VILNIUS UNIVERSITY

Jolanta Patamsytė

THE STUDY OF GENETIC DIVERSITY OF RED RASPBERRY (*Rubus idaeus* L.) IN LITHUANIA

Summary of doctoral dissertation
Biomedical science, biology (01 B)

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VILNIAUS UNIVERSITETAS

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PAPRASTOSIOS AVIETĖS (*Rubus idaeus* L.) GENETINĖS ĮVAIROVĖS TYRIMAI LIETUVOJE

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INTRODUCTION

Red raspberry (*Rubus idaeus* L.) is widespread in the northern hemisphere and exhibits a high level of morphological, phenological, genetic and habitat variation (Marshall et al., 2001; Ryabova, 2007). The genus *Rubus* belongs to the economically important Rosaceae family of perennial fruit-bearing crops (Alice, Campbell, 1999). Red raspberry is a wild relative to the multitude of commercial raspberry cultivars. The process of domestication is usually associated with the loss of genetic diversity because of predefined breeding targets and limited number of genotypes used in breeding programs (Singh, 2001). Modern cultivars of red raspberry are morphologically and genetically similar and have a narrow genetic base (Jennings, 1988; Graham, McNicol, 1995). Therefore, the maintenance and study of 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 (Jennings, 1988; Marshall et al., 2001; Graham et al., 2003; Ryabova, 2007).

Red raspberry is widely spread in Lithuania and plays measurable roles from both economic and ecological points of view. In Lithuania, red raspberry populations are located in rather varying habitats which differ with regard to ecological factors such as soil properties, light, water and others. The habitats are usually fragmented. Wild raspberry is tolerant of acid, infertile and poorly drained soils.

The collecting of wild raspberry germplasm (clonal samples) was commenced in Lithuania in 2001. The collection of the Botanical Garden of Vilnius University (VU) includes 116 accessions of wild red raspberry from different regions of Lithuania and a few accessions from abroad. The primary evaluation of this collection was carried out by Balčiūnienė et al. The authors assessed the field performance of wild raspberry accessions (Balčiūnienė et al., 2005). Knowledge of the level of genetic variation in plant germplasm collections is important for the effective utilisation and conservation of species. The effective management of this continuously increasing collection requires the use of modern molecular marker methods. Different techniques of molecular markers can assist in the evaluation of germplasm collections and gene banks as potential gene pools for plant breeding (Chebotar et al., 2002; Virk et al., 2000). The technique of RAPD (*Random Amplified Polymorphic DNA*) has a number of advantages that make it highly applicable for an efficient detection of DNA polymorphisms (Williams et al., 1990). RAPD markers have been demonstrated to be useful for the studies of parentage identification, genetic mapping, taxonomic identities, systematic relationships, species hybridization and genetic structure of population.

An understanding how genetic variation is partitioned among populations, the knowledge of relationships between germplasm genetic diversity and the level of genetic variation in natural populations localized at different habitats are important for the conservation of species genetic resources. According to previous studies, discrete spatially separated red raspberry populations are adapted to local conditions, which may result in an effective reproductive isolation in the absence of geographic barriers (Marshal et al., 2001; Graham et al., 2003). It is believed that such local adaptation of raspberry has resulted in its genetic diversity. On the other hand, all ecological characteristics of organisms are the result of the interaction between the genomes and the external factors in their environments. The RAPD method has been successfully used

to analyze population genetic structure and differentiation among plant populations from ecologically different habitats (Fahima et al., 1999; Nevo 2001; Graham et al., 2003).

THE MAIN OBJECTIVE

To study genetic diversity in the germplasm collection of the Vilnius University Botanical Garden and of wild red raspberry populations in Lithuania.

THE OBJECTIVES OF THE STUDY WERE:

1. Phenotypic (morphological) and molecular (RAPD) evaluation of wild raspberry germplasm collection of the Botanical Garden of Vilnius University.
2. To study the influence of edaphic characteristics of original habitats on the RAPD-based diversity of wild raspberry accessions.
3. To analyze the influence of geographic factors on the morphological variation in the wild raspberry germplasm collection.
4. To assess the level of DNA polymorphism among and within Lithuanian populations of red raspberry.
5. To evaluate the influence of some ecological, climatic and geographic variables on the genetic diversity of red raspberry populations.

THE NOVELTY OF THE RESEARCH

Previous studies of red raspberry in Lithuania were carried out on the commercial raspberry cultivars. The morphological diversity, biological and commercial properties of cultivars were studied. Wild red raspberry is widely spread in Lithuania, but it is poorly studied genetically. The main objective of this study was to fill this gap and assess the level of genetic diversity in the germplasm collection of Vilnius University and nineteen natural wild red raspberry populations in Lithuania.

The influence of some environmental (soil acidity) and geographical (altitude and latitude) factors on the morphological variation and pattern of RAPD diversity in red raspberry has been shown.

This study has shown that most of the genetic variation was found within populations. A rather high level (26%) of genetic differentiation among red raspberry populations was also established. A low but significant level of differentiation was observed among populations from different agroclimatic regions. RAPD analysis suggests that local site differences can promote the genetic differentiation of populations.

Our results show that the level of genetic diversity in the germplasm collection represents rather well the level of genetic variation in natural red raspberry populations.

PRACTICAL RELEVANCE

Wild raspberry has a potential to be used as a source of genetic variation for useful traits in the breeding program. The molecular characterization of the level of genetic diversity in the germplasm collection, genotyping of wild raspberry accessions, development of an optimal combination of primers for studying the polymorphic RAPD loci, evaluation of the representativeness of the collection can be valuable for the conservation and utilization of wild raspberry genetic resources. Our study of genetic structure of red raspberry populations provides new information about the biological and genetic properties of this species.

THE APPROVAL OF THE RESEARCH

The results of the work presented in this summary have been published in eight scientific papers, three of them in a peer-reviewed Lithuanian journal, and one paper was published in the international "Journal of Fruit and Ornamental Plant Research": all are included into the Master Journal List of Institute of Scientific Information (ISI). Four papers were published in other international journals.

The study results have been presented and discussed at three international conferences.

VOLUME AND STRUCTURE OF THE THESIS

The dissertation is written in Lithuanian. The work consists of Introduction, Literature Review, Materials and Methods, Results, Discussion, Conclusions, References, List of Publications, Acknowledgements and Appendices.

MATERIALS AND METHODS

The object of investigation – red raspberry. Red raspberry (*Rubus idaeus* L.) (Figure 1) is a deciduous, erect or arching, thicket-forming shrub, which has two types of stems – primocanes (first-year stems) and floricanes (second-year stems, which bear fruits). A typical raspberry rootstock system contains at least one floricane and several primocanes. Raspberry grows 1.5 to 2.5 m in height. Woody stems are light green to brownish, bristly or prickly. Leaves are alternate and pinnately compound in leaflets of tree (in floricanes) or five (primocanes). Leaves are green and glabrous to hairy above. Small white flowers are borne in clusters of one to four in a compound cyme. A red raspberry fruit is made up of many to several, red or pinkish-purple drupelets (Tirmenstein, 1990).

A B

Figure 1. Red raspberry (*Rubus idaeus* L.). A – a shrub; B – a branch with fruits (caliban.mpiz–koeln.mpg.de/~stueber/koehler/)

Red raspberry blooms in May and June, and bears fruit in July–August. It is pollinated by bees and flies. Raspberry seeds are relatively large, with a hard, thick and impermeable coat. Seeds are dispersed by birds and small animals and may become

dormant for a long time (Murkaitė, 1971). Red raspberry is capable of vigorous sprouting, thus forming clumps of genetically identical plants.

Distribution. Red raspberry is widespread in temperate regions of the northern hemisphere (Marshall et al., 2001). In Lithuania, wild raspberry is found in forests, outskirts, clearings, parks, along roadsides, etc.

Practical use. Red raspberry is well known as a popular small fruit plant (especially in northern countries), and as a medicinal and melliferous plant. Raspberry is also very important as food for small mammals and birds.

Plant material for analysis of red raspberry collection. Clonal samples of red raspberry along with soil samples were collected in the wild during the growth season in 2001 for the collection analysis (Balčiūnienė et al., 2005). Each clonal sample contained three separated 1- or 2- cane plants. The samples were planted into the field collection of the Botanical Garden of Vilnius University and managed according to common horticultural practices.

Table 1. Red raspberry accessions studied

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

* Accessions, which included into initial RAPD analysis and SOD polymorphism estimation.

Plant material for analysis of red raspberry wild populations. 315 plants of 19 populations of red raspberry were sampled for genetic diversity investigation (Table 2). Plants were sampled during 2003–2005.

Morphological characterization. The morphological characterization of raspberry accessions employing 13 quantitative morphological characters (leaf length and width, length and width of terminal leaflet in compound leaf, length of petiole and rachis, number of drupelets in aggregate fruit, fruit weight, length and diameter, height and diameter of floricane and flower diameter) was carried out within 2–5 years during the period 2003–2007.

Table 2. Red raspberry populations studied

Population	Number of plants studied	Coordinates		Population	Number of plants studied	Coordinates	
		Longitude, E	Latitude, N			Longitude, E	Latitude, N
Juodkrantė	20	21º06‘42“	55º33‘04“	Baltoji Vokė	19	25º11‘13“	54º31‘31“
Šėta	20	24º15‘10“	55º15‘21“	Melekonys	20	25º11‘19“	54º31‘43“
Vilkiautinis	20	24º01‘40“	54º06‘13“	Švarcgiris	17	22º44‘40“	55º03‘07“
Vilnius	19	25º14‘47“	54º40‘48“	Zokniai	22	23º23‘43“	55º53‘57“
Ilgiai	10	26º06‘48“	55º48‘12“	Margiai	10	25º43‘46“	54º37‘36“
Salos	10	26º00‘14“	55º21‘29“	Šiulgos	10	25º43‘45“	54º37‘45“
Prienai	19	23º55‘40“	54º36‘41“	Nemirseta	9	21º03‘40“	55º52‘06“
Linkuva	18	23º56‘01“	56º05‘50“	Anaičiai	10	21º04‘35“	55º51‘47“
Dieveniškės	20	25º33‘43“	54º11‘45“	Rokai	23	24º33‘01“	55º13‘13“
Kurtuvėnai	19	22º59‘06“	55º47‘30“				

DNA extraction and PCR amplification. DNA was purified from fresh young leaves (100 mg) using a genomic DNA purification kit #K0512 (Fermentas). Each amplification was performed in 25 µl of a reaction mixture containing 1×PCR buffer (Fermentas), 3.0 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of the primer, 1.0 u of Taq DNA polymerase (Fermentas) and 50 ng of raspberry DNA. RAPD reactions were carried out in a thermo cycler (Biometra) under conditions described earlier (Žvingila et al., 2002). The amplification products were analysed by electrophoresis in 1.6% TBE agarose gel. All reactions were repeated at least twice. Only clear and reproducible DNA bands were scored.

Superoxide dismutase (SOD, EC 1.15.1.1.1) extraction and assay. Leaf discs of plants were homogenized in a pre-colded extraction buffer consisting of 50 mM potassium buffer, pH 7.8 (1 g plant fresh weight: 1 ml buffer). The homogenate was centrifuged at 12 500 rpm for 15 min at 4ºC. The supernatant was used on the same day for electrophoretic analysis. The native polyacrylamide gel electrophoresis was performed at 4ºC using a 4% stacking gel and 9% separating gel (200 V, 40 mA) using Tris-glycine buffer (pH 8.3) (Davis, 1964). 20 µl of crude extract from leaves was loaded in each line. SOD isozymes were localized on the gels by the method of NBT reduction. After electrophoresis, the gels were incubated with a staining solution for an hour at 37ºC in the dark (Fridovich, 1978).

Data analysis. RAPD data were scored as the presence (1) or absence (0) of a given amplification product in each genotype. A resulting binary matrix was used to calculate the genetic distance coefficients: Nei and Li’s – GD_{NL} (Nei, Li, 1979); Link’s –

GD_L (Link et al., 1995) simple matching – GD_{SM} (Sokal, Michener, 1958) to estimate genetic diversity among the genotypes. The genetic distance matrices were computed from polymorphic RAPD loci (bands). The correlation between the morphological Euclidean and molecular GD_{NL} matrices, morphological Euclidean and geographical Euclidean matrices, between the GD_{NL} matrix and the geographical Euclidean distance matrix was estimated using STATISTICA 7. 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 was used for estimating the effect of presence or absence of a locus band on the geographic or morphological traits (SAS Institute Inc., 1999). The UPGMA (unweighted pair-group method using an arithmetic average) dendrogram based on morphological data was generated using STATISTICA 7 (StatSoft Inc., 2004). The UPGMA dendrogram based on molecular data (GD_{NL}) was constructed using the TREECON computer program (Van de Peer, De Wachter, 1994). The method of principal components in factor analysis was applied to analyze the data from RAPD fingerprints by the FACTOR procedure. The STEPDISC SAS procedure was applied to select RAPDs contributing most to the differentiation of individuals grouped by soil acidity properties.

Intrapopulational genetic diversity was evaluated using POPGENE version 1.31 (Yeh et al., 1999). We calculated Shannon's information index (I), Nei's (1973) gene diversity (h), the number of alleles (n_a), the effective number of alleles (n_e) and the genetic distance among populations. To evaluate the degree of genetic subdivision among populations, we calculated Nei's coefficient of gene differentiation, G_{ST}. The principal coordinate analysis and AMOVA (analysis of molecular variance) were performed using GenAlEx v.6 software (Peakall et al., 2006).

The 19 populations and the habitats of collected samples were described by their ecogeographic variables: longitude, latitude, altitude, average mean temperature, average mean precipitation. The relationships of the ecogeographic variables of populations with the genetic diversity values were tested using the Spearmen r correlation coefficient.

RESULTS

Study of genetic diversity in red raspberry collection using RAPD markers. We initially tested 44 primers (30 purchased from Carl Roth GmbH (Random Primer kits 270, 380, 470) and 14 primers from Fermentas) on 20 red raspberry accessions; 36 primers yielded informative, polymorphic products (Table 3).

A total of 284 reproducible bands were obtained; 80.64% of the identified loci were polymorphic. The RAPD primers amplified 5 to 13 scorable DNA bands per genotype. The size of amplification products ranged from 190 bp to 3275 bp. The values of genetic distance GD_{xy} were calculated for pairwise comparisons of these 20 genotypes. The genetic distance for all 190 pairs ranged from 0.28 to 0.51.

Table 3. Number of RAPD bands and RAPD patterns determined per primer in twenty red raspberry accessions with 36 oligonucleotide primers

Primer	Analyzed bands	Polymorphic bands	Polymor-phism, %	Size of analyzed bands (bp)	Genotype-specific bands, size (bp)	Number of patterns	
						Total	%
A3	11	7	64	190–1065	0	14	70
A4	11	9	91	355–1035	1 (530_0)	18	90
A5	9	7	78	270–1100	1 (795_1)	9	45
A6	7	4	57	520–1080	0	13	65
A7	7	7	100	250–730	0	16	80
B6	6	5	83	300–1100	0	13	65
B7	8	6	75	480–1570	0	14	70
MP2	8	8	100	605–2700	0	16	80
MP3	7	6	86	380–1370	0	12	60
MP4	7	6	86	545–1125	0	13	65
MP5	5	3	60	610–1540	2 (610_1, 1090_0)	4	20
MP7	6	5	83	700–2060	1 (910_1)	11	55
270-1	7	7	100	430–1155	0	11	55
270-3	8	7	88	410–3275	3 (600_0, 770_0, 790_1)	9	45
270-5	9	6	67	540–2200	0	12	60
270-6	9	5	56	515–2450	0	10	50
270-7	6	5	83	850–1800	0	6	30
270-8	8	6	75	500–1330	1 (805_1)	10	50
270-9	7	7	100	420–2500	2 (420_1, 580_1)	13	65
270-10	7	7	100	780–2980	1 (2980_0)	13	65
380-1	6	3	50	520–2370	0	6	30
380-2	11	10	91	830–2530	0	20	100
380-3	13	10	77	400–2010	2 (700_0, 820_0)	20	100
380-4	7	5	71	220–1720	1 (450_0)	10	50
380-6	9	6	67	685–1100	2 (685_1, 850_0)	6	30
380-7	7	6	86	470–1140	0	11	55
380-8	6	6	100	500–1440	1 (1000_1)	9	45
380-9	6	2	33	530–1300	0	4	20
470-1	6	6	100	560–1180	1 (650_0)	13	65
470-3	11	10	91	450–2930	0	16	80
470-4	7	6	86	630–1450	0	11	55
470-6	9	6	67	630–2370	0	10	50
470-7	6	6	100	610–2530	3 (610_0, 630_1, 800_0)	8	40
470-8	11	9	82	380–2550	1 (590_1)	19	95
470-9	10	7	70	670–2710	1 (670_1)	14	70
470-10	6	6	100	745–1860	0	11	55
Average	**7.89±1.95**	**6.33±1.83**	**80.64±16.74**			**11.81**	**59.03**
Total	**284**	**228**					

* % of different RAPD patterns in the group of 20 genotypes studied.

$_1$ genotype-specific DNA band, $_0$ genotype-specific null allele of RAPD locus.

For genotyping and a more representative analysis of the collection another 29 samples (49 raspberry accessions in total) were analyzed. The results obtained using six primers are summarized in Table 4. These primers generated 63 scorable RAPD bands in the range of 390–2900 bp; 77.83% of amplification products (48 bands) were polymorphic.

On the basis of these 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}). The 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; $p \leq 0.001$ for all cases).

Table 4. Number of RAPD bands and RAPD patterns determined per primer on 49 red raspberry accessions

Primer	Analyzed bands	Polymorphic bands	Polymorphism, %	Size of analyzed DNA bands (bp)	Number of patterns	
					Total	%∗
A3	10	8	80	450–1800	22	45
MP4	9	8	89	550–2500	39	80
270-6	14	9	64	500–2900	30	61
380-3	12	9	75	390–2100	30	61
470-8	11	8	73	440–3100	37	76
470-9	7	6	86	750–2000	21	43
Average	**10.5 ± 2.43**	**8.00 ± 1.10**	**77.83 ± 9.15**		**29.83**	
Total	**63**	**48**				

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

The UPGMA dendrogram based on Nei and Li's genetic distance matrix is shown in Figure 2. The dendrogram clearly demonstrates that all the wild raspberry samples were genetically different, which means that the primers used were suitable for genotyping 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, 470-8, 270-6 and 470-8. The use of these primers in pairs allowed to genotype all the individuals studied.

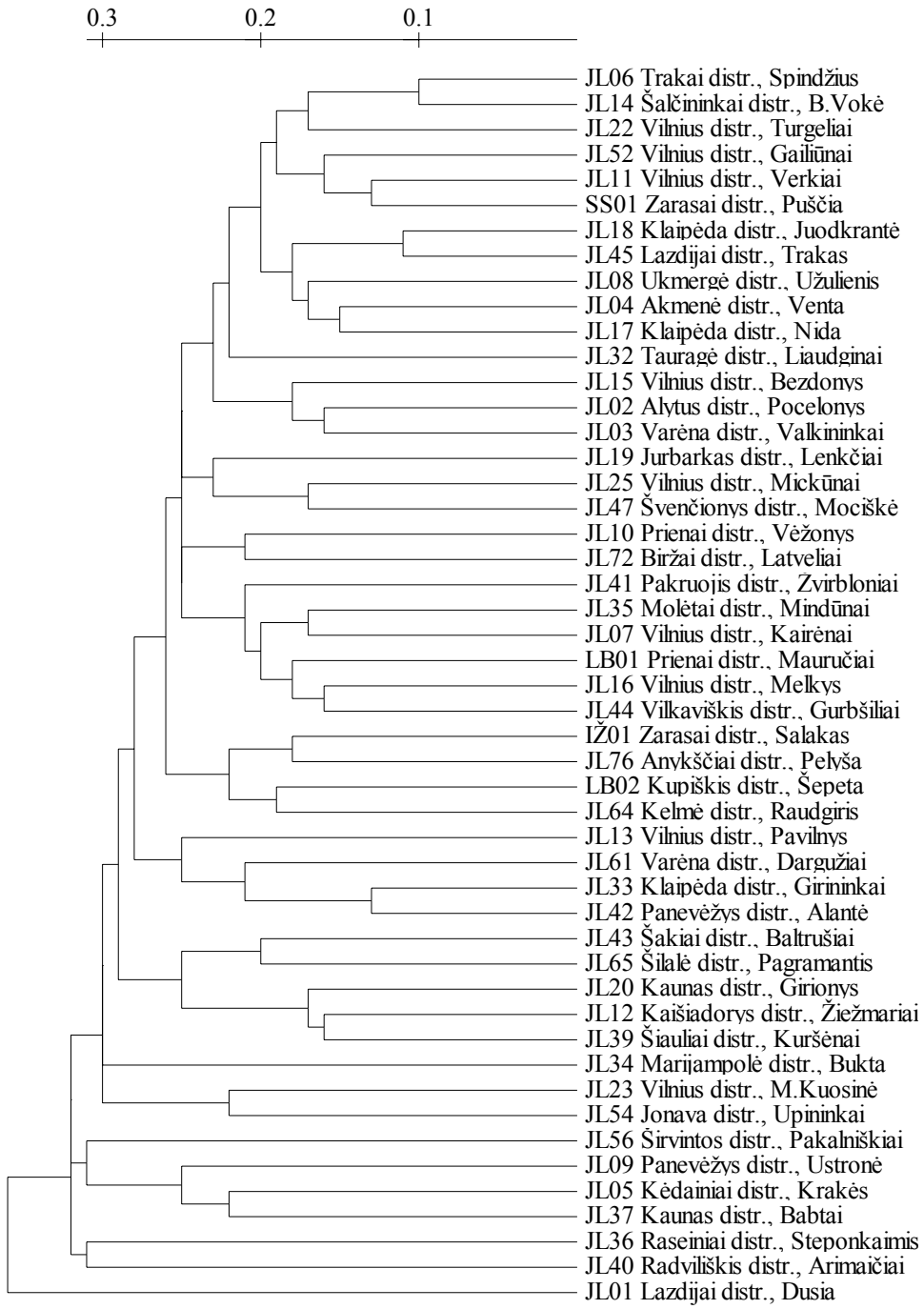

Figure 2. Dendrogram of the relative distance among red raspberry accessions obtained using the Nei and Li genetic distances and the UPGMA clustering method. The scale indicates genetic distances among the individuals

The study of influence of edaphic characteristics of original habitats on the RAPD-based diversity of wild red raspberry accessions. The fact that all genotypes – both in groups of 20 and in 49 accessions of the red raspberry collection – were genetically distinct and did not cluster by geographic distances suggests that geographic distance alone is not enough to explain the genetic divergence. The clustering can be influenced also by the peculiarities of ecological conditions of the original habitats of the plants. Thus, the next step in our work was to test this hypothesis by assessing a possible correlation between the RAPD patterns and the variation of soil properties at the sites of origin of the genotypes studied. The principal component method in factor analysis was

applied to analyze data from RAPD fingerprints to detect a possible correlation between RAPD markers and soil characteristics. The distribution of 230 polymorphic RAPD loci among 20 accessions was compared with the following soil variables: nitrogen, phosphorus, potassium and humus content, and soil acidity. A significant correlation was found only with soil acidity (-0.65). The curvilinearity test revealed the significance of the linear model (Figure 3, Table 5).

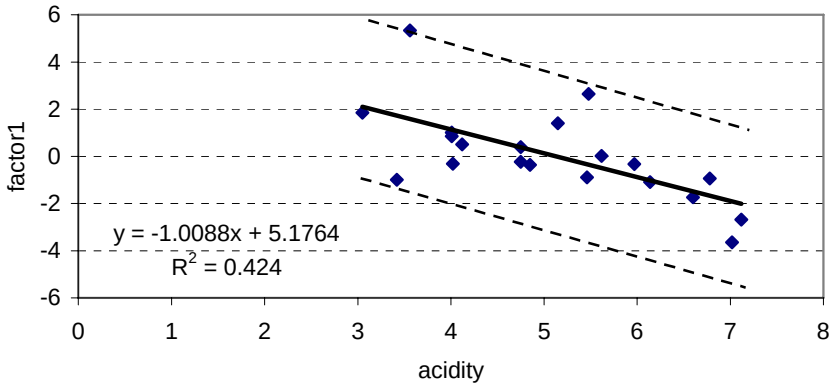

Figure 3. Scatter plot of *Rubus idaeus* genotypes based on values from factor analysis (factor1) and soil acidity of origin site. Dash lines show the upper and lower limits 95% confidence

Table 5. Correlation of factor analysis estimates with soil characteristics

Variable	Acidity	Nitrogen	Phosphorus	Potassium	Humus
Factor1	-0.65**	-0.01	-0.35	-0.18	-0.03
Factor2	-0.26	-0.03	0.22	-0.07	-0.03
Factor3	-0.30	-0.01	-0.08	-0.05	-0.05

** Significance at 1% level.

About 22 RAPD markers scored from 230 RAPD bands were mainly responsible for the correlation between RAPD diversity pattern and soil acidity. After applying STEPDISC procedure, a stepwise selection revealed 9 RAPD markers contributing most to the separation of individuals by soil acidity properties: $A4_{470}$, $A4_{530}$, $A7_{730}$, $B6_{1000}$, $MP2_{605}$, MP41015, $380\text{-}3_{1390}$, $380\text{-}3_{1550}$, $470\text{-}7_{800}$. These results show that the study plants could be adapted to soil acidity at the original growing site.

Morphological characterization of red raspberry collection. The mean values and standard deviations of the morphological characters studied show a rather high morphological variation among the accessions. The UPGMA dendrogram based on the average Euclidean distances estimated from morphological data is shown in Figure 4.

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 the characters that are strongly expressed. Among them, six accessions (JL32, SS01, JL16, JL45, JL35, JL33) have larger and heavier fruits and four genotypes have larger leaves (JL72, JL10, 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. No correlation between matrices computed on the basis of molecular (RAPD) and overall morphological data was observed (r = 0.073; p = 0.01).

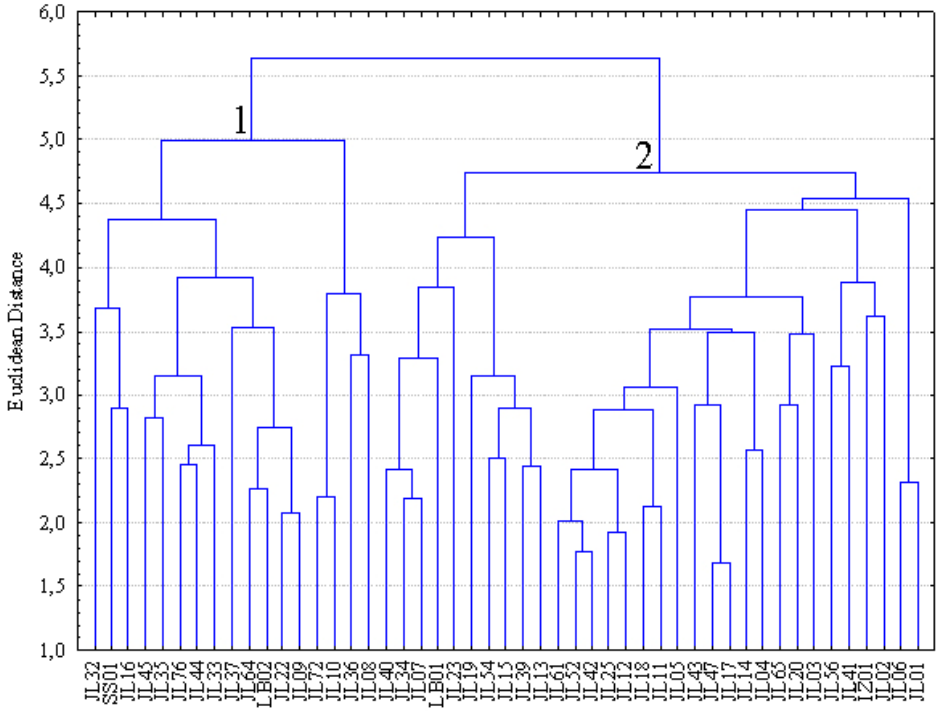

Figure 4. UPGMA dendrogram showing relationships among 49 accessions of *Rubus idaeus*, based on morphological Euclidean distance matrix

Study of the influence of geographic factors on the morphological and genetical variations in wild raspberry. The effect of presence or absence of a RAPD locus allele (band) was also assessed for geographic or morphological traits. The results presented in Table 6 show that the genetic differences of red raspberry genotypes have some longitudinal or west–east cline.

Table 6. Significant differences in RAPD loci revealed using chi-squared test and some morphological and ecological 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
470-8750		**	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.

Significant differences in loci were also established for some morphological traits in 7 cases. The correlation coefficient between the morphological Euclidean distance matrix and the Euclidean geographical distance matrix was very low, r=0.100 (p=0.01). According to this result, the hypothesis of a null correlation between the matrices can not be rejected, i.e. there was no correlation between Euclidean morphological and geographical distance. However a significant negative correlation was determined between the two geographical parameters (altitude and latitude) and the floricane height of red raspberry accessions.

The red raspberry collection consists of individuals collected from geographically different locations. The geographic distance was smallest between accessions JL25 (Vilnius distr., Mickūnai) and JL52 (Vilnius distr., Gailiūnai) – 2.14 km, the largest being between accessions JL17 (Klaipėda distr., Nida) and JL47 (Švenčionių distr., Mociškė) – 361.77 km and accessions JL18 (Klaipėda distr., Juodkrantė) and JL47 – 353.39 km. We did not reveal a clear clustering of red raspberry accessions by geographical regions (Figure 2). No correlation between matrices computed on the basis of molecular (RAPD) and Euclidean geographical data was observed (r = 0.123; p = 0.001).

Study of superoxide dismutase polymorphism in 20 accessions. To reveal a possible genetic adaptation to soil acidity in red raspberry, we studied the genetic variability in SOD isozyme profiles in various accessions of this plant species. The same plant genotypes as in RAPD study were analyzed (Table 1, 20 accessions marked by *). Significant differences were detected in isozyme patterns of different accessions. Eight SOD activity bands were detected in red raspberry leaf extracts. According to the number and mobility of these bands, six main SOD enzyme profiles were distinguished (Figure 5).

The main differences among these six SOD profiles were found in the region of gel which includes the fastest zones of SOD activity, and in the second (II) SOD enzyme profile. The disappearance of isoform 1 was detected in leaves of most plants originally grown in acidic soils (pH 3.05–4.85) (Figure 5). However, two plants (JL14 and JL10), former habitants of acidic soil, showed isoform 1 activity. A plant (JL05) that in natural habitat had grown in soil with neutral pH (pH 7.02) had the most polymorphic pattern (II) of SOD isozymes.

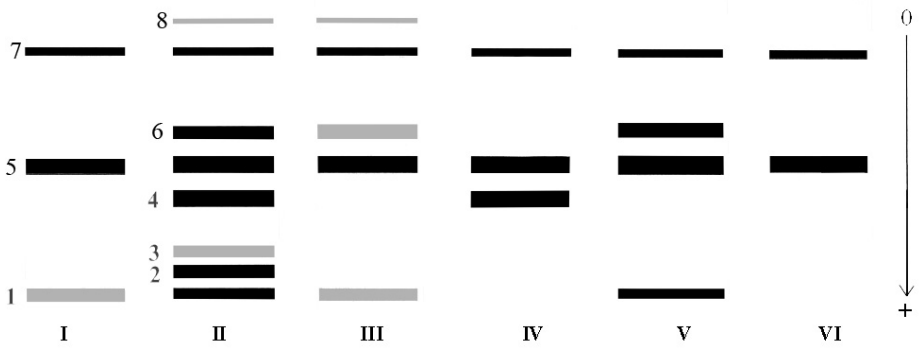

Figure 5. Superoxide dismutase (SOD) isozyme profiles (I–VI) in red raspberry leaves: I – isozyme profile typical of accessions JL01, JL04, SS01, JL06; II – JL05; III – JL09; IV – JL03, JL08, JL11, JL12; V – JL14, JL10, VI – JL02, JL15, JL16, JL18, JL22, JL36, JL45, JL52

Analysis of RAPD patterns among individual plants of red raspberry populations. Nineteen populations of red raspberry were sampled in Lithuania (Table 2). A total of 315 plants were analyzed (the sample size for a population varied from 9 (Nemirseta population) to 23 plants (Rokai population). The fragmentation and size of a population were not considered in this study. In the RAPD analysis of red raspberry populations with 8 decamer oligonucleotide primers, 113 reproducible fragments were amplified with a varying number per primer (Table 7). Ninety-six (84.31%) of RAPD bands were polymorphic. The size of the fragments ranged from 390 bp to 3100 bp. All plants showed specific RAPD phenotypes.

Table 7. Primers used in RAPD analysis of red raspberry populations and the number of generated bands

Primer	Analyzed bands	Polymorphic bands	Size of analyzed bands, bp	Polymorphism, %
P1	19	19	420–2800	100
MP4	13	12	520–2500	92,31
270-1	12	12	490–1100	100
270-6	16	13	500–2900	81,25
380-3	15	11	390–2100	73,33
380-6	13	10	600–1800	76,92
380-9	12	7	520–2230	58,33
470-8	13	12	390–3100	92,31
Average	**14,13±2,42**	**12,00±3,38**		**84,31±14,54**
Total	**113**	**96**		

On the basis of 96 polymorphic RAPD bands, Nei and Li's (1979) genetic distances were calculated among all the 315 raspberry genotypes, and an UPGMA dendrogram was generated (Figure 6). The dendrogram showed that all the red raspberry individuals were genetically different. The average Nei and Li genetic distance among individuals of the populations was 0.267 and ranged from 0.210 in the Dieveniškės to 0.329 in the Salos populations.

Intra- and interpopulation variability. Lithuanian populations of red raspberry included in this study showed different levels of intrapopulational diversity (Table 8). For example, 87.50% polymorphic DNA bands were found in the Švarcgiris population, versus only 66.67% in the Šiulgos and Margiai populations. Nei's gene diversity across all loci ranged from 0.226 (Margiai) to 0.306 (Švarcgiris). The mean values for Shannon's information index ranged from 0.341 (Margiai) to 0.455 (Švarcgiris) (Table 8). A pair-wise comparison revealed the average distance among populations to be 0.177 ± 0.04. Interpopulational genetic distances varied from 0.045 (between Salos and Ilgiai) to 0.285 (between Margiai and Dieveniškės).

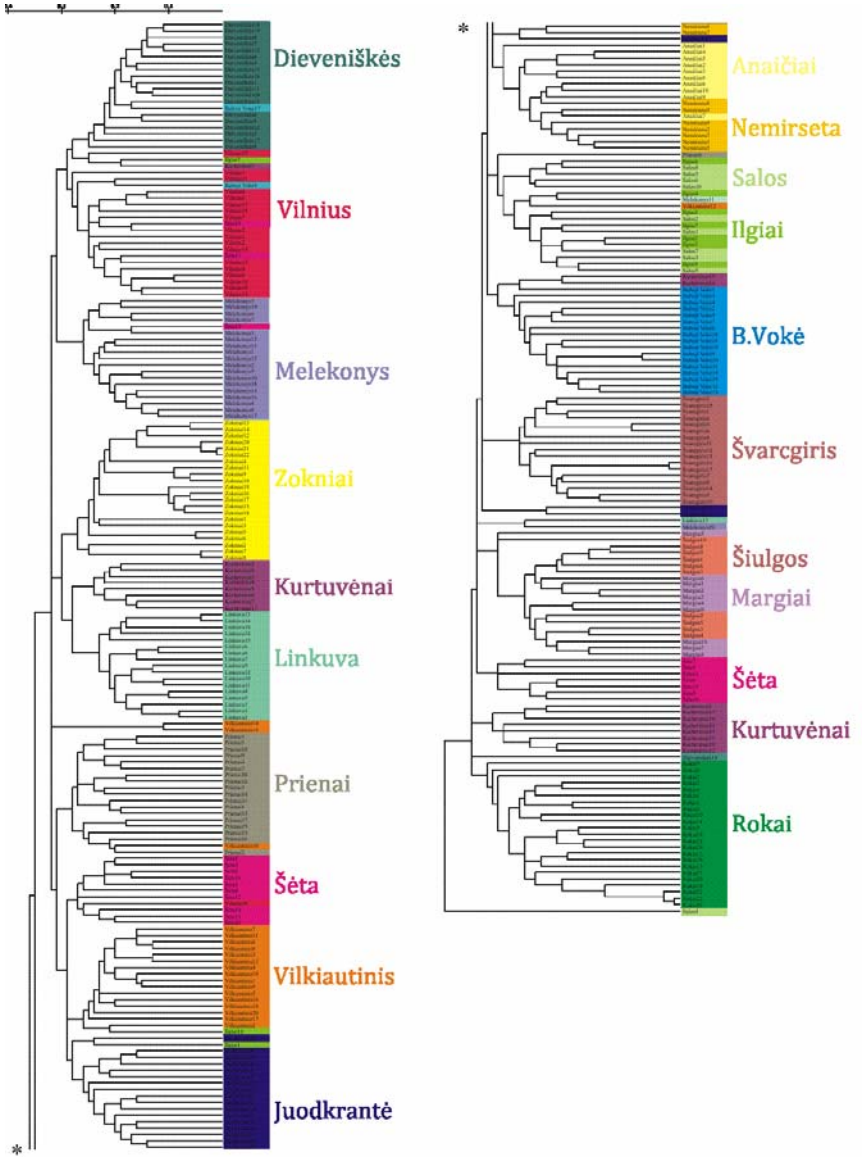

Figure 6. UPGMA dendrogram of individuals of nineteen red raspberry populations, obtained using Nei and Li's genetic distance. The scale indicates genetic distances among the individuals. * – cut place of the dendrogram

Table 8. Parameters of genetic diversity among red raspberry populations studied

Population	Polymorphism, %	n_a	n_e	h	I
Juodkrantė	79.17	1.792 ± 0.408	1.508 ± 0.369	0.292 ± 0.188	0.433 ± 0.260
Šėta	81.25	1.813 ± 0.392	1.546 ± 0.385	0.306 ± 0.193	0.450 ± 0.263
Vilkiautinis	77.08	1.771 ± 0.423	1.464 ± 0.368	0.271 ± 0.190	0.404 ± 0.264
Vilnius	79.17	1.792 ± 0.408	1.507 ± 0.370	0.291 ± 0.189	0.431 ± 0.261
Ilgiai	72.92	1.729 ± 0.447	1.469 ± 0.391	0.267 ± 0.201	0.395 ± 0.279
Salos	76.04	1.760 ± 0.429	1.437 ± 0.358	0.259 ± 0.186	0.390 ± 0.260
Prienai	77.08	1.771 ± 0.423	1.486 ± 0.376	0.279 ± 0.193	0.414 ± 0.269
Linkuva	76.04	1.760 ± 0.429	1.466 ± 0.390	0.266 ± 0.199	0.396 ± 0.275
Dieveniškės	72.92	1.729 ± 0.447	1.425 ± 0.390	0.245 ± 0.200	0.367 ± 0.277
Kurtuvėnai	79.17	1.792 ± 0.408	1.466 ± 0.345	0.276 ± 0.182	0.414 ± 0.254
Baltoji Vokė	79.17	1.792 ± 0.408	1.439 ± 0.352	0.261 ± 0.186	0.393 ± 0.259
Melekonys	79.17	1.792 ± 0.408	1.478 ± 0.375	0.276 ± 0.191	0.412 ± 0.264
Švarcgiris	87.50	1.875 ± 0.333	1.531 ± 0.361	0.305 ± 0.180	0.455 ± 0.242

Table 8 continued

Zokniai	70.83	1.708 ± 0.457	1.415 ± 0.367	0.244 ± 0.194	0.366 ± 0.274
Margiai	66.67	1.667 ± 0.474	1.385 ± 0.375	0.226 ± 0.196	0.341 ± 0.277
Šiulgos	66.67	1.667 ± 0.474	1.395 ± 0.384	0.229 ± 0.202	0.343 ± 0.283
Nemirseta	69.79	1.698 ± 0.462	1.405 ± 0.361	0.240 ± 0.191	0.362 ± 0.270
Anaičiai	73.96	1.740 ± 0.441	1.444 ± 0.368	0.260 ± 0.191	0.390 ± 0.268
Rokai	84.38	1.844 ± 0.365	1.483 ± 0.354	0.267 ± 0.181	0.405 ± 0.246
Average	**76.26 ± 5.50**	**1.763 ± 0.060**	**1.461 ± 0.045**	**0.2664 ± 0.020**	**0.398 ± 0.030**
	315 individuals	**2.0 ± 0.001**	**1.660 ± 0.270**	**0.380 ± 0.110**	**0.561 ± 0.130**

n_a – number of observed aleles; n_e – number of effective aleles; h – Nei‘s genetic diversity; I – Shannon‘s information index.

The principal coordinates analysis (PCoA) also revealed relationships among individuals and populations. Of the total variation, 43.59% was described by the first two axes. The geographically close populations are grouped nearby. Our results showed a rather high level of genetic differentiation among the red raspberry populations studied. An AMOVA revealed that 26% of the total genetic diversity occurred among populations (Φ_{PT}= 0.258) and 74% among individuals within populations. A similar level of interpopulation diversity was shown by the gene differentiation coefficient G_{ST}=0.297.

The influence of geographical and climatic factors on the genetic diversity of red raspberry populations. The following environmental variables were included in the analysis: geographical – longitude, latitude, altitude and climate means – perennial temperature and precipitation.

Geographical distances among the populations ranged from several hundred meters (Baltoji Vokė and Melekonys, Šiulgos and Margiai) to 343 km (among Dieveniškės and Nemirseta). Genetic distances among pairs of population correlated with geographical distances (r = 0.232, p = 0.002).

The number of effective alleles established in the populations showed a correlation with summer temperature (r = 0.483, p = 0.04), and the fraction of different RAPD phenotypes in populations correlated with autumn (r = 0.480, p = 0.04), winter (r = 0.631, p=0.02) and annual (r = 0.582, p = 0.01) precipitation.

We also assessed the level of genetic differentiation of populations from different agroclimatic regions of Lithuania. AMOVA showed a low but significant level of differentiation among populations from different regions (3%).

Some populations included in our study are located in ecologically different habitats. Plants of these populations colonize cuttings, peat bogs, meadows, dunes, etc. They grow in polluted military areas and in the shade of forest trees. We studied the genetic diversity pattern of populations situated in the neighborhood, but in ecologically different habitats.

The highest level of genetic divergence was shown by populations from the Melekonys forest and a population from Baltoji Vokė, colonizing an unexploited part of a peat bog. The habitats of their plants were separated by a narrow (~100 m) cutting and a road. The B. Vokė population habitat could be characterized by good lighting, soil and moisture conditions. Plants of the Melekonys population grew in the shade of dense forest trees (mainly Scots pine and juniper) in a poor soil. This population is situated about 20 m higher than the first one. Because of different microclimatic conditions, plants of the B. Vokė population usually burst into blossom earlier. Our results demonstrate a strong genetic divergence between these populations. The estimates of

Φ_{PT} from AMOVA (Φ_{PT} = 0.197) and G_{ST} (G_{ST} = 0.141) indicated a rather high genetic differentiation among these populations. Some population-specific alleles were detected in this study. PCoA shows also the divergence of these populations (Figure 7). This analysis revealed no overlap between these populations.

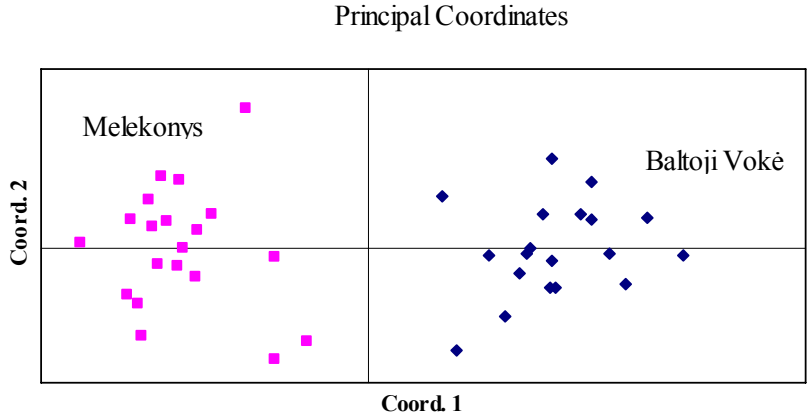

Figure 7. Two-dimensional representation of principal coordinate analysis of red raspberry populations, based on genetic distances

DISCUSSION

The management of an *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 a maximum of variability of certain species (Bretting, Widerlechner, 1995; Khadari et al., 2003). To achieve those goals, first of all the phenotypic characterization and molecular genotyping of accessions have to be performed. In this study, we carried out the genotyping of wild raspberry samples representing geographically different locations of Lithuania (Table 1). We used 6 informative primers previously tested and selected from 44 primers, and assessed them on a higher number of genotypes. About 42% of the wild raspberry collection of Vilnius University were included in this study. Our results demonstrate that RAPD markers can be effectively used in genotyping and genetic diversity studies of this collection. Application of two primers (MP4 and 470-08) was sufficient to genotype all 49 accessions. Such individual fingerprint as an absolute measure of the genetic makeup of an individual can be used to distinguish it from other accessions of the collection.

The study of adaptive diversity is one of the most important objectives for conservation of plant genetic resources (Nevo, 2001). The details of the genetic basis of adaptive variation in natural population are still largely unknown. It is discussed whether few genes with large effects or many genes with small effects underlie genetic variation in adaptive traits. The development of molecular marker technologies gave a new impulse for more detailed studies of plant genomes. RAPD and isozyme are among the most popular types of molecular markers. Usually, RAPDs show much higher values of diversity than isozymes. It is accepted that most RAPD loci consist of noncoding sequences and are pure indicators of adaptive traits (Karhu et al., 1996; Volis et al., 2001). On the other hand, some authors have pointed out that the RAPD technique provides a powerful tool for obtaining loci of both coding and non-coding sequences (Williams et al., 1990; Fahima et al., 1999; Li et al., 1999). They offer evidence that RAPD polymorphisms are, at least partly, adaptive and are determined by natural diversifying selection. This point of view is

also supported by the fact that RAPD diversity patterns in natural populations are similar to those of the allozymic differentiation (Nevo, 2001). Protein polymorphism, considered to be correlated with ecology, is produced by the coding sequences of the genome. We studied the influence of edaphic characteristics of original habitats on the RAPD diversity pattern of the accessions studied. A significant correlation ($r = -0.65$; $p = 0.01$) was found with soil acidity. The principal component analysis (PCO) supports these results (Figure 8).

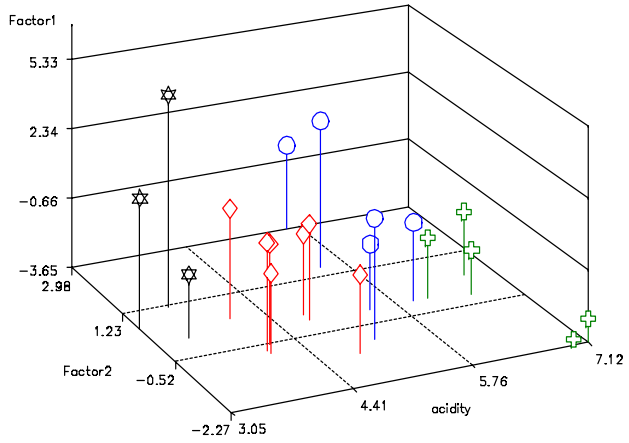

Figure 8. Plotting of red raspberry genotypes values in tridimensional perspective on the axis of soil acidity of origin site and two axes from factor analysis of polymorphic RAPD DNA fingerprints. Different shapes indicate individuals grouped by soil acidity properties: star for acidity 3 to 4, diamond for 4 to 5, ball for 5 to 6, cross for 6 to 7.12

The first three principal components from the principal component analysis explained a total of 64.5% of the variation among the samples. PCO showed genetic differences among individuals with regard to the soil acidity of their original habitats. The PCO plot indicates some slope in the spatial grouping of accessions, starting from the location of samples possibly adapted to acidic soils towards samples originally grown in grounds with a higher pH.

Differences among SOD spectra could be related to the local adaptation and survival mechanism of different genotypes under natural environmental stress. It has been shown by previous studies that different stress factors (NaCl salinity, osmotic stress, heavy metals, chilling, fotodestruction) lead to a different regulation of SOD isozymes (Watson et al., 1980; Ren et al., 1999). It is very interesting that in some cases the disappearance of isoforms and a decline in SOD activities in leaves were detected. As Ren et al. have reported (Ren et al., 1999), the activity of one SOD isoform was greatly diminished both in leaves and roots with increasing the altitude and disappeared at a high altitude in *Plantago major*. The authors consider this SOD polymorphism in *P. major* as an adaptation of alpine plants to abiotic stress. SOD polymorphism of red raspberry in our study is more complicated and did not show such a clear adaptive dependence.

Our results demonstrate that there is no correlation between the genetic and geographical distences of the accessions studied ($r = 0.123$; $p = 0.001$). In spite of these our results, Table 6 also shows that the genetic differences of red raspberry genotypes have some longitudinal or west–east cline (only once both geographic characters were significant for differences in the same locus). Genetic diversity as a relative measure of the genetic distances among the genotypes is represented by a dendrogram (Figure 2)

which can be used in creating a core collection and in the future breeding experiments. According to Graham et al., daughter seedlings of red raspberry are about 90% and more similar to the mother plant (Graham et al., 1997). Based on this assumption, samples that show such similarity could be excluded from the collection. In our study, there were only two such genotypes – JL06 and JL14 (Figure 2). The genetic distances among the majority of accessions are relatively large, demonstrating a high level of genetic divergence among them. 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. To save all types of genetic diversity and to have a more representative collection, various types of markers have to be used (Wen, Hsiao, 1999, Wang et al., 2006). 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 red raspberry were assessed in previous studies (Jennings, 1988; Balčiūnienė et al., 2005; Ryabova, 2007). Marshall et al. analyzed the morphological variation of samples collected from seven sites of the Tayside region, Scotland and grown over two years in two environments (the greenhouse and under nylon mesh) (Marshall, 2001). The primary evaluation of the phenotypic diversity of the wild raspberry collection of VU Botanical Garden was carried out by Balčiūnienė et al. (2005). The authors assessed the field performance (some fruiting peculiarities, vegetative growth pattern and resistance to fungal diseases) of wild raspberry accessions. In our current work, we have studied morphological and molecular variation in a wild raspberry *ex-situ* collection and assessed a correlation between phenotypic and molecular (RAPD) data. No correlation was detected between them ($r = 0.073$; $p = 0.012$). The independence between these two kinds of data was noted also in some other studies (Karhu et al., 1996; Wang et al., 2006). On the other hand, a correlation between phenotypic and molecular markers was also reported (Wen, Hsiao, 1999; Sensoy et al., 2007). Differences between patterns of morphological and RAPD marker variation in our study can be explained by the quantitative nature of the morphological data. These traits are more strongly affected by the environment than qualitative morphological characters and molecular markers. Although the data analysis showed an overall independence between the phenotypical and the other two kinds of data (genetic and geographical), a certain effect of the presence or absence of a RAPD band (allele) was established for geographical data and some morphological traits (Table 6). In 7 of 9 cases, the same differences in loci were also significant for a certain morphological trait. So, if even a comparison of the distance matrices for geographic characters and morphological traits did not reveal any correlation, we can see from Table 6 that a certain relation was possible.

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

The RAPD method can be a sensitive tool for determination of genetic structure and in genetic diversity studies of plant populations (Nybom, Bartish, 2000). Genetic diversity studies in red raspberry populations have revealed a high level of DNA polymorphism of this species (Graham et al., 2003). The same tendency was also observed in nineteen Lithuanian populations of wild raspberry. On the average, 76.26% of RAPD bands per population were polymorphic and this value is very close to those estimated in the red raspberry collection (77.83%). Besides, 98% of RAPD loci established by seven oligonucleotide primers in natural populations of *R. idaeus* were detected also in the germplasm collection. This fact suggests that the genetic diversity of the red raspberry collection fully represent the genetic diversity found in natural populations. The polymorphism of red raspberry populations was similar to the one determined in perennial outcrossing plant populations whose seeds are dispersed by animals (Bartish et al., 1999; Jordano, Godoy, 2000).

The proportion of total diversity found within populations based on the Φ_{PT} value was 74%, while 26% was due the variation among populations. This result is in agreement with the information that outcrossing plants retain a considerable variability and that most variation is exhibited within populations (Nybom, Bartish, 2000; Garkava-Gustavsson, 2005).

All individuals studied in one population were genetically different, and the genetic distances among red raspberry plants in separate populations were quite high. This fact suggests the importance of sexual reproduction in red raspberry populations.

Genetic distances among red raspberry populations positively correlated with geographical distances ($r = 0.232$, $p = 0.002$). Such a correlation is frequent in outcrossing plants (Nybom, Bartish, 2000) and implies that isolation by distance may have an impact on the genetic differentiation among populations.

The problem of RAPD-based adaptive variation was studied in various plant species: *Pinus sylvestris* (Karhu et al., 1996), *Hordeum spontaneum* (Volis et al., 2001; Owuor et al., 2003), *Triticum dicoccoides* (Fahima et al., 1999), *Phytolacca dodecandra* (Semagn et al., 2000), *Sesleria albicans* (Reish et al., 2003). The possible adaptive role of RAPD polymorphisms was also shown in some studies (Fahima et al., 1999; Semagn et al., 2000; Zhao et al., 2006 etc.). A spatially dependent genetic variation was established by RAPDs within and among wild raspberry colonies (Graham et al., 1997). Red raspberry seems to be well adapted to local ecological conditions (Marshall et al., 2001). Our studies of Lithuanian red raspberry populations also support this point of view. The high level of genetic differentiation among unisolated populations, presence of population-specific alleles, results of principal coordinate and UPGMA analyses demonstrate that the gene flow among Baltoji Vokė and Melekonys populations is overridden by natural selection.

CONCLUSIONS

1. A high level of DNA polymorphism was established in accessions of the germplasm collection and natural populations of red raspberry ($77.83 \pm 9.15\%$ and $76.26 \pm 5.50\%$, respectively).
2. Individual plants of the red raspberry germplasm collection show differences in their morphological and phenological characters. Some accessions could be used as donors of valuable genes in red raspberry breeding.

3. A correlation of some RAPD patterns with soil acidity in plant-collecting sites was established.

4. A significant negative correlation was determined between the altitude and latitude and the floricane height of red raspberry accessions.

5. No correlation was found between the morphological Euclidean and the genetic distance among accessions of the red raspberry collection. Both morphological and molecular markers should be used for a detailed evaluation of the genetic resources of red raspberry.

6. Local geographical and microclimatic conditions may impact red raspberry population differentiation.

7. Most of genetic variations were found within red raspberry populations (74%) and among populations from the same agroclimatic region (23%). Only a very low level of differentiation was established among populations from different agroclimatic regions (3%).

8. Rather high genetic differentiation of red raspberry populations and the presence of population-specific loci show a specific character of some of them, which could be valuable for the conservation of genetic resources.

9. The absence of identical RAPD phenotypes in the red raspberry plant material studied shows that sexual reproduction could be important for the expansion of populations.

10. The red raspberry germplasm collection of the VU Botanical Garden represents rather well the genetic diversity of natural red raspberry populations in Lithuania.

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SANTRAUKA

Šiame darbe pirmąkart Lietuvoje molekulinių ir morfologinių žymenų metodais įvertinta paprastosios avietės kolekcijos pavyzdžių genetinė įvairovė ir palyginta su gamtinėse populiacijose aptinkama genetine įvairove.

Naudojant šešių pradmenų rinkinį, genotipuoti 49 paprastosios avietės pavyzdžiai, atrinktos pradmenų poros, su kuriomis būtų galima identifikuoti visus šiuos augalus.

Kolekcijos morfologiniam įvertinimui pasirinkta 13 kiekybinių morfologinių požymių. Paprastosios avietės kolekcijoje nustatyta didelė morfologinių ir fenologinių požymių įvairovė. Kai kurie kolekcijos pavyzdžiai pasižymėjo ūkiškai svarbiomis savybėmis (pvz., derėjimu dukart per metus, ankstyva vegetacija), todėl galėtų būti naudingi vertingų genų donorai paprastosios avietės selekcijoje. Pirmąkart įvertintas ryšys tarp avietės kolekcijos pavyzdžių morfologinės įvairovės ir jų DNR polimorfizmo. Tarp morfologinių euklidinių ir genetinių atstumų koreliacijos neaptikta. Nustatyta patikima neigiama koreliacija tarp avietės pavyzdžių florikano aukščio ir augavietės aukščio virš jūros lygio ($r=-0,304$, $p=0,03$). Šiuo darbu parodyta, kad paprastosios avietės kolekcijos pavyzdžių morfologinių požymių ir molekulinių žymenų tyrimas puikiai papildo vienas kitą, abiem metodais buvo parodyta didelė įvairovė kolekcijos pavyzdžiuose. Objektyviam genetinių išteklių įvertinimui reikėtų naudoti tiek morfologinius, tiek molekulinius žymenis.

Buvo tirta augavietės edafinių savybių įtaka avietės morfologiniams požymiams ir RAPD polimorfizmui. Ištyrus 20-ies geriausiai ištirtų kolekcijos pavyzdžių 230-ies RAPD lokusų pasiskirstymą, nustatyta patikima žymi neigiama koreliacija su augaviečių dirvožemio rūgštumu ($r=-0,65$, $p\leq0,01$). Tačiau tiriant tą pačią priklausomybę tarp 49–ių kolekcijos pavyzdžių su žymiai mažesniu RAPD lokusų skaičiumi (48), priklausomybės tarp dirvožemio rodiklių ir RAPD pasiskirstymo dėsningumo negauta, nors buvo nustatyta kai kurių RAPD lokusų pasiskirstymo tirtuose augaluose priklausomybė nuo dirvožemio cheminių savybių (rūgštumo, fosforo ir kalio kiekio).

SOD polimorfizmo tyrimas taip pat atskleidė paprastosios avietės tirtų pavyzdžių susiskirstymą į grupes pagal augaviečių dirvožemio rūgštumą. Pagal SOD izoformų judėjimo pobūdį gelyje buvo išskirti 6 SOD izofermentiniai tipai.

Norint geriau įvertinti paprastosios avietės genetinius išteklius Lietuvoje, buvo atlikti ir gamtinių populiacijų iš įvairių agroklimatinių regionų tyrimai. Ištyrus 315 augalų iš 19–os populiacijų, nustatyta aukštas DNR polimorfizmo lygis. Individų RAPD fenotipų unikalumas rodo, jog šios rūšies plitimui labai svarbus yra lytinis dauginimasis. Unikalūs RAPD lokusai, nustatyti kai kuriose populiacijose, rodo paprastosios avietės populiacijų genetinį savitumą ir išskiria jas iš kitų avietės populiacijų tarpo. Šia savybe galima pasinaudoti, atrenkant pavyzdžius į genetines kolekcijas, nes tokie saviti lokusai gali būti susiję su populiacijų prisitaikymu prie vietos sąlygų bei unikaliomis genetinėmis savybėmis. Molekulinės genetinės įvairovės analizė (AMOVA) parodė, kad paprastosios avietės populiacijų diferenciacija yra 26%. Panašus genetinės diferenciacijos lygis buvo apskaičiuotas ir programa PopGene, kur genetinės diferenciacijos koeficientas buvo 0,30. Tyrimai parodė, kad paprastosios avietės populiacijų diferenciacijai įtakos galėjo turėti ir populiacijų izoliacija, priklausanti nuo atstumų. Buvo gauta nedidelė, bet patikima koreliacija tarp paprastosios avietės populiacijų geografinių ir genetinių atstumų ($r=0,232$, $p=0,002$). Palyginus populiacijas tarpusavyje AMOVA metodu trimis lygiais (tarp agroklimatinių regionų, tarp

populiacijų pavyzdžiuose ir individų populiacijose) nustatyta, kad paprastosios avietės populiacijų genetinė diferenciacija yra 23%, o pagrindinė genetinė įvairovė pasiskirsčiusi populiacijų viduje – 74% (p=0,001). Tarpregioninei genetinei įvairovei teko tik 3% bendros genetinės įvairovės (p=0,001). Paprastosios avietės populiacijose nustatyta vidupopuliacinė įvairovė buvo labai artima literatūroje nurodomai kryžmadulkiams augalams nustatytai genetinei įvairovei. Palyginti nemažą avietės populiacijų genetinę diferenciaciją galima paaiškinti jų prisitaikymu prie lokalių sąlygų bei su tuo susijusiais genomo pokyčiais. Buvo parodyta, kad kai kurie klimato veiksniai (vidutinis metinis kritulių kiekis) gali turėti įtakos RAPD lokusų polimorfizmui paprastosios avietės populiacijose. Populiacijų genetinei diferenciacijai įtakos gali turėti ir vietinės ekologinės bei mikroklimato sąlygos. Tai parodė atlikti Baltosios Vokės ir Melekonių populiacijų genetinės diferenciacijos tyrimai.

Paprastosios avietės populiacijose nustatytas vidutinis polimorfizmas (76,26%) savo reikšme buvo labai artimas nustatytam kolekcijos pavyzdžiuose (77,83%). Tyrimai taip pat parodė, kad 98% alelių, nustatytų avietės populiacijose, buvo rasti ir VU Botanikos sodo kolekcijoje. Tai rodo, kad VU Botanikos sodo paprastosios avietės kolekcija objektyviai atspindi šios rūšies genetinę įvairovę Lietuvos teritorijoje.

Laukinė avietė yra potencialus genų donoras avietės veislių selekcijoje. Kolekcijos pavyzdžių genetinės įvairovės įvertinimas ir dalies pavyzdžių genotipavimas, pradmenų derinių RAPD polimorfizmui tirti parinkimas gali būti pritaikytas kolekcijos tvarkymui, paprastosios avietės genetinių išteklių saugojimui ir panaudojimui molekulinėje selekcijoje. Mūsų atlikti avietės populiacijų tyrimai suteikia nemažai naujos informacijos apie šio Lietuvoje paplitusio ir ekologiniu požiūriu svarbaus augalo biologinius ir genetinius savitumus.