

Selection of RAPD primers for geographically most distinct Lithuanian populations of *Impatiens parviflora*

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Surprisingly little information is available about adaptations of invasive species in the Baltic countries. Since 1934 *Impatiens parviflora* DC. was recorded in the suburbs of Vilnius, supposedly it escaped from the VU Botanical Garden. Presently *I. parviflora* belongs to naturalized species of active distribution. In Lithuania, *I. parviflora* occurs abundantly in man-disturbed localities – urban sites, roadsides or farmyards. Permanently overmoistured gleyic forest sites are also common habitats of *I. parviflora*. Our study aimed at selection of RAPD primers for evaluation of genetic variability among geographically contrasting four populations of *I. parviflora*. Populations growing near the western, southern, northern and eastern borders of Lithuania (Karklė, Ratnyčia, Žagarė, Švenčionys) were selected. Randomly Amplified Polymorphic DNA (RAPD) as the most ubiquitous for plant analysis molecular markers type was selected for evaluation of genetic diversity of *I. parviflora* populations. Among thirty RAPD primers tested, 222, 250, 269, 340, 474, 516, OPA-20, OPB-7, OPD-20, OPQ-11 generated the largest amount of DNA bands and were selected for the analyses. For each population, the percentage of polymorphic bands with ten primers was 21–27 (the lowest for Karklė and the highest for Žagarė population), and the number of polymorphic bands ranged in the interval 40–50. Molecular variance among populations was much higher (82%) than within populations. Varying geographically, populations of *I. parviflora* were sufficiently distinct according to RAPD based principal component analyses, also by UPGMA dendrograms. Pair-wise genetic distance among these populations ranged from 0.349 to 0.583. The obtained data show that distribution of invasive species might bring changes in genetic diversity.

Key words: small balsam, *Balsaminaceae*, polymorphism, invasion, alien species

INTRODUCTION

Globalisation provides vastly expanded opportunities for plant species to be transported to new locations through a wide range of pathways [1]. Alien species might have serious implications for the environment and communities. Urgent problems of Europe include pathways of invasion and elucidation of species traits in determining invasiveness [2]. After introduction of *I. parviflora* to European botanical gardens at the beginning of the 19th century (e. g.

1837, in Dresden), its intervention to the natural communities was soon observed [3]. It is supposed that in Lithuania *I. parviflora* escaped from the VU Botanical Garden and in 1934 it was recorded in the suburbs of Vilnius for the first time [4]. Within the last decades in many European countries great attention has been paid to *Impatiens glandulifera* [5, 6]. Investigations of behaviour of sister species, small balsam, are surprisingly scarce despite the fact that it belongs to naturalized species of active distribution [7], presently being very common for cities and intervening deciduous forest communities [8, 9]. There is lack of information regarding local differentiation of both alien *Impatiens*

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species of the Baltic region [7, 10]. Till now *Impatiens* spp. data available for this area mainly deal with geographical location, morphology and lists of species comprising communities [10, 11]. The present study aimed at evaluation of genetic diversity of *I. parviflora* populations growing in the most geographically contrasting areas of Lithuania. This is an initial step in evaluating populations of small balsam throughout the country.

MATERIALS AND METHODS

Plant material. *I. parviflora* was collected from the most contrasting according to the geography areas of Lithuania (Table 1): near the west border of Lithuania – Karklė, near the east border – Švenčionys (distance between Karklė and Švenčionys is 330 km), near the north border – Žagarė and near the south border – Ratnyčia (distance between Žagarė and Ratnyčia – 268 km). Sampling date was July 21–August 1, 2010. Aboveground parts of the plants were cut, sealed in separate bags, cooled and transported to the laboratory. Leaves from the top of the plants were cut and frozen under -20 °C.

DNA extraction and RAPD-PCR. Total DNA was extracted from frozen leaves. Approximately 100–150 mg of plant material was grinded with liquid nitrogen and transformed to 200 µl Tris-EDTA buffer with 400 µl lysis solution (Genomic DNA Purification Kit, #KO512, Fermentas, Lithuania). The concentration and purity of DNA samples were determined spectrophotometrically (Eppendorf Bio-Photometer, Germany), other details of DNA extraction have been described earlier [12]. 0.5 µl RNase A/T1 Mix (#ENO551, Fermentas, Lithuania) was applied. For RAPD analysis 10 oligonucleotide primers of 10 bp length were used (Biomers.net GmbH, Germany). PCR mix in a volume of 25 µl consisted of 4 µl DNA, 2.5 µl Taq reaction buffer, 0.2 µl dNTP (25 nM), 1.5 µl MgCl₂ (25 nM), 2 µl primer (10 pmol/µl), 0.25 Taq DNA polymerase (MBI Fermentas, Lithuania) and 14.55 µl deionised water.

RAPD analyses. DNA amplification was performed in Mastercycler gradient (Eppendorf, Germany) according to the following program: first denaturation for 2 min at 94 °C;

35, 40 or 45 cycles of denaturation for 30 s at 94 °C, primers annealing for 35 s at 32 °C or 34 °C (depending on primer: the same as melting temperature or lower), extension for 1 min at 72 °C and final extension for 2 min at 72 °C. The reaction products were fractionated by electrophoresis in 1.5% agarose gel with ethidium bromide and the photographs of gels in the UV light were taken using Herolab transilluminator (Germany). The length of bands were estimated according to the gene ruler (GeneRuler™ DNA Ladder Plus with the standard molecular marker of 100 bp, Fermentas, Lithuania).

Statistical analyses. Genetic distances between populations (GD_{xy}) were calculated according to Nei and Li [13], and based on these data UPGMA grouping methods were applied. Molecular genetic diversity analysis was performed using GenAlEx v 6.41 program [14].

RESULTS AND DISCUSSION

Thirty RAPD primers were tested for amplification with *I. parviflora* DNA. Ten most informative primers were applied analyzing 4 populations (Table 2). Selected RAPD primers generated from 14 to 22 bands each, most of the bands (13–20 bands in primer) were polymorphic, and percentage of polymorphic bands was 89–100, respectively. Merely a few bands in some primers (1–2 bands in primer) were monomorphic. DNA band size ranged from 210 bp to 2900 bp. The shortest bands were generated by 222 primer, while the longest one by OPA-20. More detailed analyses of separate RAPD primers generated DNA (Table 3) showed that 474 primer was very efficient in generating bands for all populations. However, all populations with 516 primer, and populations of Ratnyčia, Karklė – 340 primer, Švenčionys, Žagarė – OPQ-20 primer were monomorphic, and OPD-7 (Švenčionys 71.4% polymorphic bands) primer, and 474 primer (Švenčionys 63.2% and Ratnyčia 57.9% polymorphic bands) were most polymorphic.

Number of different bands with a frequency $\geq 5\%$ ranged in the interval 91–106 highest for Švenčionys population (Fig. 1). Number of locally common bands found in $\leq 50\%$ populations was 23–28. Expected heterozygosity was 0.071–0.092 (1.3 times differences, being the highest

Table 1. Geographical coordinates and geographical distance (km) among populations of *I. parviflora*

Population	Altitude, m	Švenčionys	Ratnyčia	Žagarė	Karklė
Švenčionys, N 56° 21'–E 23° 15'	40	–	–	–	–
Ratnyčia N 55° 49'–E 21° 04'	30	187.3	–	–	–
Žagarė N 55° 03'–E 26° 14'	170	225.8	267.7	–	–
Karklė N 54° 00'–E 24° 01'	60	330.4	277.2	149.2	–

Table 2. *I. parviflora* polymorphic DNA generated by ten RAPD primers (total number of bands – 188)

RAPD primer	Sequence 5' → 3'	Annealing T (°C)	Length of DNA bands, bp	Number of bands			Percentage of polymorphic bands
				RAPD primer	monomorphic	polymorphic	
222	AAG CCT CCC C	34	210–2500	22	1	21	95.45
250	CGA CAG TCC C	34	250–2500	21	2	19	90.48
269	CCA GTT CGC C	34	250–1200	20	0	20	100.00
340	GAG AGG CAC C	34	290–1800	20	2	18	90.00
474	AGG CGG GAA C	34	300–1700	19	2	17	89.47
516	AGC GCC GAC G	36	220–1100	18	0	18	100.00
OPA-20	GTT GCG ATC C	32	450–2900	18	0	18	100.00
OPB-7	GGT GAC GCA G	34	300–2000	14	1	13	92.86
OPD-20	ACC CGG TCA C	34	280–2200	20	0	20	100.00
OPQ-11	TCT CCG CAA C	34	300–2800	16	0	16	100.00
All primers (total)		32–36	210–2900	188			

Table 3. *I. parviflora* polymorphic DNA generated by separate RAPD primers (total number of the bands – 188)

Population	RAPD primers									
	222	250	269	340	474	516	OPA-20	OPB-7	OPD-20	OPQ-11
Number of polymorphic bands										
Švenčionys	3	4	5	2	12	0	7	10	1	0
Ratnyčia	2	8	8	0	11	0	6	3	1	2
Karklė	2	6	8	0	9	0	8	2	1	4
Žagarė	2	8	6	4	7	0	11	10	2	0
Percentage of polymorphic bands										
Švenčionys	13.6	19.1	25.0	10.0	63.2	0	38.9	71.4	5.0	0
Ratnyčia	9.1	38.1	40.0	0	57.9	0	33.3	21.4	5.0	12.5
Karklė	9.1	28.6	40.0	0	47.4	0	44.4	14.3	5	25
Žagarė	9.1	38.1	30.0	20.0	36.8	0	61.1	71.4	10.0	0

for Žagarė population and the lowest for Ratnyčia population) it might be caused by differences in the size of population – Žagarė population was scattered and big, while Ratnyčia population – the smallest in size and very compact. For each population the percentage of polymorphic

bands with all primers was 21–27 (the lowest for Karklė and the highest for Žagarė population) and the number of polymorphic band ranged in the interval 40–50. Molecular variance among populations was much higher (82%) than within populations (18%; Fig. 2).

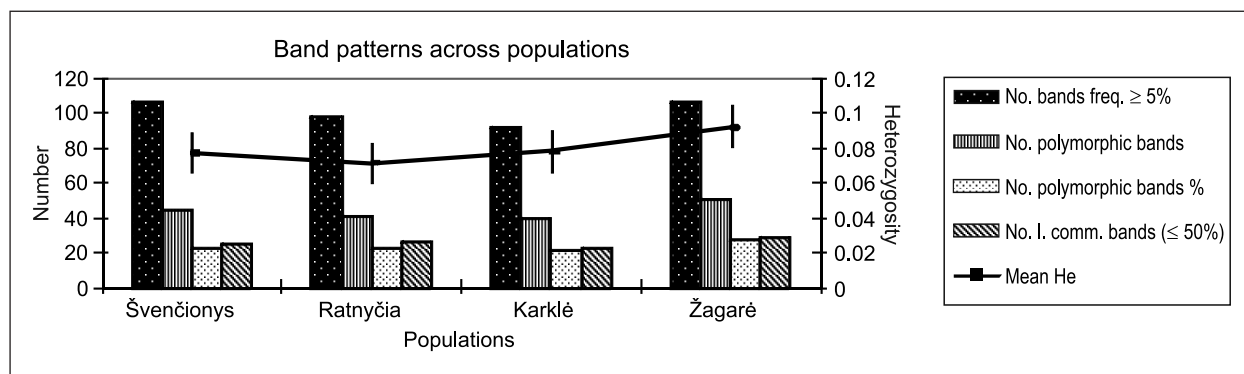


Fig. 1. Total DNA band patterns for geographically contrasting 4 populations of *I. parviflora* growing in Lithuania (No. bands freq. – number of different bands with a frequency $\geq 5\%$. No. polymorphic bands – number of polymorphic bands in all primers. No. polymorphic bands % – number percentage polymorphic bands in all primers. No. l. comm. bands – number of locally common bands found in $\leq 50\%$ populations. He – expected heterozygosity)

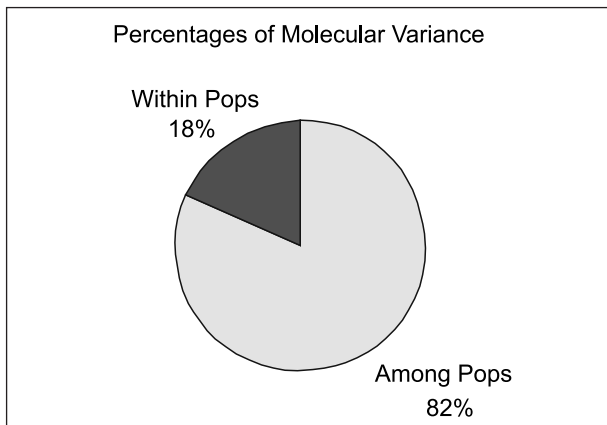


Fig. 2. Results with 10 primers of molecular variance of four *I. parviflora* populations in Lithuania

Relationships between populations according to the bands generated by different RAPD primers gave two types of dendrograms (Fig. 3). One type was an asymmetric tree with one branch – one of populations was very distinct from the other branch with three populations. The tree of other type consisted of two more or less equal branches with 2 populations in each. It was true for 250, OPD-20 and OPQ-11 primers. In two equal branch trees always the same pairs of populations separated: east–south and north–west population. In case of asymmetric tree the most distinct population depended on the primer: for 222 and OPD-20 primers the most different population was from the southern part of Lithuania (Ratnyčia), for primers 340 and OPB-7 the most distinct population was from the west (Karklė), for 269 and 516 primers the east part

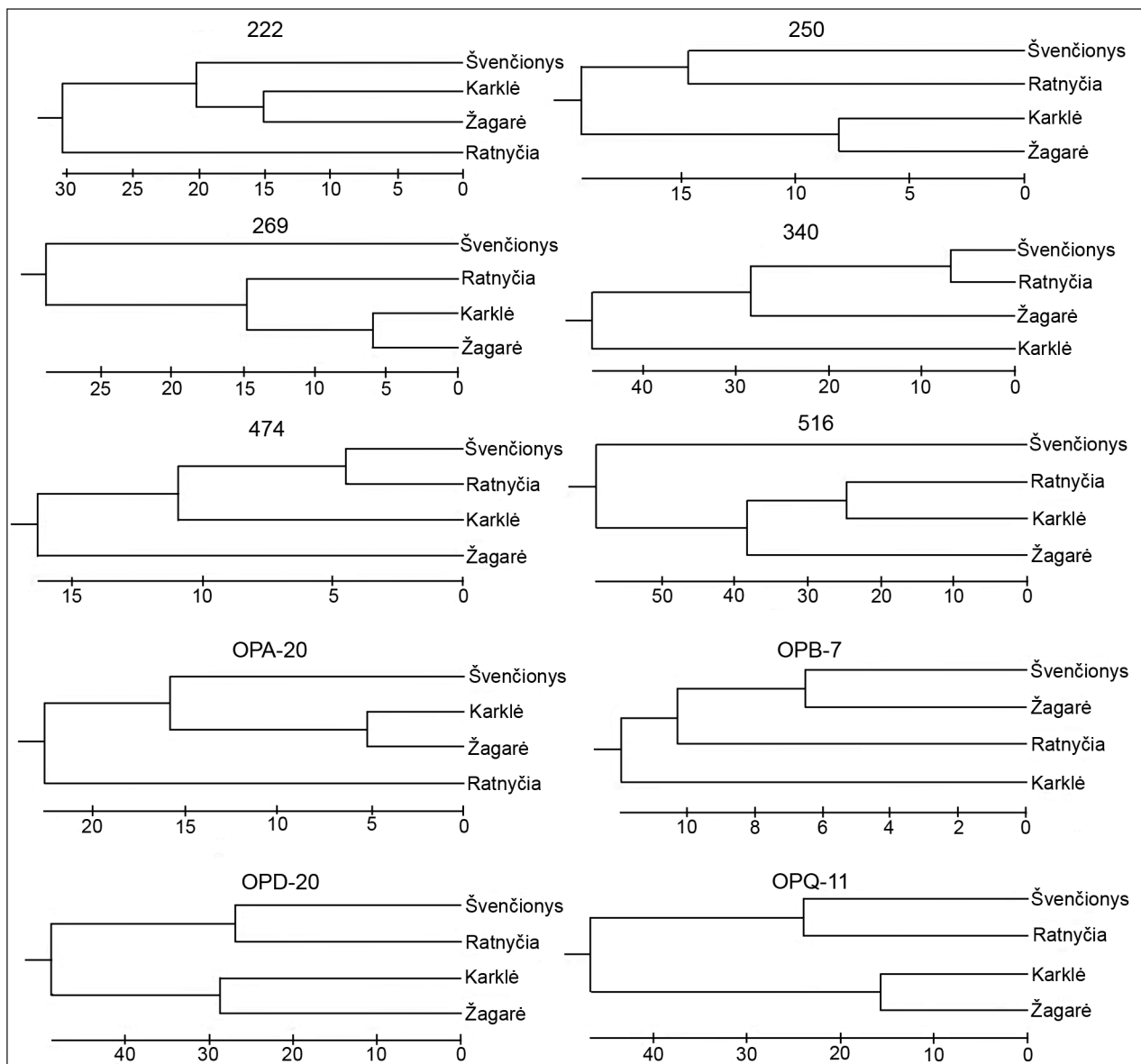


Fig. 3. Genetic relationships among 4 populations of *I. parviflora* based on ten separate RAPD primers using the UPGMA algorithm and the genetic distance (GD_{ij} according to Nei and Li [13])

population (Švenčionys) was distinguished from the others and for one primer (474) the north population (Žagarė) was most distinct. It shows that different DNA RAPD primers might be useful markers specifying populations. The classifications obtained by 250, OPD-20 and OPQ-11 primers reflected relationships between individuals of 4 populations according to all examined primers using Nei and Li [13] genetic distance matrix (bootstrap values obtained after 1 000 iterations) and UPGMA method (Fig. 4). Each

of 80 examined individuals revealed different RAPD phenotype (i. e. 80 RAPD phenotypes). Principal coordinate analyses confirmed UPGMA data (Fig. 5) and reflected genetic distances among populations very well: Švenčionys–Žagarė 0.583, Švenčionys–Karklė 0.572, Ratnyčia–Žagarė 0.560, Ratnyčia–Karklė 0.444, Švenčionys–Ratnyčia 0.443. The shortest genetic distance Karklė–Žagarė 0.349 corresponded to the shortest geographical distance (149 km).

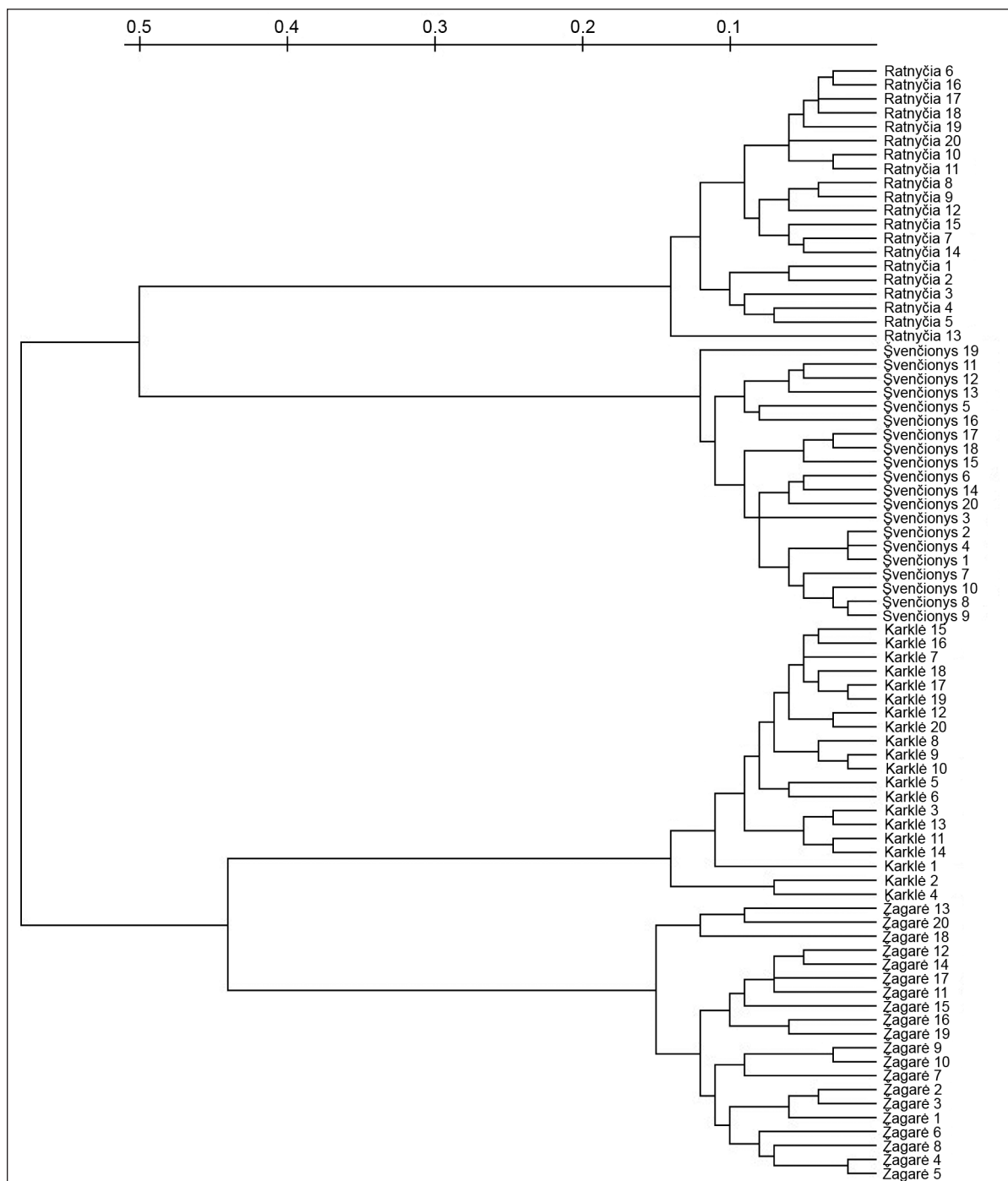


Fig. 4. Genetic relationships among 4 populations of *I. parviflora* based on ten RAPD primers using the UPGMA algorithm and the genetic distance (GD_y according to Nei and Li [13])

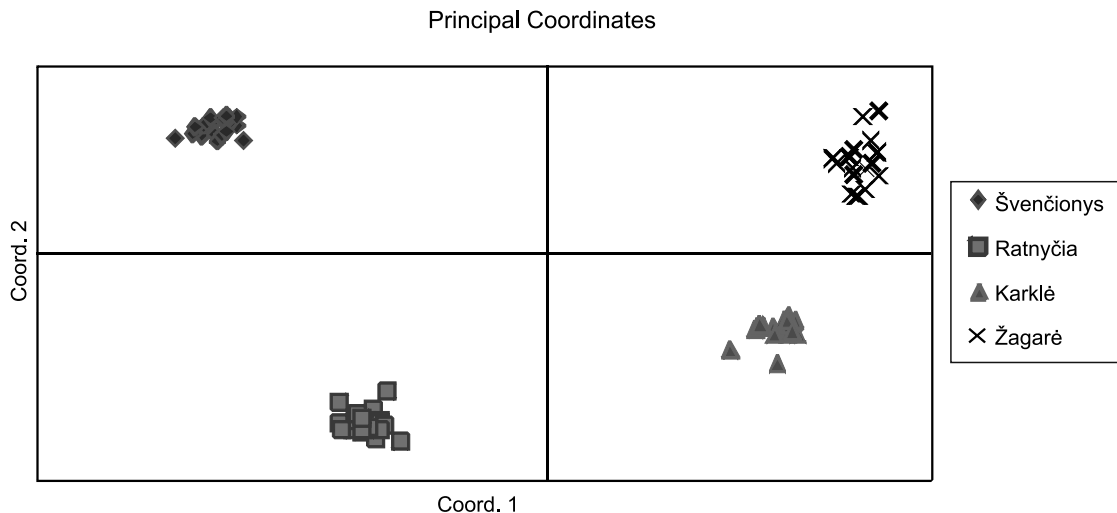


Fig. 5. Principal coordinate analyses of genetic distance of four *I. parviflora* populations from Švenčionys, Ratnyčia, Karklė, Žagarė with 10 RAPD primers

CONCLUSIONS

The obtained data show that distribution of invasive species might bring changes in genetic diversity. Selected RAPD primers generated from 14 to 22 bands each, most of the bands (13–20 bands in primer) were polymorphic, and percentage of polymorphic bands was 89–100, respectively, merely a few bands in some primers (1–2 bands in primer) were monomorphic. For each population the percentage of polymorphic bands with all primers was 21–27 (the lowest for Karklė and the highest for Žagarė population). UPGMA dendrograms (both for populations and for individuals) revealed genetic differentiation among populations. In two equal branch trees always the same pairs of populations separated: east–south and north–west population. In case of an asymmetric tree, the most distinct population depended on the primer: for 222 and OPD-20 primers the most different population was from the southern population (Ratnyčia). The shortest genetic distance Karklė–Žagarė corresponded to the shortest geographical distance (149 km). Principal coordinate analyses of RAPD data confirmed UPGMA dendrogram results. Based on different RAPD primers, principal component analyses provided the best information about geographical location of populations.

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GEOGRAFIŠKAI LABIAUSIAI NUTOLUSIŲ *IMPATIENS PARVIFLORA* POPULIACIJŲ LIETUVOJE APPD PRADMENŲ PARINKIMAS

Santrauka

Nedaug tyrimų atlikta aiškinantis invazinių augalų rūšių plitimo būdus Baltijos šalyse. Manoma, kad smulkiažiedė sprigė (*Impatiens parviflora* DC.) pirmą kartą buvo pastebėta Vilniaus priemiesčiuose 1934 m. ir kad ji paplito iš VU Botanikos sodo. Šiuo metu smulkiažiedė sprigė išplitusi žmogaus dažnai lankomose, gyvenamose vietose – miestuose, pakelėse ar kaimo sodybose. Perteklinio drėgnio miškai taip pat yra dažnos smulkiažiedės sprigės augavietės. Mūsų tyrimo tikslas – palyginti prie Lietuvos vakarinės, pietinės, šiaurinės ir rytinės sienų (Karklė, Ratnyčia, Žagarės, Švenčionių) augančios smulkiažiedės sprigės populiacijų genetinę įvairovę su geografine padėtimi. *I. parviflora* populiacijų genetinės įvairovės vertinimui buvo pasirinktas atsitiktinai pagausintos polimorfinės DNR (APPD) metodas. Iš tirtų trisdešimt APPD pradmenų daugiausiai DNR atkarpų pagamino 222, 250, 269, 340, 474, 516, OPA-20, OPB-7, OPD-20, OPQ-11 pradmenys. Atrinkta ir iširta dvidešimt individų populiacijoje. Keturiuose *I. parviflora* populiacijose su dešimčia pradmenų susidarė 21–27 % polimorfinių fragmentų, o jų skaičius siekė nuo 40 iki 50. Tarppopuliacinė genetinė įvairovė buvo daug didesnė (82 %) nei populiacijos viduje. Genetinis atstumas tarp populiacijų svyravo nuo 0,349 iki 0,583. Geografiškai skirtingų vietų populiacijų tarpusavio skirtumai atsispindėjo pagal APPD metodą susiformavusiose UPGMA dendrogramose, taip pat analizuojant principines koordinates. Gauti duomenys rodo, kad plintant svetimžemėms, invazinėms rūšims galimi genetinės įvairovės pokyčiai.

Raktažodžiai: smulkiažiedė sprigė, balzaminių šeima, polimorfizmas, invazija, svetimžemės rūšys