BIOLOGIJA. 2005. Nr. 4. P. 24–28

Assessement of ecological impact on genetic diversity among populations of *Rubus idaeus* L.

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The genetic diversity of *Rubus idaeus* L. both within and among the populations in different ecological conditions was compared. The diversity of RAPD markers was studied in 136 plants of the wild raspberry from seven populations sampled in Lithuania. The results showed a high level of DNA polymorphism in *R. idaeus* populations. The seven primers used in this study amplified 73 reproducible DNA fragments of which 89.04% were polymorphic. The size of these fragments ranged from 400 bp to 2530 bp. Eight RAPD loci were monomorphic for all the genotypes studied.

We found no correlation between the genetic and geographic distances of the study populations. A relatively high genetic differentiation (G_{ST} = 0.164) among the populations was established.

Assessment of the correlation between the genetic diversity values of the populations and the measures of ecological factors showed a significant negative association of the former values only with annual accumulated precipitation. Our results show that genotypic variability is greater under conditions differing from the ecological optimum of the species.

Key words: RAPD, population differentiation, *Rubus idaeus*

INTRODUCTION

Rubus idaeus is a Lithuanian native plant species of the *Rosaceae* family. The wild form of this species is widely distributed in temperate areas of Europe, North America and Asia. Red raspberry reproduces by vegetative expansion and by seed, which is usually dispersed by birds. The plant is pollinated by insects, mainly bees [1].

In Lithuania, populations of *R. idaeus* are located in rather varying habitats differing with regard to ecological factors such as soil properties, light, water and others. Besides, red raspberry habitats are usually fragmented. The best developed colonies grow in nutrient-rich and well-lit habitats with normal moisture regimes. Wild *R. idaeus* is tolerant of acid, infertile and poorly drained soils.

According to previous studies, spatially separated populations of *R. idaeus* are adapted to local conditions, which may result in an effective reproductive isolation in the absence of geographic barriers [1, 2]. It is believed that such local adaptation of raspberry has resulted in its genetic diversity. The highly variable phenotypes found in plants of this species have developed as a result of adaptation. On the other hand, all ecological characteristics of organisms result from the interaction between the genomes and the external factors in their environments [3]. The studies of RAPD phenotypes in *R. idaeus* also revealed a high level of variability [2, 4, 5]. The RAPD method has been successfully used to analyze genetic differentiation among plant populations from ecologically different habitats [2, 3, 6–9]. Because data on the population structure of *R. idaeus* in Lithuania are missing, the aim of our work was to measure genetic similarity and differentiation among Lithuanian populations of *Rubus idaeus* located in different environmental conditions.

MATERIALS AND METHODS

Plant material. Seven populations of *R. idaeus* were sampled in Lithuania (Table 1). A total of 136 plants were analyzed (the sample size for a population varied from 18 to 20 plants). The distance between sampled plants was approximately 30–50 meters. The fragmentation and size of a population was not considered in this study.

Table 1. **The seven native populations of *Rubus idaeus* L. studied**

Population	N*	Latitude, N	Longitude, E	Altitude, m
Juodkrantë	20	55°31'35"	21°06'25"	30
Ðëta	20	55°15'33"	24°14'40"	87
Vilkiautinis	20	54°06'21"	24°01'29"	125
Vingis	19	54°41'20"	25°14'23"	112
Prienai	19	54°36'32"	23°55'40"	131
Linkuva	18	56°05'31"	23°55'30"	56
Dieveniðkës	20	54°11'59"	25°33'49"	194

N* – the number of individuals per population studied.

te continentality index of the population origin was calculated using Chromov's method [15]. The relationships of the eco-geographic varieties of the populations to the genetic diversity values were tested using the Spearmen r correlation coefficient.

The Spearmen's correlation and its p-value were calculated to relate the genetic diversity measures to climatic and geographic data (CORR procedure; SAS V8 program package) [16].

Table 2. **Primers used in RAPD analysis among *Rubus idaeus* L. populations and number of generated bands**

Primer	Sequence (5'→3')	Fragment size range (bp)	Total number of bands	Numer of polymorphic bands
P1	TAGCGGCTAC	420–450	2	2
MP4	GGTGAACGCT	550–2530	13	13
Roth 270-1	GTCTCGTCGG	460–1920	14	14
Roth 270-6	CAGGGGCATC	420–2400	14	11
Roth 380-3	GGCCCCATCG	400–2140	13	11
Roth 380-9	ACGGCGGCTC	520–2110	10	8
Roth 470-8	GAGAGGGAGG	420–1100	7	6
Total			73	65

DNA extraction and RAPD analysis. DNA was extracted from fresh leaf tissue collected in seven populations. Extraction and PCR-amplification were carried out as described in Þvingila et al. [10]. Fragments generated by amplification were separated according to size on a 1.5% agarose (Top vision™, Fermentas) gel run in TBE, stained with ethidium bromide, and visualized by illumination with UV light.

RAPD profiles were photographed using the BioDocAnalyse gel documentation system (Biometra). The reproducibility of amplification profiles was tested for each primer. Only clear and consistently reproduced DNA bands were considered. The bands with the same molecular weight and mobility were treated as identical fragments.

Statistical analysis. A matrix of different RAPD phenotypes was assembled. Intrapopulational genetic diversity was evaluated using POPGENE version 1.21 [11]. We calculated Shannon's information index (*I*) [12], Nei's (1973) gene diversity (*h*), observed number of alleles (n_a), effective number of alleles (n_e) and genetic distance between populations. To evaluate the degree of genetic subdivision among populations, we calculated Nei's coefficient of gene differentiation, G_{ST}. From these G_{ST} values the estimate of gene flow (*Nm*) was derived. The principal coordinate analysis was performed using GenAlEx V5 software [13].

The seven population-collecting locations were described by their ecogeographic variables: longitude, latitude, altitude, average temperature, average precipitation, duration of vegetation [14]. The climate

RESULTS

Analysis of RAPD patterns in individual plants. In the RAPD analysis of 136 plants from seven populations of red raspberry with seven decamer oligonucleotide primers, 73 reproducible fragments were amplified with a varying number per primer (Table 2). For example, primers Roth 270-1 and Roth 270-6 produced 14 scorable bands each, while primer P1 amplified only two fragments; 65 (89.04%) RAPD bands were polymorphic. The size of the fragments ranged from 400 bp to 2530 bp. Eight RAPD bands were common to all individuals studied. All plants showed specific RAPD phenotypes.

Intra- and interpopulation variability. The Lithuanian populations of *R. idaeus* studied showed different levels of intrapopulational diversity. For example, 76.71% polymorphic RAPD bands was found in the Prienai population and only 63.01% in the Juodkrantë population. Nei's gene diversity across all loci ranged from 0.238 (Juodkrantë) to 0.273 (Prienai). Ranges of mean values for Shannon's information index were from 0.351 (Juodkrantë) to 0.407 (Prienai) (Table 3). Pair-wise comparisons revealed an average [17] distance among populations of 0.074 ± 0.027 (Table 4). Interpopulational distances ranged from 0.042 (Prienai and Vingis) to 0.146 (Juodkrantë and Dieveniðkës). The evaluation of genetic distances between individual plants in each population showed that Prienai population was most heterogeneous (GD_{xy} = 0.235). Contrary to Prienai population, Juodkrantë population was most homogenous

Table 3. **Intrapopulational genetic diversity of *Rubus idaeus***

Population	P* (%)	Effective number of alleles, n_e	Nei's gene diversity, h	Shanon information index, I	GDxy**
Juodkrantë	63.01	1.414	0.238	0.351	0.186
Ðëta	73.97	1.462	0.267	0.396	0.225
Vilkiautinis	64.38	1.437	0.249	0.365	0.188
Vingis	73.97	1.451	0.261	0.388	0.198
Prienai	76.71	1.479	0.275	0.407	0.235
Linkuva	71.23	1.445	0.254	0.376	0.198
Dieveniðkës	71.23	1.451	0.257	0.380	0.200
Mean	70.64 ± 4.74	1.448 ± 0.018	0.257 ± 0.011	0.380 ± 0.017	0.204 ± 0.017

P* – the percentage of polymorphic RAPD bands.
Gdxy** – genetic distances among individual plants in population.

Table 4. **Nei's genetic distance between *Rubus idaeus* L. populations based on RAPD data**

	Juodkrantë	Ðëta	Vilkiautinis	Vingis	Prienai	Linkuva
Ðëta	0.070					
Vilkiautinis	0.060	0.053				
Vingis	0.087	0.062	0.069			
Prienai	0.091	0.046	0.061	0.042		
Linkuva	0.081	0.047	0.055	0.072	0.047	
Dieveniðkës	0.146	0.062	0.131	0.111	0.086	0.070

Table 5. **Spearmen's correlation between genetic and climatic characteristics of *Rubus idaeus* L. populations. The significance of an estimate is given in italics below the correlation value**

Ecogeographic variable	Percentage of polymorphic loci	Observed number of alleles	Effective number of alleles	Nei's gene diversity	Shannon's information index
Latitude	–0.07	–0.07	–0.18	–0.14	–0.14
	0.88	*0.88*	*0.70*	*0.76*	*0.76*
Longitude	0.36	0.36	0.45	0.43	0.43
	0.42	*0.42*	*0.31*	*0.34*	*0.34*
Altitude	0.4	0.4	0.52	0.46	0.46
	0.37	*0.37*	*0.23*	*0.29*	*0.29*
Average annual temperature	–0.13	–0.13	–0.09	–0.09	–0.09
	0.78	*0.78*	*0.85*	*0.85*	*0.85*
Average January temperature	–0.07	–0.07	–0.05	–0.05	–0.05
	0.88	*0.88*	*0.91*	*0.91*	*0.91*
Average July temperature	–0.32	–0.32	–0.28	–0.28	–0.28
	0.48	*0.48*	*0.54*	*0.54*	*0.54*
Temperature amplitude	0.07	0.07	0.05	0.05	0.05
	0.88	*0.88*	*0.91*	*0.91*	*0.91*
Average annual precipitation	–0.77	–0.77	–0.79	–0.78	–0.78
	0.04	*0.04*	*0.03*	*0.04*	*0.04*
Precipitation during vegetation period	–0.40	–0.40	–0.20	–0.25	–0.25
	0.37	*0.37*	*0.67*	*0.59*	*0.59*
Continentality	0.36	0.36	0.34	0.36	0.36
	0.42	*0.42*	*0.45*	*0.43*	*0.43*
Duration of vegetation	–0.53	–0.53	–0.33	–0.41	–0.41
	0.22	*0.22*	*0.47*	*0.36*	*0.36*

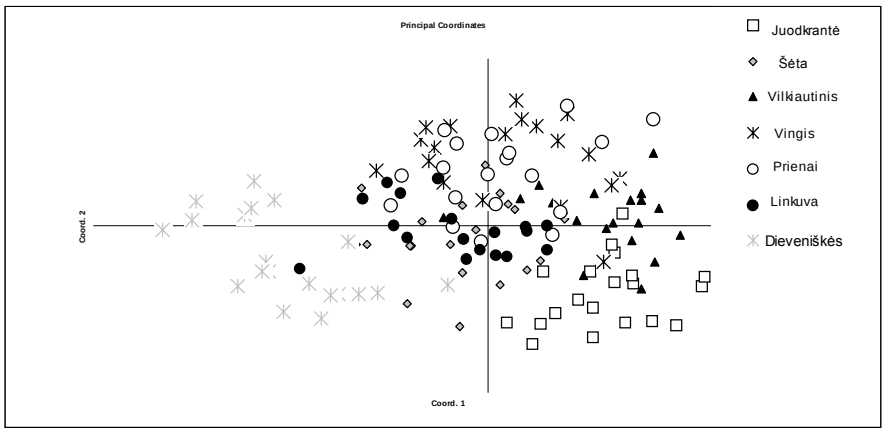

Figure. Principal coordinate analysis of genetic similarity of 136 plants from seven wild raspberry (*Rubus idaeus*) populations

(GD_{xy} = 0.186). The coefficient of genetic differentiation among populations was estimated G_{ST} = 0.164. For different RAPD loci G_{ST} ranged from 0.015 ($MP4_{580}$) to 0.54 (Roth $380\text{-}9_{870}$). The mean gene flow (Nm = 2.5) among the populations calculated using the G_{ST} value was rather high.

Genetic diversity of *R. idaeus* populations and climatic factors in their habitats. The following environmental variables were included in the analysis: geographical (longitude, latitude, altitude); climatic (temperature: annual, January, July, seasonal temperature amplitude); precipitation (annual rainfall, precipitation during vegetation period); continentality index; duration of vegetation period (Table 5). Only annual accumulated precipitation showed a correlation with population genetic diversity (Table 5).

DISCUSSION

Genetic diversity studies in the populations of *R. idaeus* revealed a high level of DNA polymorphism of this species [2, 4]. The same tendency was observed in seven Lithuanian populations of wild raspberry. On average 70.6% of RAPD bands per population were polymorphic (Table 3). Eight monomorphic markers were also taken into account. Such DNA fragments are considered by some authors as possible species-specific markers [18] and can be used in population diversity studies. When AMOVA was used for the evaluation of inter-population diversity, the inter-population variance component was 0.184, while the intra- population variance component was 2.171. Results of the random permutation test showed that the inter-population variance component was highly significant (P = 0.001).

The proportion of the total diversity found within the populations based on the G_{ST} value was 83.6% *versus* 16.4% of variation among the populations. This result is in agreement with the information that outcrossing plants retain a considerable variability and that most of variation is exhibited within populations [19]. According to these authors, the G_{ST} value for outcrossing animal-pollinated plants was about 0.197 when the allozyme analysis was used for the evaluation of population genetic structure. In spite of the neutrality of RAPD markers, it is possible that some of RAPD loci are influenced by natural selection [20]. These could be the markers that showed high G_{ST} values ($270\text{-}6_{1390}$ – 0.434, $470\text{-}8_{850}$ – 0.456, $380\text{-}3_{480}$ – 0.469, $380\text{-}9_{870}$ – 0.540).

The principal coordinate analysis (PCO) demonstrated that individuals from the populations studied formed rather compact groups. Some populations showed considerable overlapping between each other. Some of them, for example, Dieveniðkës and Juodkrantë, are presented on the PCO plot as separated and distantly remoted clusters. However, the geographic distances among population location sites was not associated with the genetic distance among the populations (r = 0.33, p = 0.146). Such situation was also observed by other authors [20] who tried to explain them by the impact of similar ecological factors in the habitats of different populations [9, 21–23]. We examined a correlation between the estimates of genetic diversity and climatic conditions in the habitats of the study populations. The genetic diversity data based on RAPD analysis showed a significant negative correlation only with annual accumulated precipitation (*e.g.*, r = –0.79; p = 0.03 for an effective number of alleles). The percentage of polymorphic alleles, Nei's gene diversity, the number of alleles, the effective number of alleles and the Shannon index increased when the amount of accumulated precipitation went down. Other climatic and geographic variables showed no correlation with population genetic data. Of course, the number of the populations studied was not large enough and the obtained results can only illustrate the tendencies according to the possible associations of climatic and genetic factors. A careful examination of data presented in Table 5 leads to a conclusion that the highest genetic diversity in *R. idaeus* tends to be associated with a more stressful environment. This tendency was noted also by other authors [3, 24].

Our results demonstrated a relatively high genetic differentiation of the Lithuanian population of *Rubus idaeus*. The knowledge of the genetic variation in this species under different ecological conditions is important for developing appropriate strategies for conservation of genetic resources of *R. idaeus* in Lithuania.

ACKNOWLEDGMENTS

This work was supported by Grant T-43/05 of Lithuanian State Science and Studies Foundation and the Lithuanian State program "Genefund".

Received 14 October 2005
Accepted 8 December 2005

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EKOLOGINIØ VEIKSNIØ ÁTAKA *Rubus idaeus* L. POPULIACIJØ GENETINEI ÁVAIROVEI

Santrauka

Genetinë ávairovë septyniose *R. idaeus* populiacijose ið ávairiø Lietuvos vietø buvo tirta atsitiktinai pagausintos polimorfinës DNR metodu (RAPD). Ðios populiacijos egzistuoja gana ávairiomis klimato sàlygomis. Iðtyrus 136 augalus nustatytas didelis paprastosios avietës populiacijø DNR polimorfizmas. Panaudojus septynis pradmenis, gauti 73 RAPD þymenys, ið kuriø 89,04% buvo polimorfiðki. DNR fragmentø dydis svyravo nuo 400bp iki 2530bp. Aðtuoni RAPD lokusai buvo bendri visiems tirtiems individams. Koreliacijos tarp populiacijø genetiniø ir geografiniø atstumø (r = 0,33; p = 0,146) nenustatyta. Tiriant ávairiø ekologiniø veiksniø reikðmæ genetinës ávairovës lygiui populiacijose, nustatyta patikima koreliacija tarp apskaièiuotø genetiniø rodikliø (Shannon'o indekso, genø ávairovës pagal Nei ir kt.) ir vidutinio metinio krituliø kiekio. Gauti rezultatai patvirtina jau anksèiau pastebëtà dësningumà, kad stresinë aplinka didina genetinæ ávairovæ.