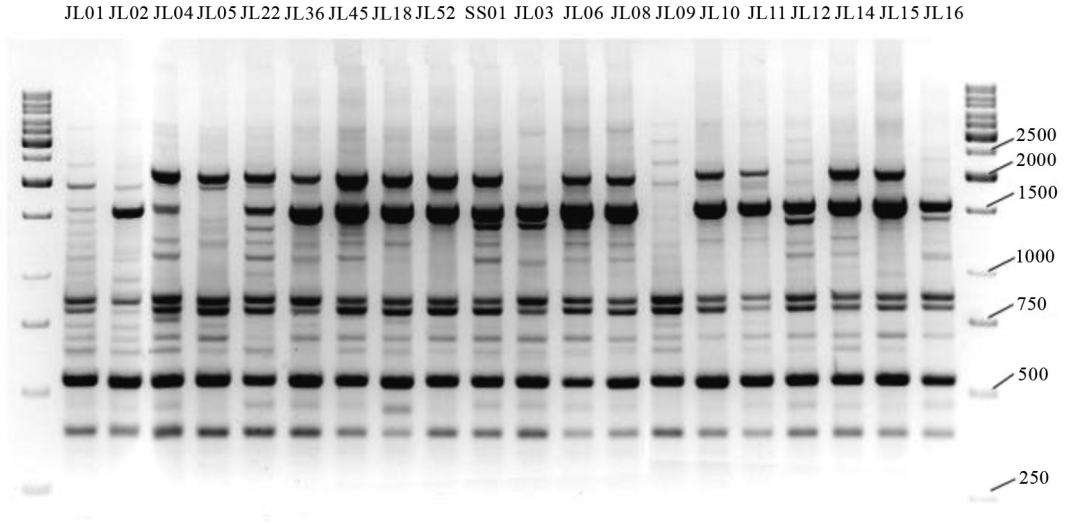

Fig. 2. RAPD phenotypes of 20 wild raspberry accessions generated by the primer Roth 380-3. The first and the last lines of the agarose gel – DNA fragment ladder (GeneRuler™1kb DNA Ladder, Fermentas). Size of DNA fragments is shown in base pairs

Table 3. **Number of RAPD bands and RAPD patterns determined per primer on 49 accessions of *R. idaeus***

RAPD primer	Analysed RAPD bands	Polymorphic RAPD bands	Polymorphism, %	Size of analysed DNA bands (bp)	Number of RAPD patterns	
					Total	%*
A3	10	8	80	450–1800	22	45
MP4	9	8	89	550–2500	39	80
Roth 270-6	14	9	64	500–2900	30	61
Roth 380-3	12	9	75	390–2100	30	61
Roth 470-8	11	8	73	440–3100	37	76
Roth 470-9	7	6	86	750–2000	21	43
Total	63	48				
Average	10.5	8	77.83		29.83	

* % of different RAPD patterns in the group of 49 genotypes studied.

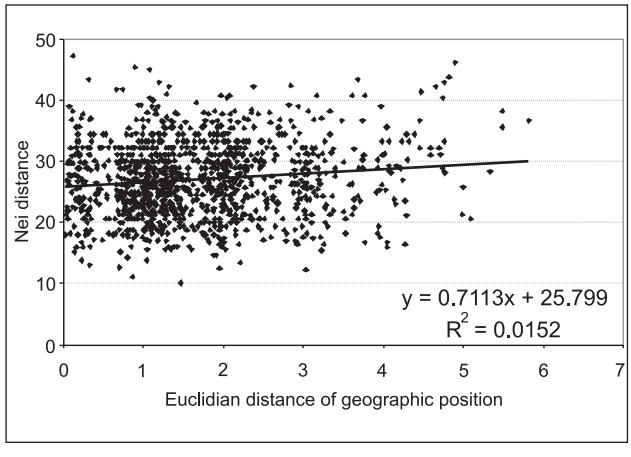

Fig. 3. Interdependence among genetic and geographical distances of *R. idaeus* accessions in pairwise comparison

Table 4. **Significant differences among loci revealed using chi-squared test and some morphological traits**

Locus (size of DNA fragment, bp)	Latitude, N.	Longitude, E.	Morphological trait# (5% significance level)
270-6 (1050)		*	–
270-6 (1100)	*	***	3
270-6 (1200)		*	2
380-3 (950)		*	3
470-8 (750)		**	1
470-8 (920)		*	4, 7, 8, 11, 12, 13
MP4 (820)		*	–
A3 (890)	**		5, 6, 9, 10
A3 (900)	*		1, 2

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

1 – leaf length; 2 – leaf width; 3 – length of terminal leaflet in compound leaf; 4 – width of terminal leaflet in compound leaf; 5 – length of petiole; 6 – length of rachis; 7 – number of drupelets in the fruit; 8 – fruit weight; 9 – fruit length; 10 – fruit diameter; 11 – floricane height; 12 – floricane diameter at the height of 30 cm; 13 – flower diameter.

A weak but significant correlation between matrices computed on the basis of molecular (RAPD) and overall morphological data was observed (r = 0.073; p = 0.012). The effect of presence or absence of the locus allele (RAPD band) was also assessed for geographic and morphologic traits (Table 4). The results presented in Table 4 show that genetic differences of *Rubus idaeus* genotypes have some longitudinal or west–east cline. Significant differences of loci were also established for some morphological traits in 7 of 9 cases.

The UPGMA dendrogram based on Nei and Li's genetic distance matrix is shown in Fig. 4. The dendrogram clearly demonstrates that all the wild raspberry samples studied are genetically different, which means that the used primers were suitable for the genotyping of the study material. All clusters of the dendrogram are rather heterogeneous and do not show any clear geographically dependent pattern. Based on these data, the most similar genotypes are JL06 and JL14 (90% of similarity), the most dissimilar being JL43 and JL40 (51% of similarity). The average similarity among all the genotypes studied was 71.9%. The highest potential in the genotyping procedure had the primers MP4 and Roth 470–8. The use of these primers allowed identifying 39 and 37 RAPD haplotypes respectively among the 49 accessions studied (Table 3), while the DNA fingerprints generated by these two primers identify all the individuals studied (data not shown).

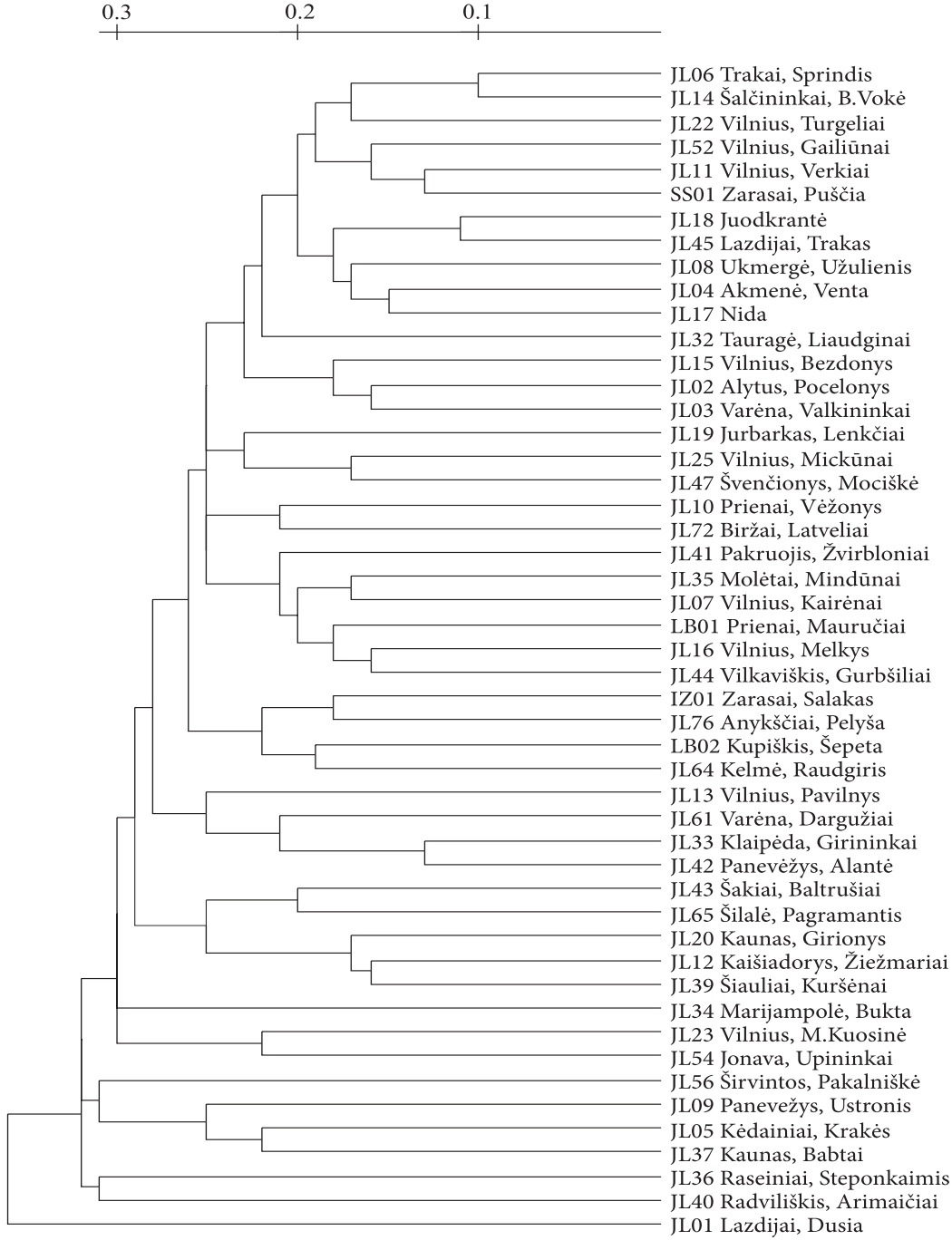

Fig. 4. Dendrogram of the relative genetic distance among *Rubus idaeus* accessions obtained using Nei and Li's genetic distance coefficient and the UPGMA clustering method. The scale indicates genetic distances among the individuals

DISCUSSION

The management of *ex-situ* plant germplasm collection includes the characterization of samples in order to eliminate cases of mislabeling and redundancies and development of a core collection representing the maximum of variability of certain species [27]. To achieve those goals, first of all the phenotypic characterization and molecular genotyping of accessions should be performed. In this study, we carried out a genotyping of wild raspberry samples from geographically different locations of Lithuania (Table 1). We used primers previously tested and selected from 44 informative primers [18] and assessed them on a higher number of genotypes. About 40% of the wild raspberry collection of Vilnius University were included in this study. Our results demonstrate that RAPD markers may be effectively used in genotyping and genetic diversity studies of this collection. Application of two primers (MP4 and Roth 470-08) was sufficient to genotype all 49 accessions. Such individual fingerprint as an absolute measure of the genetic makeup of an individual may be used to distinguish it from other accessions of a collection [28].

Our results demonstrate that there is some association between genetic and geographical parameters of the accessions studied ($r = 0.123$; $p = 0.001$). Table 4 also shows that the genetic differences of *Rubus idaeus* genotypes have some longitudinal or west–east cline (only once both geographic characters were significant for the same locus differences). We could suppose that some climate factors such as precipitation or snow cover influence the genetic adaptation of wild raspberry populations [29]. Genetic diversity as a relative measure of the genetic distances among the genotypes studied is represented by a dendrogram (Fig. 4) which can be used in creating the core collection and in the future breeding experiments. According to Graham et al. [11], the daughter seedlings of *R. idaeus* are about 90% similar to the mother plant. Thus, samples that show such similarity could be excluded from the collection. In our study, there are only two such genotypes – JL06 and JL14 (Fig. 4). The genetic distances among most of accessions are relatively large, demonstrating a high level of genetic variation. These results indicate that the field collection consists of genetically divergent material which is important in the conservation of genetic resources. In addition, RAPD markers are not genes, and most of them are located in the areas of non-coding DNA [30]. To save all types of genetic diversity and to have a more representative collection, various types of markers should be used [16]. In our study, we also assessed the phenotypic diversity of raspberry accessions according to 13 quantitative morphological traits. Different aspects of the phenotypic variation of *R. idaeus* were assessed in previous studies [1, 2, 4, 17, 31, 32]. In some of these works, the morphological diversity of wild raspberry was assessed *in situ*. In the others [2, 3, 32], the phenotypic and genotypic variations were studied in experimental plots and greenhouse. Marshall et al. [3] analysed the morphological variation of samples collected from seven sites of Tayside region, Scotland and grown over two years in two environments (greenhouse and under nylon mesh). The primary evaluation of the phenotypic diversity of the wild raspberry collection of the VU Botanical Garden was carried out by Balčiūnienė et al. [17]. The authors assessed a field performance (some fruiting peculiarities, the vegetative growth pattern and resistance to fungal diseases) of wild raspberry accessions. In our current work, we have studied the morphological and molecular variation in a wild raspberry *ex situ* collection and assessed a correlation between phenotypic and molecular (RAPD) data. A weak but significant correlation was detected between them ($r = 0.073$; $p = 0.012$). The independence between these two kinds of data was noted in some other studies [33–36]. On the other hand, a correlation between the phenotypic and molecular markers was also reported [37–39]. Differences between patterns of morphological and RAPD marker variation in our study may be explained by the quantitative nature of the used morphological data. These traits are more strongly affected by the environment than qualitative morphological (phenotypic) characters and molecular markers. One more explanation could be the nature of molecular markers which are usually considered phenotypically neutral and may not reflect variations in ecologically important traits or adaptation to local environmental conditions [40–43]. Although the data analysis showed an overall independence of phenotypical and other two kinds of data (genetic and geographical), the effect of the presence or absence of a certain RAPD band (allele) was established for geographic data or some morphological traits (Table 4). In seven of nine cases, the same differences of loci were also significant for one or another morphological trait. And it was in half of all cases when significant differences of loci in morphological traits were calculated. So, even if a comparison of the distance matrices for geographical characters and morphological traits did not reveal any significant correlation, we can see from Table 4 that there could be some relation.

The information obtained in this study can be useful in the future management of wild raspberry collection. It demonstrates that morphological and RAPD markers have to be analysed separately because of the lack of a pronounced congruence between them. The identity of raspberry accessions is difficult to establish using morphological traits. In this situation, RAPD fingerprints of 49 genotypes and selected optimal informative primer combinations may be used to recognize certain accessions and their clones in the *R. idaeus* collection of Vilnius University. Information about genetic distances among the genotypes could be used in the breeding process introducing genes from wild relatives into red raspberry cultivars.

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PAPRASTOSIOS AVIETĖS (*RUBUS IDAEUS* L.) KOLEKCIJOS GENETINĖS ĮVAIROVĖS TYRIMAS NAUDOJANT MORFOLOGINIUS POŽYMIUS IR RAPD ŽYMENIS

Natūraliai gamtoje paplitusios rūšys, giminiškos kultūriniams augalams, gali būti vertingų genų donorai kuriant naujas augalų veisles. Viena tokių rūšių – paprastoji avietė (*Rubus idaeus* L.). Kadangi šiuolaikinėse aviečių veislėse genetinė įvairovė kur kas mažesnė palyginus su laukinėmis rūšimis, tai paprastoji avietė gali būti potencialus vertingų genų šaltinis naujoms veislėms. Norint įvertinti ir išsaugoti šios rūšies genetinius išteklius, 2001 m. VU Botanikos sode pradėta auginti paprastosios avietės pavyzdžių, surinktų iš įvairių šalies vietų, kolekcija.

Darbo tikslas – įvertinti šios kolekcijos 49-ių pavyzdžių (apie 40% kolekcijos) morfologinę ir genetinę įvairovę. Morfologinė įvairovė įvertinta pagal 13 kiekybinių požymių, o molekulinė – pagal 48 polimorfinius RAPD (*angl.* atsitiktinai pagausintos polimorfinės DNR) lokusus. Remiantis šiais morfologiniais ir molekuliniais žymenimis, buvo sudarytos euklidinių ir genetinių atstumų tarp tirtų individų lentelės ir nubraižyti genotipų gimininguмo medžiai. Genetiniai atstumai buvo apskaičiuoti trimis būdais: 1) pagal Nei ir Li, 2) pagal Link, 3) paprastai suderinant (angl. *simple matching*). Nustatyta stipri koreliacija ($r \geq 0,95$) tarp visais trimis būdais apskaičiuotų genetinių atstumų. Palyginę morfologinius euklidinius atstumus su genetiniais, nustatėme patikimą, tačiau labai silpną jų koreliaciją ($r = 0,073$; $p = 0,012$). Taigi, norint įvertinti paprastosios avietės tinkamumą selekcijoje, reikia naudoti tiek morfologinius, tiek DNR žymenis, kurie papildo vieni kitus. Naudojant RAPD žymenis buvo genotipuoti visi 49 tirti paprastosios avietės kolekciniai pavyzdžiai. Šiame darbe nustatyti RAPD profiliai gali būti naudojami konkrečiam genotipui ar jo klonui identifikuoti, o ištirti paprastosios avietės morfologiniai požymiai yra mažiau informatyvūs. Nustatyta silpna koreliacija tarp genetinių ir geografinių atstumų ($r = 0,123$, $p = 0,001$).