

# European *Impatiens* species differences at RAPD and ISSR loci

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Information concerning comparison of three widely spread European species of *Impatiens* along wider geographical areas is still missing. The present study is aimed at comparing genetic variability at RAPD and ISSR loci of *Impatiens noli-tangere*, *I. parviflora*, and *I. glandulifera*, covering a marked geographic area. Twenty four populations of these *Impatiens* (eight populations of each species) from two countries (Lithuania and Czech Republic) were examined. Eight randomly amplified polymorphic DNA (RAPD) and 5 inter simple sequence repeat (ISSR) markers were chosen considering the lack of data on the general molecular characteristics of *Impatiens*. The highest genetic differentiation at RAPD loci ( $G_{ST} = 0.81$ ) was characteristic of *I. parviflora*, and the highest genetic differentiation at ISSR loci ( $G_{ST} = 0.73$ ) was documented for *I. glandulifera*. According to Nei's genetic distances between two species populations, significant correlations were determined for *I. noli-tangere* and *I. parviflora* ( $r = 0.79$ ;  $p < 0.05$ ) and for *I. parviflora* and *I. glandulifera* ( $r = 0.76$ ;  $p < 0.05$ ) based on RAPD loci and for *I. parviflora* and *I. glandulifera* ( $r = 0.89$ ;  $p < 0.05$ ) based on ISSR loci. According to the mean values of polymorphism, genetic differentiation and Nei's genetic distances between two species populations at RAPD loci, the closest were *I. noli-tangere* and *I. parviflora*, while at ISSR loci, the most similar were invasive species, *I. parviflora* and *I. glandulifera*. UPGMA dendrograms revealed that the closest species were *I. noli-tangere* and *I. parviflora* by both RAPD and ISSR data. In conclusion, our study did not show unambiguous results about similarity between *Impatiens* species.

**Key words:** *Balsaminaceae*, balsams, alien species, genetic variability

## INTRODUCTION

The touch-me-not balsam (*Impatiens noli-tangere* L.) is present as a native species in almost all European countries. This *Impatiens* species is

found mainly in the temperate zone, that is why it is absent in Spain, Greece, and northern part of Scandinavia (Hatcher, 2003). It is naturally growing in many Asian countries, like Russia, Japan and Mongolia. In North America, *I. noli-tangere* is present in regions from south Alaska

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to Washington State. Invasive areas of this species have not been described, with exception of one note that in some places of Great Britain it is alien (Hatcher, 2003). Such statement has no further support from other studies.

The small balsam (*Impatiens parviflora* DC) is native in Afghanistan, Kazakhstan, Turkmenistan, Uzbekistan, Tajikistan (Tanner, 2008). It is an invasive species across Europe, including northern countries of Scandinavia and southern countries near the Mediterranean Sea (Coombe, 1956); also it is an invader of North America, being documented for west Canada and almost all United States (Tanner, 2008).

The native region of the Himalayan balsam (*Impatiens glandulifera* Royle) is limited to a small area of Asia, covering a 40 km belt of the Himalays mountains belonging to four neighbouring countries (India, Pakistan, Nepal, and China) (Beerling, Perrins, 1993). In the remainder part of Asia and in other continents like North America, Australia and Europe, it is an invasive species documented for Russia, Canada (Clements, 2008), USA (Tanner, 2008), New Zealand, Central Europe to northern countries, Britain and Ireland (Beerling, Perrins, 1993).

Hereby, *I. glandulifera* and *I. parviflora* are characterized as invasive plants growing in most parts of Europe (Coombe, 1956; Helmisaari, 2010).

There are overlapping areas of *I. noli-tangere*, *I. parviflora*, and *I. glandulifera* in Europe and North America, so this fact may lead to a native *I. noli-tangere* colonization by alien species of the same genera.

Favourable conditions for invasion of these two alien *Impatiens* species are created by natural and mainly anthropogenic factors such as ground water level change and flooding of rivers (Csiszar, Bartha, 2008), destroyed vegetative cover (Dobravolskaitė, 2012), neglected and unattended territories, destruction of forests, planting in gardens, and other favourable factors (Gudzinskas, 1998). A very important reason of invasion is the fact that an alien species may colonize new eroded places faster due to its better capabilities for adaptation. The small balsam could exist in areas which are too dry

and shaded for *I. noli-tangere*, and *I. glandulifera* could survive in open light-demanding areas, which are not suitable for a native one (Čuda et al., 2014). There are some areas of co-existence of all three *Impatiens* species in Central Europe (Čuda et al., 2014). There are some habitats where both native and alien species can coexist naturally in the Eastern Baltic area (Kupcinskiene et al., 2015). These circumstances help indicate what differences are between invasive and native species (Vervoort, Jacquemart, 2012) and what specific characters are beneficial for invaders. *I. parviflora* and *I. glandulifera* are more adaptive to unfavourable environment conditions than *I. noli-tangere* (Godefroid, Koedam, 2010). Similarities of these territories led to an evolutionary plant adaptation to the local climate (Skalova et al., 2011) and might get reflection on the molecular level.

The randomly amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) marker methods are applicable to many species of plants around the world (Nybom et al., 2014), because they are inexpensive and fast, do not require special information about the species; in addition, DNA loci cover various places of genome. Studies using these methods have been successfully conducted since 1990 (Williams et al., 1990). Nowadays, these methods are still widely used in comparing crop varieties or populations (Dabkevičienė et al., 2011; Yilmaz et al., 2012). Some investigations of invasive plants in Lithuania have been done by RAPD and ISSR markers: *Bunias orientalis* L. (Patamsytė et al., 2011), *Lupinus polyphyllus* Lindl. (Vyšniauskienė et al., 2011), *Medicago sativa* L. (Vyšniauskienė et al., 2013). The methods mentioned above are chosen considering the lack of data on the general molecular characteristics of *Impatiens*. As invasive Lithuania species, *I. glandulifera* (Zybartaitė et al., 2011) or *I. parviflora* (Kupcinskiene et al., 2011; Kupcinskiene et al., 2015) populations were separately investigated by dominant markers, but information covering comparison of three *Impatiens* species along wider geographical areas is still missing. The wider the geographical area, the bigger differences might be expected

concerning adaptation to the local climate and edaphic conditions. These differences might get reflection in DNA loci examined by RAPD and ISSR markers.

Interspecies studies are needed to check if the species might be separated employing some genetic markers. Interspecific interactions between balsam species are important to know when solving plant invasion problems. There are favourable conditions to compare *Impatiens* of a wider geographical region of Europe, because there are many places where three *Impatiens* species are growing beside or in close vicinity.

The present study is aimed at comparing genetic variability at RAPD and ISSR loci of three European species of *Impatiens* covering a marked geographic area.

## MATERIALS AND METHODS

### Plant material

*I. noli-tangere*, *I. parviflora*, and *I. glandulifera* were collected in 2012. Eight populations of each 3 *Impatiens* species growing beside or at a small distance were sampled in Lithuania (Anykščiai – Any, Varėna – Var, Kaunas – Kau, Juodkrantė – Juo) and in Czech Republic (Potschtein – Pot, Polabí, Velký Osek – Pol, Celina – Cel, Cernetice by Volyne – Vol). The population sampling included territories along 56°20' – 49°14' latitude (N) and 25°30' – 13°89' longitude (E); location details are described earlier (Kupcinskiene et al., 2015). Each plant sample included the top of the shoot without any abiotic or biotic damage. Detached plant parts were transferred to the bags with silica gel. Top leaves were taken and cooled at –20 °C temperature in the laboratory of the Department of Biology (Vytautas Magnus University).

### DNA extraction, RAPD and ISSR analyses

For DNA extraction, the DNA Purification Kit (#KO512, Thermo Scientific, Lithuania) was used. To measure concentration and to determine purity of DNA, a spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, USA) was used. RAPD and ISSR primers were used

as it was described earlier (Kupcinskiene et al., 2015). Markers employed were OP-A20, OP-D20, 222, 250, 269, 340, 474, 516 (RAPD) and ISSR2, ISSR3, ISSR4, ISSR5, and ISSR6 (ISSR). For polymerase chain reaction, a thermo-cycler Mastercycler gradient (Eppendorf, Germany) was used. PCR products were realized in 1.5% agarose gel electrophoresis. RAPD data was collected based on Williams et al. (1990) method, with some modifications.

### Statistical analysis

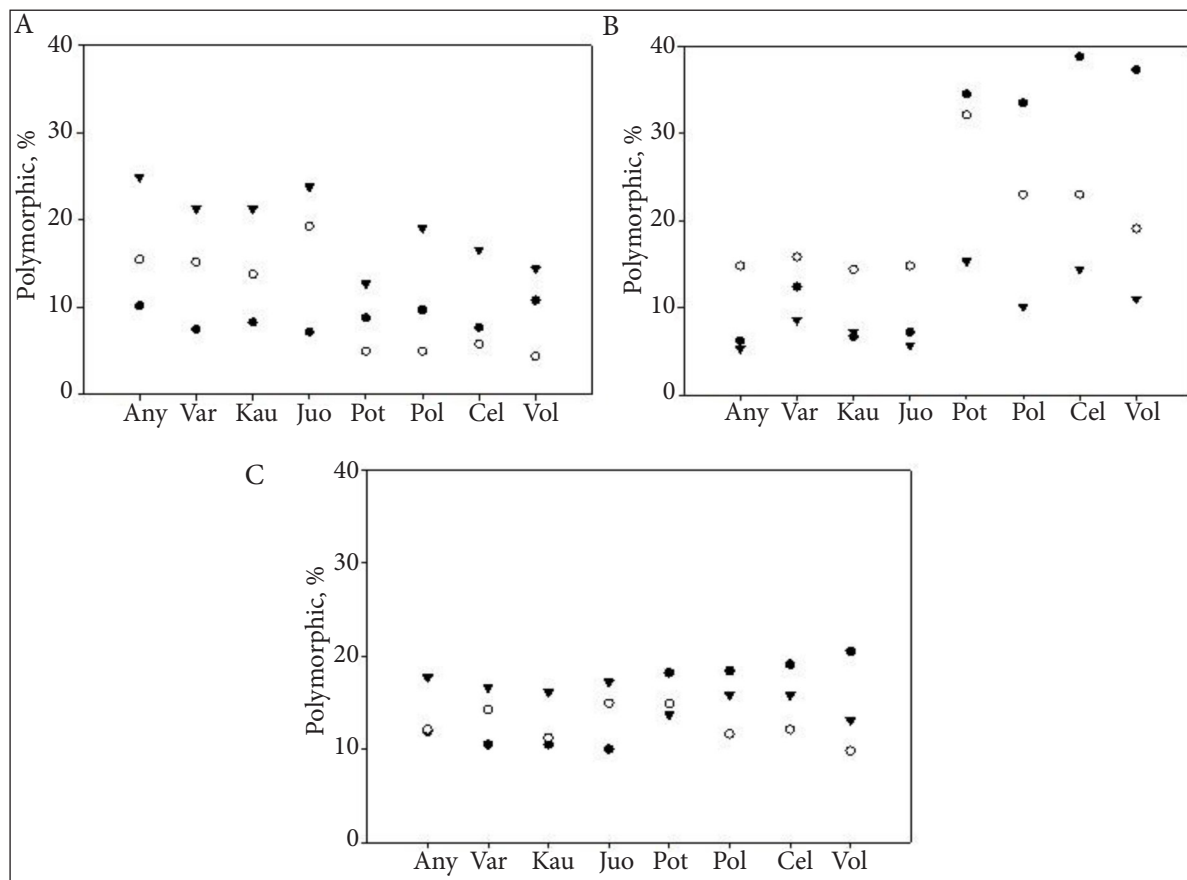
Electrophoresis images were analyzed using the GeneRuler™ 1 kb DNA Ladder Plus (Thermo Scientific, Lithuania) ruler. The presence (1) or absence (0) of DNA fragments of a particular fragment size was determined and a data sheet was composed.

DNA polymorphic loci ( $P\%$ ) were counted and analyzed using Genetic Analysis in Excel (GenALEX) version 6.4. Genetic differentiation ( $G_{ST}$ ), genetic flow between populations ( $N_m$ ), and Nei's genetic distances ( $GD$ ) were calculated with *PopGene*, version 1.32 (Nei, 1978). According to Nei's genetic distances ( $GD$ ), populations were grouped into the clusters using an unweighted pair group method with arithmetic mean (UPGMA).

## RESULTS AND DISCUSSION

The highest polymorphism ( $P\%$ ) for all three *Impatiens* species was documented at ISSR loci compared to RAPD loci or RAPD + ISSR loci (Fig. 1). According to the mean values (for all populations of each species) of this parameter at RAPD loci, the closest were *I. noli-tangere* and *I. parviflora* (mean  $P\% = 13.9\%$  and  $P\% = 17.3\%$ , respectively). The minimum difference of average of polymorphic loci based on ISSR data was between *I. parviflora* and *I. glandulifera* (mean  $P\% = 26.5\%$  and  $P\% = 22.0\%$ , respectively).

The greatest genetic differentiation according to RAPD markers was in *I. parviflora* populations ( $G_{ST} = 0.81$ ) and the lowest in *I. glandulifera* populations ( $G_{ST} = 0.61$ ; Table 1). The results of gene flow between populations were opposite



**Fig. 1.** Percentage of polymorphic loci (P%) of three *Impatiens* species, each represented by eight populations. A – P% at RAPD loci, B – P% at ISSR loci, C – P% at RAPD + ISSR loci, ● – *I. noli-tangere*, ○ – *I. parviflora*, ▼ – *I. glandulifera*. Any, Var, Kau, Juo, Pot, Pol, Cel, Vol – abbreviated titles of populations

**Table 1.** *I. noli-tangere*, *I. parviflora*, and *I. glandulifera* genetic differentiation ( $G_{ST}$ ) and genetic flow between populations ( $N_m$ )

DNA marker type	$G_{ST}$	$N_m$
<i>I. noli-tangere</i>		
RAPD	0.79	0.13
ISSR	0.36	0.89
<i>I. parviflora</i>		
RAPD	0.81	0.12
ISSR	0.68	0.23
<i>I. glandulifera</i>		
RAPD	0.61	0.32
ISSR	0.73	0.18

to genetic differentiation: the lowest value was for *I. parviflora* ( $N_m = 0.12$ ), while the highest for *I. glandulifera* ( $N_m = 0.32$ ). According to

ISSR markers data, the highest genetic differentiation ( $G_{ST} = 0.73$ ) and the lowest gene flow ( $N_m = 0.18$ ) were visible between *I. glandulifera* populations. However, the lowest genetic differentiation was in *I. noli-tangere* populations ( $G_{ST} = 0.36$ ), where gene flow was the highest ( $N_m = 0.89$ ). To sum up these results, *I. noli-tangere* and *I. parviflora* were similar species at RAPD loci, while invasive balsams were similar at the ISSR markers. There is evidence to argue about genetic differentiation importance for the invasion process in rapidly changing conditions (Bossdorf et al., 2005), and this is noticeable in our studies. Both invasive species, *I. parviflora* and *I. glandulifera*, had, respectively, the highest genetic differentiation at RAPD loci and the highest genetic differentiation at ISSR loci. This could cause these species'

**Table 2.** Nei's genetic distances for populations of three *Impatiens* species at RAPD (lower triangle) loci and ISSR loci (upper triangle)

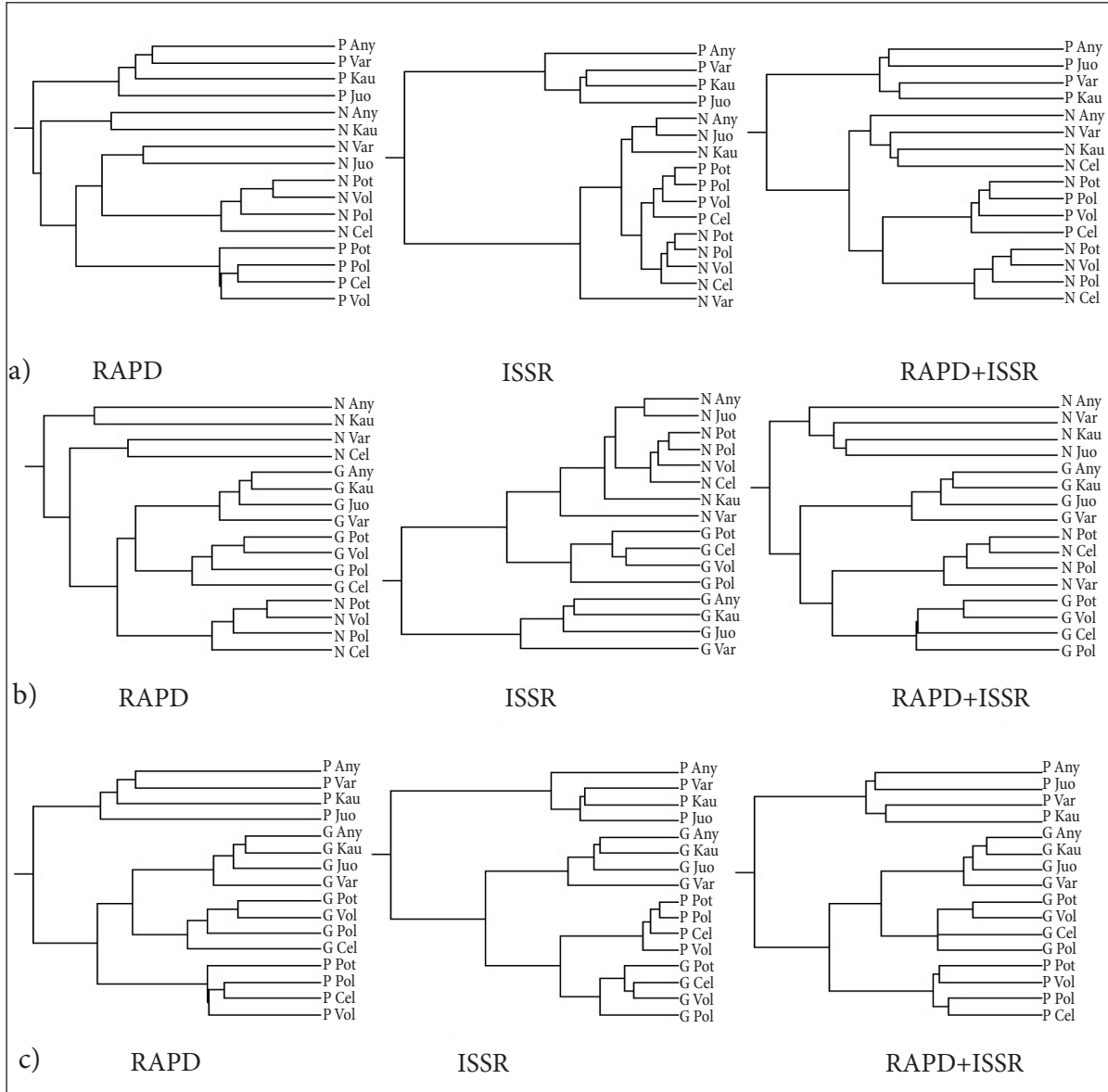
<i>I. noli-tangere</i>	<b>Any</b>	X	0.180	0.116	0.059	0.093	0.107	0.103	0.071
	<b>Var</b>	0.368	X	0.125	0.165	0.144	0.123	0.140	0.115
	<b>Kau</b>	0.295	0.299	X	0.062	0.097	0.110	0.094	0.104
	<b>Juo</b>	0.334	0.250	0.303	X	0.086	0.101	0.079	0.074
	<b>Pot</b>	0.333	0.295	0.350	0.284	X	0.037	0.053	0.054
	<b>Pol</b>	0.378	0.322	0.399	0.322	0.125	X	0.053	0.042
	<b>Cel</b>	0.405	0.328	0.373	0.322	0.123	0.152	X	0.066
	<b>Vol</b>	0.374	0.304	0.378	0.274	0.081	0.117	0.167	X
<i>I. parviflora</i>	<b>Any</b>	X	0.166	0.190	0.157	0.348	0.348	0.338	0.388
	<b>Var</b>	0.254	X	0.126	0.138	0.268	0.286	0.278	0.303
	<b>Kau</b>	0.289	0.269	X	0.125	0.321	0.344	0.318	0.355
	<b>Juo</b>	0.272	0.358	0.283	X	0.314	0.327	0.321	0.358
	<b>Pot</b>	0.362	0.409	0.403	0.451	X	0.031	0.035	0.036
	<b>Pol</b>	0.349	0.379	0.351	0.382	0.131	X	0.056	0.053
	<b>Cel</b>	0.408	0.421	0.393	0.460	0.172	0.129	X	0.072
	<b>Vol</b>	0.410	0.409	0.409	0.398	0.153	0.137	0.163	X
<i>I. glandulifera</i>	<b>Any</b>	X	0.231	0.151	0.154	0.360	0.391	0.326	0.368
	<b>Var</b>	0.162	X	0.176	0.244	0.420	0.438	0.373	0.419
	<b>Kau</b>	0.122	0.148	X	0.171	0.339	0.364	0.309	0.374
	<b>Juo</b>	0.150	0.198	0.132	X	0.463	0.499	0.425	0.445
	<b>Pot</b>	0.291	0.314	0.292	0.316	X	0.140	0.128	0.093
	<b>Pol</b>	0.275	0.309	0.254	0.294	0.159	X	0.133	0.205
	<b>Cel</b>	0.284	0.294	0.291	0.273	0.188	0.207	X	0.092
	<b>Vol</b>	0.309	0.343	0.305	0.298	0.129	0.196	0.226	X
	<b>Any</b>	<b>Var</b>	<b>Kau</b>	<b>Juo</b>	<b>Pot</b>	<b>Pol</b>	<b>Cel</b>	<b>Vol</b>	

successful invasiveness in the studied areas of Europe. According to genetic differentiation, *I. parviflora* was in the intermediate position compared to all three *Impatiens* species.

The pairs of *Impatiens* species were compared according to correlations between population Nei's genetic distances defined for RAPD and ISSR loci. Nei's genetic distances at RAPD loci significantly correlated for *I. noli-tangere* and *I. parviflora* ( $r = 0.79$ ;  $p < 0.05$ ) and for *I. parviflora* and *I. glandulifera* ( $r = 0.76$ ;  $p < 0.05$ ); correlation was not observed in case of *I. noli-tangere* and *I. glandulifera* comparison ( $r = 0.49$ ;  $p > 0.05$ ). Nei's genetic distances at ISSR loci significantly correlated for *I. parviflora* and *I. glandulifera* ( $r = 0.89$ ;  $p < 0.05$ ); correlation was not observed in case of *I. noli-tangere* and *I. parviflora* comparison ( $r = 0.36$ ;  $p > 0.05$ ) and for *I. noli-tangere* and *I. glandulifera* ( $r = 0.38$ ;  $p > 0.05$ ). Relying on the men-

tioned data, the highest correlations were between *I. parviflora* and *I. glandulifera* at ISSR loci. Our data suggested that among three pairs of *Impatiens* species, the paired *I. parviflora* and *I. glandulifera* (invasive species) were significantly the closest to each other according to correlations of Nei's genetic distances defined at ISSR loci.

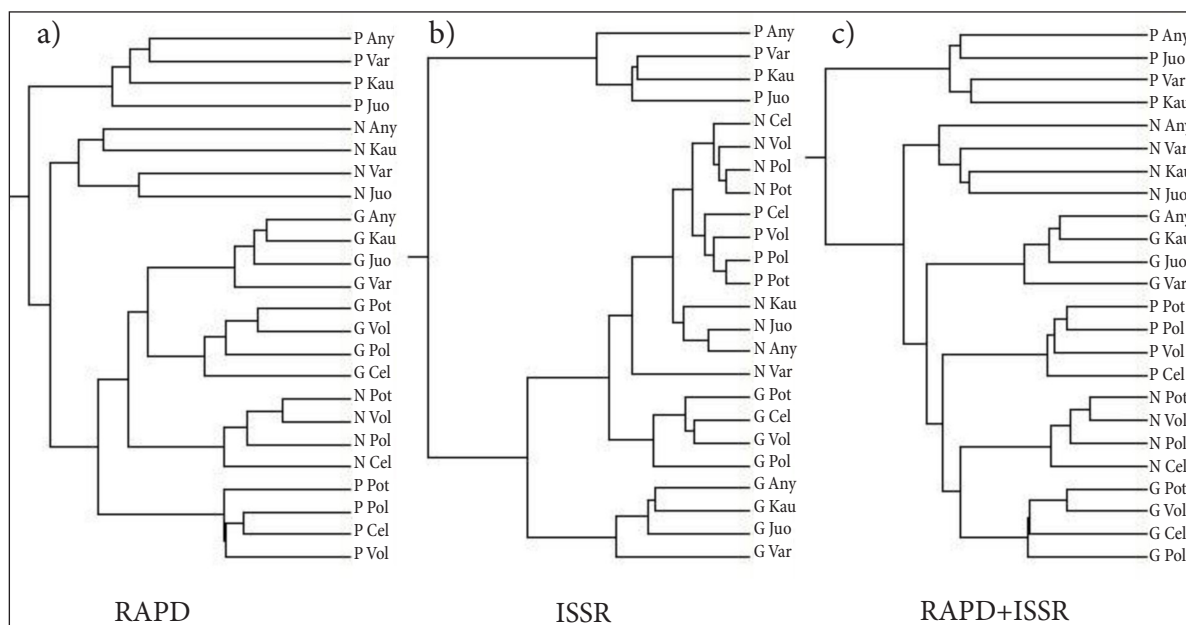
According to the UPGMA dendrogram of genetic relations among populations of *I. noli-tangere* and *I. parviflora* based on RAPD data, the species did not branch into different clades, either species separated similarly based on ISSR markers and RAPD + ISSR markers data (Fig. 2a). *I. noli-tangere* and *I. glandulifera* species population comparison UPGMA dendrograms based on RAPD, ISSR, and RAPD + ISSR data showed that species were clearly detached by the RAPD and ISSR markers, but were not by conjugated markers data (Fig. 2b).



**Fig. 2.** UPGMA dendrograms of genetic relations among populations of *I. noli-tangere*, *I. parviflora*, and *I. glandulifera* (8 populations for each species) based on RAPD, ISSR, and RAPD + ISSR data, using UPGMA algorithm and Nei's genetic distances: a) – dendrograms of *I. parviflora* and *I. noli-tangere*, b) – dendrograms of *I. glandulifera* and *I. noli-tangere*, and c) – dendrograms of *I. parviflora* and *I. glandulifera*. In the dendrograms, the title of each population comprises species name (N – *I. noli-tangere*, P – *I. parviflora*, G – *I. glandulifera*) and abbreviated titles of populations (Any, Var, Kau, Juo, Pot, Pol, Cel, Vol)

Nevertheless, we suggested segregating *I. noli-tangere* and *I. glandulifera* as different species. UPGMA dendrograms based on RAPD, ISSR, and RAPD + ISSR markers revealed *I. parviflora* and *I. glandulifera* populations as distinct groups by RAPD data and by conjugated markers data, while ISSR markers data showed scattered populations (Fig. 2c).

The UPGMA dendrogram of genetic relations among populations of *I. noli-tangere*, *I. parviflora*, and *I. glandulifera* (8 populations for each species) based on RAPD data revealed *I. glandulifera* as the most distinct species, all populations of which formed separate clades, while *I. noli-tangere* and *I. parviflora* were scattered, *I. parviflora* in particular (Fig. 3).



**Fig. 3.** UPGMA dendrograms of genetic relations among populations of *I. noli-tangere*, *I. parviflora*, and *I. glandulifera* (8 populations for each species) based on a) RAPD data, b) ISSR data, c) RAPD and ISSR data, using UPGMA algorithm and Nei's genetic distances. In the dendrograms the title of each population comprises species name (N – *I. noli-tangere*, P – *I. parviflora*, G – *I. glandulifera*) and abbreviated titles of populations (Any, Var, Kau, Juo, Pot, Pol, Cel, Vol)

Similarly, the UPGMA dendrogram based on ISSR data revealed *I. glandulifera* as the most distinct species, the populations of which formed two related clades, and the populations of the other two *Impatiens* species did not behave in the same manner. For separation of *Impatiens* species, the UPGMA dendrogram based on combined RAPD and ISSR data was not helpful at all.

Our dendrograms based on RAPD or ISSR data for three *Impatiens* species showed bigger similarity between *I. noli-tangere* and *I. parviflora*: they did not form separate clades. This was also true when comparing the mean values of polymorphism at RAPD loci of these two species. It is in agreement with a bigger morphological similarity (height of the plant and size of the flowers) between *I. noli-tangere* and *I. parviflora* and indicator values of their habitats (values for each factor ranging between 1 and 9; Ellenberg et al., 1992). *I. noli-tangere* and *I. parviflora* were determined as similar species by several features of their environ-

ment: light demand (4), climate type (5), and nitrogen quantity in soil (6). *I. glandulifera* and *I. parviflora* were characterised as similar only in the requirement for soil reaction (7).

According to correlations between population Nei's genetic distances defined for ISSR loci, *I. parviflora* and *I. glandulifera* were more similar to each other, compared to the other pairs of *Impatiens* species. It is in support to phylogenetic similarity between *I. glandulifera* and *I. parviflora* according to phylogenetic trees of combined ITS and *atpB-rbcL* spacer data (Janssens et al., 2009).

Our study did not show unambiguous results. It might be due to the fact that selected RAPD and ISSR markers triggered *Impatiens* loci responsible for different features and functions. *Balsaminaceae* species, including three *Impatiens* species studied by us, were compared using DNA sequencing (Janssens et al., 2006; Janssens et al., 2009). Relations between three *Impatiens* species differed depending on the scope of loci selected for sequencing. In

the phylogenetic tree based on *atpB-rbcL* spacer data (Janssens et al., 2006), *I. noli-tangere*, *I. parviflora*, and *I. glandulifera* were allocated in parallel clades.

Molecular markers are important to determine invasion pathways and genetic variation of introduced plants (Bossdorf et al., 2005). For further comparisons of three European species of *Impatiens*, extension of population geography and molecular markers is required.

## CONCLUSIONS

1. The present study revealed that *I. noli-tangere* and *I. parviflora* were the most similar species by polymorphic loci ( $P\%$ ), genetic differentiation ( $G_{ST}$ ), UPGMA dendrograms based on RAPD and ISSR data, also based on RAPD data correlation between Nei's genetic distances among pairs of populations of these two species.

2. Among three pairs of *Impatiens* species, the paired *I. parviflora* and *I. glandulifera* (invasive species) were the closest to each other according to significant correlations of Nei's genetic distances defined at ISSR loci.

3. Our data suggested that *I. noli-tangere* and *I. glandulifera* were the most different species by almost all investigated parameters based on RAPD and ISSR markers.

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### **EUROPOS *Impatiens* RŪŠIŲ SKIRTUMAI PAGAL APPD IR PKSİ ŽYMENIS**

#### *Santrauka*

Tyrimų tikslas yra palyginti pagal atsitiktinai pagausintos polimorfinės DNR (APPD) ir paprastųjų kartotinių sekų intarpų (PKSI) žymenis tris Europos *Impatiens* rūšis, kurios surinktos skirtingose geografinėse zonose. Tai aktualu, nes trūksta informacijos, kaip visos trys *Impatiens* rūšys gali būti palyginamos platesnėje geografinėje teritorijoje. Iš viso ištirtos kiekvienos rūšies – *Impatiens noli-tangere*, *I. parviflora* ir *I. glandulifera* – 8 populiacijos iš dviejų šalių (Lietuvos ir Čekijos Respublikos). APPD ir PKSİ metodai pasirinkti atsižvelgiant į duomenų trūkumą vertinant bendras molekulinės *Impatiens* rūšių savybes. Buvo taikomi 8 APPD ir 5 PKSİ žymenis. Lyginant polimorfizmo procento vidurkius, pagal APPD žymenis artimiausios buvo *I. noli-tangere* ir *I. parviflora* rūšys (atitinkamai  $P\% = 13,9\%$

ir  $P\% = 17,3\%$ ), o pagal PKSİ duomenis – *I. parviflora* ir *I. glandulifera* (atitinkamai  $P\% = 26,5\%$  ir  $P\% = 22,0\%$ ). *I. parviflora* pasižymėjo didžiausia genetinė diferenciacija pagal APPD lokusus ( $G_{ST} = 0,81$ ) ir *I. glandulifera* – pagal PKSİ žymenis ( $G_{ST} = 0,73$ ). Atsižvelgiant į genetinės diferenciacijos rezultatus, panašiausios rūšys pagal APPD duomenis yra *I. noli-tangere* ir *I. parviflora*, o pagal PKSİ žymenis – invazinės sprigės rūšys. Genetinių atstumų palyginimas tarp suporuotų rūšių populiacijų rodo reikšmingą koreliaciją tarp *I. noli-tangere* ir *I. parviflora* ( $r = 0,79$ ;  $p < 0,05$ ) bei *I. parviflora* ir *I. glandulifera* ( $r = 0,76$ ;  $p < 0,05$ ) pagal APPD lokusus, taip pat *I. parviflora* ir *I. glandulifera* ( $r = 0,89$ ;  $p < 0,05$ ) pagal PKSİ žymenis. UPGMA dendrogramos atskleidė, kad panašiausios rūšys yra *I. noli-tangere* ir *I. parviflora* pagal APPD duomenis, *I. parviflora* ir *I. glandulifera* pagal PKSİ žymenis, o *I. noli-tangere* ir *I. glandulifera* labiausiai skiriasi pagal minėtus molekulinį žymenų rodiklius.

**Raktažodžiai:** *Balsaminaceae*, invazinės rūšys, genetinė įvairovė, sprigė