Assessment of Genetic Variation among Elite and Wild Germplasm of Blue Honeysuckle (*Lonicera caerulea* L.)

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Abstract

Honeysuckles (*Lonicera* L.) are a frequent element of northern boreal forest undergrowth and a rather new horticultural plant. Random amplified polymorphic DNA (RAPD) analysis was used to assess the genetic variation of 51 accessions of blue honeysuckle (*Lonicera caerulea* L.), including 19 elite cultivars and 32 genetic lines derived from seeds collected in wild populations. Twelve selected primers revealed 149 reproducible RAPD bands, 83.2 % of them being polymorphic. AMOVA and UPGMA analyses have shown that the group of genetic lines derived from wild populations is significantly different from the group of elite cultivars and can be used as a source of additional diversity in honeysuckle breeding programs. RAPD analysis identified RAPD band specific to the *L. caerulea* 4× complex. Cloning and sequencing of a species-specific RAPD marker revealed DNA sequence polymorphism in this locus of *L. caerulea* L., *L. caerulea* L. subsp. *stenantha* (Pojark.) and *L. caerulea* L. subsp. *kamtschatica* (Pojark.) accessions.

Key words: RAPD, *Lonicera caerulea*, forest genetic resources, genetic diversity

Introduction

Forests have a number of natural trees and shrubs that still are of minor interest to forest managers, but could be highly valuable in terms of genetic resources and future use (FAO, DFSC, IPGRI 2002). Honeysuckle (*Lonicera* L.) is a frequent element of forest undergrowth, and represents a group of species that have good potential for future usage. *Lonicera xylosteum* L. is only autochthonous *Lonicera* species in Lithuanian forests. In neighbouring Latvian forests there is one more species, blue honeysuckle (*Lonicera caerulea* L.). This taxonomically complex circum Holarctic polymorphic species belongs to the family *Caprifoliaceae* Juss. section *Isica* Lebed subsection *Caeruleae* Rehd. (Плеханова и Ростова 1994) and is native to northern boreal forests in Asia, Europe and North America. Tetraploid forms (2n=4x=36) of blue honeysuckle occupy most of the species area in northern Eurasia, while diploids (2n=2x=18) occur only in the scattered regions at the southern edge of the species range (Tetsuo et al. 2007). Under conditions of global warming there is possibility for changing of native borders of this species. Several species of *Lonicera* are known as invasive (Miller and Gorchov 2004, Schierenbeck 2004). On the other hand *L. caerulea*, unlike invasive *Lonicera* species, occupies a rather narrow ecological niche (Bors, personal communication). In the latest and comprehensive analysis, Plekhanova and Rostova (1994) consider *L. caerulea* to be one polymorphic 4× complex consisting of eight subspecies (Плеханова и Ростова 1994). Blue honeysuckle is a deciduous shrub; its hermaphrodite, mostly self-incompatible flowers are pollinated by insects. As an important component of forest ecosystems blue honeysuckle provides a food and cover for many wildlife species. Moreover, berries of blue honeysuckle have long been harvested from wild plants in some regions of Russia, China and Japan where superior edible forms are native (Thompson and Chaovanalikit 2003). Blue honeysuckle berries are an excellent source of dietary phytochemicals (phenolic acids, flavonoids, anthocyanins, etc.) and can be used as natural antioxidants and colorants (Plekhanova 2000, Chaovanalikit et al. 2004).

However, this species as a fruit crop is relatively rare in Lithuania and neighbouring countries (Poland, Russia, Latvia and Byelorussia). The commercial breeding of blue honeysuckle was started in the former Soviet Union in the 1950s (Janick and Paull 2008). More than 100 cultivars have been developed in Russia since then (Куклина 2007). Breeding programs from the local genetic material of *Lonicera* sp. have been also

initiated in Japan and China. Selection programmes using the Russian plant material are carried out in Romania, Czech Republic, Latvia, Sweden, Canada and Oregon (USA) (Janick and Paull 2008).

Studies of blue honeysuckle in Lithuania started in 1974 at the Botanical Garden of Vilnius University (Žilinskaitė et al. 2007). The collection of blue honeysuckle of the Vilnius University Botanical Garden contains accessions of four subspecies, 28 cultivars and 35 genetic lines. Plants of genetic lines were previously grown from seeds of wild populations (collected in forests of the Russian Altay region) and selected according to superior fruit flavour, size and productivity. These lines were characterized morphologically; the properties of superior lines of this collection were compared with cultivar-standard properties (Žilinskaitė et al. 2007). However, for a more effective management of this genetic material and its use in germplasm enhancement, for prognosis of possibility for changing of native borders of this species, especially in changing climate conditions, detailed molecular studies are necessary, because morphological traits are known to be influenced by the environment and the processes of domestication and breeding. DNA markers are a useful tool for the studying genetic relationships among cultivars and their wild relatives (Wünsch and Hormaza 2002, Carelli et al. 2006). Despite some progress, there is a lack of information about blue honeysuckle genome sequences (Theis et al. 2008). In this situation, the RAPD technique is most convenient (relatively simple and economical) and effective because it does not require the prior knowledge of primer target sequence and provides a sufficient number of informative markers. RAPD markers are also used in plant taxonomical studies (Shimada et al. 2001, Román et al. 2003, Yamagishi et al. 2005), identification of species-specific markers (Sanchez et al. 1998, Scheepers et al. 2000, Nkongolo et al. 2002, Zhang and Zhou 2009) and marker-assisted selection (Masojć 2002, Francia et al. 2005). We have already shown that the RAPD technique is applicable to genotype *L. caerulea* accessions (Naugžemys et al. 2007). RAPD can be used to develop markers for the identification of *L. caerulea* and its hybrids. Since blue honeysuckle is a taxonomically problematic species, the development of species-specific markers could enable a more efficient utilization of the genetic resources of this species.

Based on the above considerations, the objectives of this study were to use the RAPD technique:

- to determine the genetic diversity and relationships within and among *L. caerulea* accessions (cultivars and the genetic lines raised from wild germplasm);

- to assess the potential of genetic lines in expanding the genetic base of blue honeysuckle breeding material;

- to identify RAPD markers specific to this taxonomically complex polymorphic *L. caerulea* species.

Materials and methods

Plant material: 51 blue honeysuckle accessions including 19 elite cultivars (Table 1) and 32 genetic lines (2R, 3-5, 2-1, 3-79, 3R, 3U, 3-80, 10-32, 1L, 1G16,

Table 1. Cultivars of *Lonicera caerulea* L. used in the study, year of release, catalogue number in – N.I Vavilov All-Russian Scientific Research Institute of Plant Industry and available pedigree information

No.	Year of release	VIR*	Name	Parentage
1	1982	30049	'Narymskaya'	Selected from open pollinated seedlings of cv 'Delfin'
2	1962	25783	'Desertnaya'	Selected from open pollinated seedlings of plants from Kamtschatka
3	1986	32254	'Salyut'	Derived from *L. caerulea* subsp. *altaica*
4	1982	30042	'Sinyaya Ptitca'	Selected from open pollinated seedlings of cv. 'Start'
5	1982	31386	'Lazurnaya'	Selected from open pollinated seedlings of cv. 'Start'
6	1972	25799	'Start'	Selected from open pollinated seedlings of plants from Kamtschatka
7	1992	35900	'Fialka'	Selected from open pollinated seedlings of cv. 'Roksana'
8	1982	30054	'Roksana'	Selected from open pollinated seedlings of plants from Kamtschatka
9	1992	35919	'Morena'	Hybrid of elite forms derived from Kamtschatka and Primorje
10	1982	30041	'Goluboje Vereteno'	Selected from open pollinated seedlings of cv. 'Start'
11	1962	31608	'Kuvshinovidnaya'	Selected F2 seedlings of plants from Petropavlovsk–Kamtschatsky region
12	1982	31385	'Zolushka'	Selected from open pollinated seedlings of plants from Kamtschatka
13	1982	30044	'Bakcharskaya'	Selected from open pollinated seedlings
14	1994	38157	'Viola'	Hybrid of 'Leningradskii Velikan' and 'Sayanskaya-322'
15	1979	29822	'Vasyuganskaya'	Selected from open pollinated seedlings of cv. 'Delfin'
16	1979	29825	'Tomichka'	Selected from open pollinated seedlings of cv. 'Delfin'
17	1979	25793	'Leningradskii Velikan'	Selected from F3 seedlings of wild plant from Petropavlovsk–Kamtschatsky region
18	2000	-	'Volshebnica'	Selected from open pollinated seedlings of cv. 'Smolinskaya'
19	1994	-	'Chernichka'	Selected from open pollinated seedlings of cv. 'Smolinskaya'

*VIR - N.I Vavilov All-Russian Scientific Research Institute of Plant Industry of Russian Academy of Science original catalogue number

1E, 2D, 2C63, 1B43, 2K, 2S, 19, 1N73, 22T12, 1U, 1V, 2E, 1T, 1M, 1P, 96-1, 96-2, 96-3, 96-4, 69-3, 10, 32) were used for RAPD analysis in the first step of the study. The next step was to assess the species-specificity of monomorphic RAPD bands analysing additional accessions of four *L. caerulea* subspecies [*L. caerulea* L. subsp. *kamtschatica* Sevast., *L. caerulea* L. subsp. *stenantha* Pojark., *L. caerulea* L. subsp. *altaica* (Pall.), *L. caerulea* L. subsp. *pallasii* (Ledeb.) Browicz], and 12 *Lonicera* L. species [*L. caerulea* L., *L. alpigena*, *L. vesicaria* Kom., *L. praeflorens* Lam., *Lonicera* x *bella* f. candida Zabel, *L. xylosteum* L., *L. chrysantha* Turcz. ex Ledeb., *L. venulosa* (Maxim.) Worosh., *L. edulis* Turcz. ex Freyn, *L. emphyllocalyx* (Maxim.) Nakai, *L. boczkarnikowae* Plekhanova, *L. orientalis* Lam.] with the same set of primers.

DNA extraction: Total DNA was extracted from fresh young leaves, employing the Genomic DNA purification kit (*Fermentas*, Vilnius, Lithuania). DNA quantity and quality were measured with a spectrophotometer and electrophoretically. Only high-quality DNA was used for the RAPD-PCR analysis.

PCR amplification: Amplifications were carried out in 25 µl of reaction mixtures containing the following components: l× PCR buffer (*Fermentas*, Vilnius, Lithuania), 3.0 mM MgCl2, 0.2 mM dNTPs, 0.4 µM of the primer, 1.0 u of *Taq* DNA polymerase (*Fermentas*, Vilnius, Lithuania) and 20 ng of total DNA. The decameric primers used in this study were purchased from *Karl Roth GmbH & Co. KG* (Karlsruhe, Germany). All amplifications were carried out in *Eppendorf Mastercycler® ep gradient* (*Eppendorf AG*, Hamburg, Germany) programmed for 35 cycles: 4 min at 94 °C initial DNA denaturation step, followed by 35 cycles of 60 s at 94 °C, 60 s at 35 °C, 120 s at 72 °C. The last cycle was followed by the final extension step for 5 min at 72 °C. The reaction mixture without DNA was used as a negative control. Amplification products were fractionated in 1.5 % agarose gel (l× TBE) and visualized with ethidium bromide. RAPD-PCR reproducibility was assessed by comparing at least two reactions. Primers generating RAPD profiles of low reproducibility were excluded from the further analysis.

Cloning and sequencing *L. caerulea* specific RAPD fragments: Three DNA fragments representing the same monomorphic *L. caerulea* 4× complex specific RAPD locus were isolated from genomic DNA amplification spectra visualized in ethidium bromide stained agarose gel of three accessions *L. caerulea* L., *L. caerulea* L. subsp. *kamtschatica* Sevast. and *L. caerulea* L. subsp. *stenantha* Pojark. using *NucleoSpin® Extract II* kit (*Macherey-Nagel GmbH&Co. KG*, Düren, Germany). The isolated DNA fragments were cloned into the pTZ57R/T vector using the *InsTAclone™* PCR Cloning Kit (*Fermentas*, Vilnius, Lithuania). Recombinant clones were selected using lacZ' system on the *FastMedia™ LB Agar Amp IPTG/X-Gal* (*Fermentas*, Vilnius, Lithuania). Plasmid DNA was isolated using *NucleoSpin® Plasmid* kit (*Macherey-Nagel GmbH&Co. KG*, Düren, Germany). The cloned inserts were sequenced at the Sequencing Center of the Institute of Biotechnology (Lithuania) with the *3130xl* Genetic Analyzer (*Applied Biosystems*, Forster City, USA) using *BigDye® Terminator v3.1 Cycle Sequencing* Kit (*Applied Biosystems*, Forster City, USA).

The nucleotide sequence data reported in this article have been submitted to the GenBank nucleotide sequence database (accession no.: GS928137, GS928138 and GS928139).

SCAR design and analysis. A pair of SCAR primers was derived from nucleotide sequence data of cloned species-specific RAPD marker using programme *PrimerSelect* of software package *DNASTAR, Inc.USA,Lasergene®8*. Amplification of genomic DNA with SCAR primers was performed using reaction components identical to those of RAPD-PCR except that two designed primers were used (each 0.2 µM of final concentration) and PCR protocol was changed. The PCR was carried out for 25 cycles composed of a 94 °C denaturation step for 1 min, a 56 °C annealing step for 1 min, and a 72 °C extension step for 2 min. The final extension step was carried out at 72 °C for 5 min. The SCAR bands were analyzed on 1 % agarose gel in 1.0× TBE buffer.

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 Nei and Li (1979) genetic distance (GDxy) estimates. The GDxy matrices were computed from polymorphic RAPD loci. Cluster analysis based on the genetic distance matrix was performed by the unweighted pair-group method of arithmetic averages (UPGMA) of the TREECON for Windows computer programme (Van de Peer and De Wachter 1994). The confidence of dendrogram branches was determined by the bootstrap analysis using 1000 replications. The analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was used to estimate the variation within and among cultivars and genetic lines. AMOVA was performed using *GenAlEx v.6.3* software (Peakall and Smouse 2006).

Sequences data of cloned fragments were evaluated with *ChromasPro v.1.5* (*Technelysium*, Queensland, Australia) and *MEGA 4.1*. (Kumar et al. 2008). A homology search was carried out with the *BLAST* (Altschul et al. 1990) at the *NCBI* database.

Results

A total of 50 primers had been initially tested to select the ones producing reliable RAPD profiles. Twelve selected primers generated distinct, easily scorable DNA bands. These RAPD primers generated 149 DNA bands suitable for analysis in the 51 accessions studied (Table 2). 124 (83.2 %) bands were polymorphic. The number of amplified bands ranged from 10 to 14 per primer. The size of bands used for genotyping and diversity analysis was in the range 270-2500 bp.

Table 2. DNA polymorphism established with informative primers used for the RAPD-PCR analysis of cultivars and genetic lines of *Lonicera caerulea* L.

Primer code	Primer sequence 5'→3'	Number of scored bands	Polymorphic bands	Polymorphic bands [%]	Size range of DNA bands [bp]
170-08	CTGTACCCCC	14	13	92.8	490 – 2100
170-10	CAGACACGGC	14	14	100	470 – 1800
380-01	ACGCGCCAGG	12	9	75	490 – 1700
380-02	ACTCGGCCCC	10	10	100	510 – 1800
380-07	GGCAAGCGGG	14	12	85.7	560 – 1960
A-01	CAGGCCCTTC	11	11	100	700 – 2300
A-02	TGCCGAGCTG	11	6	54.5	570 – 2500
A-03	AGTCAGCCAC	10	6	60	680 – 2480
A-04	AATCGGGCTG	12	7	58.3	560 – 1900
A-05	AGGGGTCTTG	13	12	92.3	490 – 2100
A-09	GGGTAACGCC	14	13	92.8	270 – 1180
A-11	CAATCGCCGT	14	11	78.5	370 – 1400
Total:		149	124	83.2	270 –2500

The pairwise GDxy values among the 51 accessions studied ranged from 0.054 to 0.479, the mean GDxy being 0.283 ± 0.069 (data not shown). When cultivars and genetic lines were analyzed separately, the GDxy among the cultivars ranged within 0.098-0.453 and among the genetic lines 0.055-0.443. The UPGMA dendrogram based on GDxy estimates derived from RAPD analysis shows genetic relationships among the accessions studied (Fig. 1). The UPGMA analysis grouped all accessions into two main clusters. The first cluster includes all cultivars and seven genetic lines (32, 69-3, 10, 96-2, 96-3, 96-1, 96-4), and the second cluster only includes genetic lines. To assess the informativeness of this cluster analysis, we compared the genetic relationships of cultivars based on RAPD data presented in the GDxy matrix (data not shown) and the dendrogram (Fig. 1) with the pedigree information on the study cultivars available from published pedigree records (Table 1) (Куклина 2007). The subcluster Ia of cluster I contains 14 cultivars, some of which are closely related according to pedigree data. First of all, this concerns the cultivar 'Start' and its descendents. Three cultivars – 'Goluboje Vereteno', 'Lazurnaya' and 'Sinyaya Ptitca' – were derived from this cultivar at the Lisovenko Research Institute of

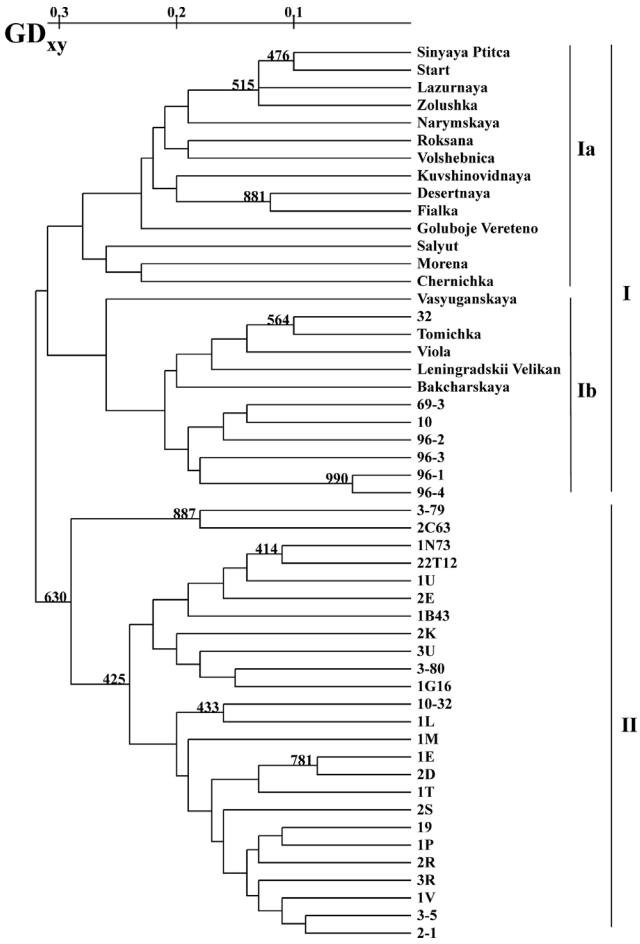

Figure 1. UPGMA dendrogram of *Lonicera caerulea* L. cultivars and genetic lines based on RAPD data and generated using Nei and Li (1979) genetic distance (GDxy) matrix. Internal branching probabilities were determined by bootstrap analysis using 1000 replications

Horticulture Russian Academy of Agricultural Sciences. All these genotypes clustered closely in the dendrogram. The genetic relationships, according to molecular data, are especially close among 'Start' and two cultivars – 'Sinyaya Ptitca' (GDxy = 0.098) and 'Lazurnaya' (GDxy = 0.143). Two closely related cultivars – 'Fialka' and 'Roksana' – are also in the same cluster (GDxy = 0.258). 'Fialka' was selected from open pollinated seedlings of 'Roksana'. The second subcluster (Ib) comprises 12 accessions, among them five elite cultivars and seven genetic lines. The UPGMA dendrogram indicates close genetic relationships between the cultivars 'Viola' and 'Leningradskii Velikan' (GDxy = 0.168). 'Viola' is one of the few among the study cultivars that were bred using controlled cross. 'Viola' is a hybrid of 'Leningradskii Velikan' and 'Sajanskaya-322'. Two other related cultivars – 'Vasyuganskaya' and 'Tomichka' (GDxy = 0.226) – are also grouped in this same subcluster.

The AMOVA applied in studying blue honeysuckle showed that although most of variation is allocated in the genetic material of accessions within cultivars and genetic lines (81 %), these two groups are rather different (Φ_{PT} = 0.189, P ≤ 0.001) (Table 3).

Table 3. Summary of AMOVA between and within groups of blue honeysuckle (*Lonicera caerulea* L.) cultivars and genetic lines

Source of variation	DF	Square sum	Percentage variation	Φ_{PT}
Between groups	1	109.183	19.0	0.189*
Within groups	49	815.327	81.0	
Total	50	924.510	100	

* – P ≤ 0,001; DF – degrees of freedom

The RAPD analysis of blue honeysuckle cultivars and lines revealed also 25 monomorphic RAPD bands in the 51 accessions studied (Table 2). Such uniformity of some bands in the accessions implies that some of them may be species-specific. To evaluate this possibility, we included in the RAPD analysis additional accessions of four *L. caerulea* L. subspecies and twelve *Lonicera* L. species and analyzed them with the same decamer primers. The RAPD analysis of *L. caerulea* L. subspecies and *Lonicera* L. species showed a higher level of the DNA polymorphism (Table 4) than in the previous case (Table 2) when cultivars and genetic lines were studied. Of the 149 bands scored, 96.6 % were polymorphic. The average RAPD-based genetic distance among the study accessions of four *L. caerulea* subspecies and twelve *Lonicera* L. species was 0.453 ± 0.148 and ranged from a minimum of 0.188 to a maximum of 0.708. When the RAPD profiles of all accessions were compared, we identified one RAPD band (about 570 bp) that was specific to all accessions (including subspecies) belonging to the *L. caerulea* 4× complex (Плеханова и Ростова 1994) (Fig. 2). This species-specific band ($A02_{570}$) was generated using the Roth A-02 primer. This marker ($A02_{570}$) was studied in more detail. Three different $A02_{570}$ bands were isolated from the agarose gel and cloned in the pTZ57R/T vector. These three bands ($A02_{570(1)}$, $A02_{570(2)}$ and $A02_{570(3)}$) of the same RAPD locus were cloned from *L. caerulea* L., *L. caerulea* L. subsp. *kamtschatica* (Pojark.) and *L. caerulea* L. subsp. *stenantha* (Pojark.) RAPD profiles, respectively. DNA sequencing showed all these fragments to be identical in size (568 bp), but a certain divergence in nucleotide sequence (less than 3.2 %) was also established (Fig. 3). A subsequent BLAST search in the GenBank did not identify any sequence homology between this 568 bp DNA fragment and the other known plant sequences.

Table 4. DNA polymorphism established with informative primers used for the RAPD-PCR analysis of twelve *Lonicera* L. species and four *L. caerulea* L. subspecies

Primer code	Primer sequence 5'⇒3'	Number of scored bands	Polymorphic bands	Polymorphic bands [%]	Size range of DNA bands [bp]
170-08	CTGTACCCCC	14	14	100	490 – 2100
170-10	CAGACACGGC	14	13	92.8	470 – 1800
380-01	ACGCGCCAGG	12	12	100	490 – 1700
380-02	ACTCGGCCCC	10	10	100	510 – 1800
380-07	GGCAAGCGGG	14	14	100	560 – 1960
A-01	CAGGCCCTTC	11	10	90.9	700 – 2300
A-02	TGCCGAGCTG	11	11	100	570 – 2500
A-03	AGTCAGCCAC	10	10	100	680 – 2480
A-04	AATCGGGCTG	12	11	91.7	560 – 1900
A-05	AGGGGTCTTG	13	13	100	490 – 2100
A-09	GGGTAACGCC	14	12	85.7	270 – 1180
A-11	CAATCGCCGT	14	14	100	370 – 1400
	Total:	149	144	96.6	270 – 2500

On the basis of $A02_{570}$ DNA sequence, we derived new primers – $A02_{570D}$ (5'ATGTTGGGCCTGTCTGATGTAG3') and $A02_{570R}$ (5'TGCCGAGCTGTTGGATGA3') (Fig. 3) – which were used to obtain a sequence-characterized amplified region (SCAR) marker. However, conversion of the RAPD marker to SCAR changed the original polymorphism. The amplified fragment is present in all the genotypes (data not shown).

Discussions and conclusions

The level of DNA polymorphism in this study was sufficient to genotype all the 51 accessions (Fig. 1). The genetic relationships among studied accessions, according to the RAPD analysis, are consistent with the known pedigree information and suggest that RAPD markers identified in our study are suitable for assessing genetic relationships among cultivars. Similar results showing a correlation between molecular and genealogical data have been reported by different authors (Doldi et al. 1997, Кудрявцев и др. 2003, Raddová et al. 2003). Based on these data, we can suppose that the UPGMA dendrogram presented here displays rather accurately genetic relationships among elite cultivars and genetic lines of *L. caerulea* L. The genetic lines included in our study were selected from seedlings of blue honeysuckle seeds collected from wild populations of the Russian Altay region. Most of these accessions form a second cluster with 63% bootstrap support. Only a small number of genetic lines were grouped together with elite cultivars. These results imply the genetic uniqueness of genetic lines as breeding material.

Some authors have used AMOVA to assess the level of genetic variation within and among different (morphologically, genetically, geographically) groups of germplasm accessions (Jakse et al. 2004, Carelli et

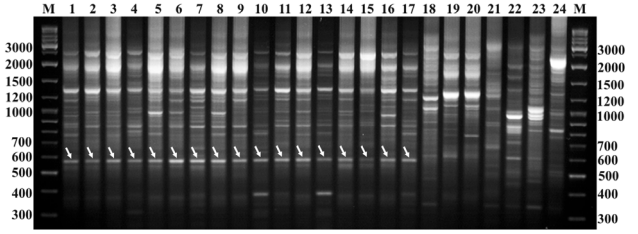

Figure 2. *Lonicera caerulea* L. 4× complex species-specific RAPD marker resulting from amplification of genomic DNA of *Lonicera* L. with Roth A02 primer. Arrows indicate species-specific RAPD band. Line 1 – *Lonicera caerulea* L. subsp. *kamtschatica* (Pojark.), 2 – *Lonicera caerulea* L. subsp. *stenantha* (Pojark.), 3 – *Lonicera caerulea* L. subsp. *pallasii* (Ledeb.), 4 – *Lonicera caerulea* L. subsp. *altaica* (Pall.), 5 – 'Desertnaya', 6 – 'Salyut', 7 – 'Sinyaya Ptitca', 8 – 'Fialka', 9 – 'Morena', 10 – 'Viola', 11 – 'Vasyuganskaya', 12 – 'Tomichka', 13 – 'Leningradskii Velikan', 14 – *Lonicera caerulea* L., 15 – *Lonicera edulis* Turcz. ex Freyn, 16 – *Lonicera boczkarnikowae* Plekhanova, 17 – *Lonicera emphyllocalyx* (Maxim.) Nakai, 18 – *Lonicera chrysantha* Turcz. ex Ledeb., 19 – *Lonicera xylosteum* L., 20 – *Lonicera orientalis* Lam., 21 – *Lonicera vesicaria* Kom., 22 – *Lonicera alpigena* L., 23 – *Lonicera* x *bella* f. candida Zabel, 24 – *Lonicera praeflorens* Batalin, M – molecular size marker (100 bp DNA Ladder, *Fermentas*, Vilnius, Lithuania)

al. 2006, Soleimani et al. 2007). This technique is frequently applied in studying population genetics when dominant molecular markers (RAPD, AFLP, ISSR) are used (Nybom 2004; Patamsytė et al. 2010). AMOVA also showed rather large differentiation (Φ_{PT} = 0.189, P ≤ 0.001) between elite cultivars and genetic lines (Table 3). These results support the UPGMA analysis and indicate that the genetic lines derived in the Botanical Garden of Vilnius University can be used as an additional source of genetic variation in the breeding process of blue honeysuckle. Significant differences between cultivated and wild germplasm or between cultivars and landraces, revealed by the AMOVA and UPGMA analyses, have been reported by other authors who used different types of markers to analyse genetic diversity (Sonnante et al. 2002, Jakse et al. 2004, Carelli et al. 2006).

The taxonomy of blue honeysuckle is rather complex. The first world taxonomic treatment of *L. caerulea* was carried out by Rehder in 1903. He included all ecogeographic races into one polymorphic species, divided into eight subspecies and eight forms. Later other researchers have assigned some or all of the described taxa to the species level. For example, Pojarkova in the Flora of the USSR (1999) described ten species (Janick and Paull 2008). According to Plekhanova and Rostova (1994), the *L. caerulea* 4× complex includes *L. stenantha* Pojark., *L. pallasii* Ledeb., *L. altaica* Pall., *L. kamtschatica* (Sevast.) Pojark., *L. emphyllocalyx* Maxim., *L. edulis* Turcz. ex Freyn. (tetraploid forms), among others. As shown in *Fig.* 2 $A02_{570}$ marker is present in the RAPD profiles of all studied cultivars and genetic lines as well in all studied subspecies. This result suggests that the RAPD marker could be species-specific. This marker was not detected in the RAPD profiles of *L. chrysantha* Turcz. ex Ledeb., *L. xylosteum* L., *L. orientalis* Lam., *L. vesicaria* Kom., *L. alpigena* L., *Lonicera* x *bella* f. candida Zabel, *L. praeflorens* Batalin. The cloning and sequencing of this DNA fragment from taxonomically different accessions of *L. caerulea* L., *L. caerulea* L. subsp. *stenantha* (Pojark.) and *L. caerulea* L. subsp. *kamtschatica* (Pojark.) revealed about 3.2 % DNA polymorphism. No homologous DNA sequence was found after BLAST search in the GenBank. It could mean that cloned DNA fragment represents the non-coding region of the *L. caerulea* L. genome. The difference among the sequences can be explained by genetic divergence of the non-coding sequence of the studied genotypes. A higher level of divergence (about 9 %) was established between two monomorphic DNA fragments of different *Vitis* spp. cultivars (Böhm and Zyprian 1998). RAPD markers specific to one or few species were identified by Nkongolo et al. (2002) in studies of eight pine species (*Pinus sylvestris*, *P. strobus*, *P. rigida*, *P. resinosa*, *P. nigra*, *P. contorta*, *P. monticola*, *P. banksiana*). This study demonstrated high sequence similarity between amplified sequences of the same molecular weight showed by several species. We did not find any similarity of the cloned RAPD band ($A02_{570}$) with plant mobile elements. In this aspect, our result is concordant with data reported by Bodénès et al. (1997) and Lu et al. (1997) who found that the majority of RAPD fragments in their study were not generated from repetitive sequences.

The conversion of species-specific RAPD band into SCAR marker was not informative, a monomorphic band was observed in all of studied genotypes (data not shown). This means that the species-specific polymorphism of the $A02_{570}$ marker was probably caused by a mismatch on the one RAPD primer A02 binding site at the 5' position. Bodénès et al. (1997) have noted that RAPD polymorphism is caused mainly by point mutations in the primer binding sequence. These authors also indicate that in about 50 % of SCARs, PCR amplification resulted in the loss of the original RAPD polymorphism. In this situation only RAPD marker $A02_{570}$ can be used for the molecular identification of blue honeysuckle. The size of this marker falls into the range of size of RAPD bands that have been qualified by various authors as the most reproducible (Fahima et al. 1994; Badenes et al. 2004).

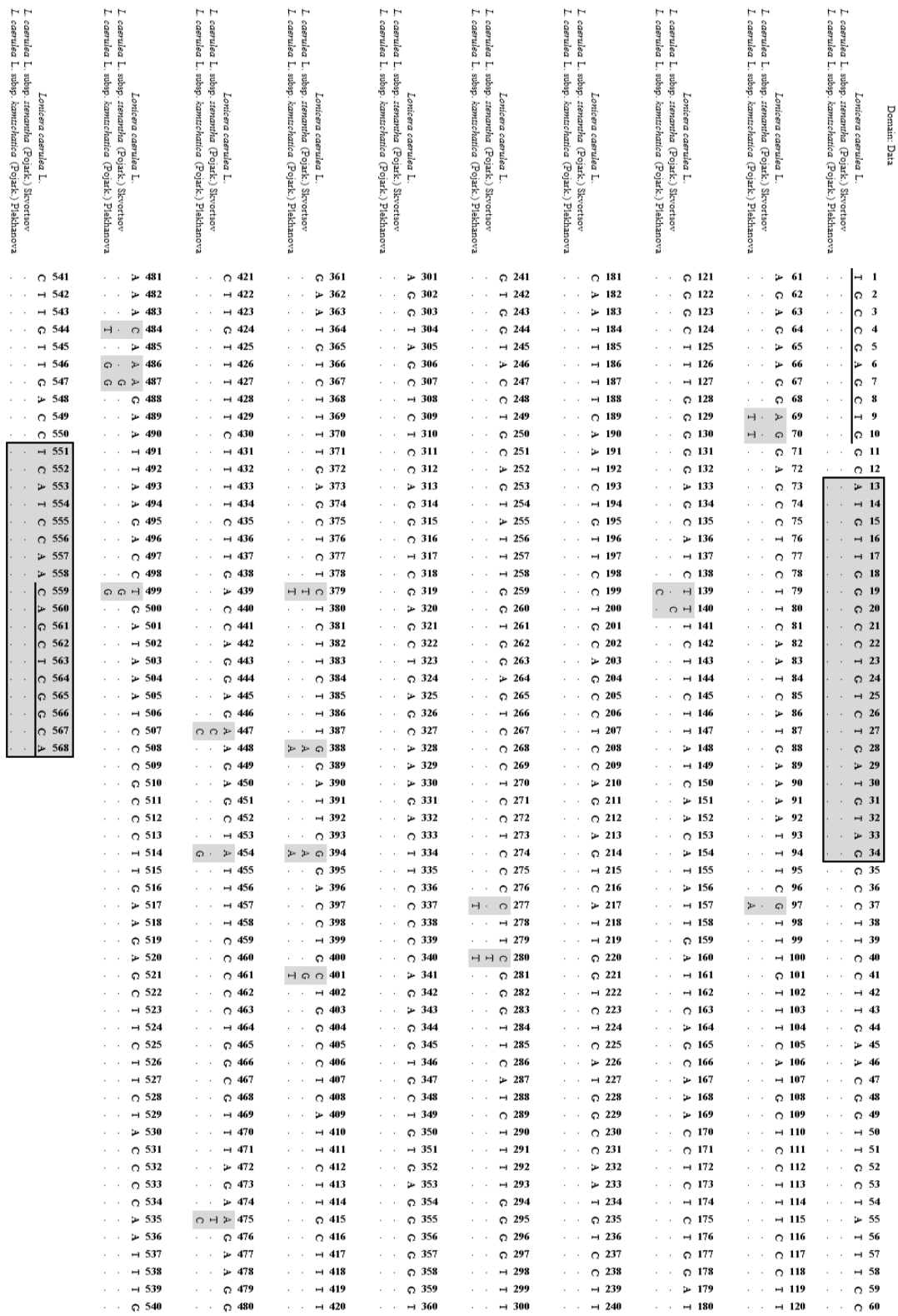

Figure 3. Sequence of cloned *Lonicera caerulea* L. 4 complex species-specific RAPD marker $A02_{570}$ from accessions of *L. caerulea* L., *L. caerulea* L. subsp. *stenantha* (Pojark.) and *L. caerulea* L. subsp. *kamtschatica* (Pojark.) Plekhanova. The location and sequence of A02 primer is underlined. The location and sequence of SCAR forward primer and the location and sequence complementary to a reverse primer are shown in gray boxes

In conclusion, our study has demonstrated a rather high level of DNA polymorphism of studied blue honeysuckle accessions – a property that could be important for the adaptation to global warming and climate change. It was also shown that the RAPD method is suitable to identify genetic relationships among *L. caerulea* L. accessions. The UPGMA dendrogram based on RAPD data showed a rather good concordance with the available pedigree information. The UPGMA and the AMOVA have revealed that the genetic lines derived from wild germplasm significantly differ ($\Phi_{PT} = 0.189$; $P \leq 0.001$) from elite cultivars and can be used as a source of genetic diversity in the blue honeysuckle breeding programme. These results suggest that *L. caerulea* L. as a new crop species retains a wide pool of genetic variation in its wild populations.

In our study, we identified a RAPD marker specific to the *L. caerulea* 4× complex. The sequencing of the cloned $A02_{570}$ species-specific marker and development of a SCAR marker on the basis of the established sequence has shown that RAPD polymorphism in the species-specific locus studied may be caused by a mutation in the primer binding sequence. The identified species-specific RAPD marker can be used for the identification of *L. caerulea* and its hybrids in the breeding process.

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ОПРЕДЕЛЕНИЕ ГЕНЕТИЧЕСКОГО РАЗНООБРАЗИЯ В ЭЛИТНЫХ СОРТАХ И ГЕНЕТИЧЕСКИХ ЛИНИЯХ ПРИРОДНОГО ПРОИСХОЖДЕНИЯ СИНЕЙ ЖИМОЛОСТИ (*LONICERA CAERULEA* L.)

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Резюме

Голубая жимолость (*Lonicera caerulea* L.) является важным компонентом подлеска таёжных и других северных бореальных лесов. Селекция этого вида начата сравнительно недавно. В данном исследовании был изучен полиморфизм в 19 элитных сортах голубой жимолости и в 32 генетических линиях, выведенных в Ботаническом саду Вильнюсского университета из семян дикорастущих популяций Алтайского края Российской Федерации. Установлен высокий уровень полиморфизма ДНК (83,2%). Методами AMOVA и UPGMA было показано, что генетические линии существенно отличаются от элитных сортов жимолости и могут быть использованы как потенциальный источник генетической изменчивости в процессе селекции. Был установлен RAPD маркер, специфический по отношению к тетраплоидному циркумголарктическому полиморфному виду *L. caerulea*. Клонирование и секвенирование этого маркера (568 п.н.) выявило полиморфизм (3,2%) на уровне ДНК последовательности в даном локусе у *L. caerulea* L., *L. caerulea* L. subsp. *stenantha* (Pojark.) и *L. caerulea* L. subsp. *kamtschatica* (Pojark.).

Ключевые слова: RAPD, *Lonicera caerulea*, генетические ресурсы, генетическое разнообразие