UDK 582.912.46: 577.213.3/575.22

ASSESSMENT OF GENETIC DIVERGENCE OF LILAC (*SYRINGA* L.) VARIETIES OF BELARUSIAN SELECTION BASED ON INTEGRATED APPLICATION OF RAPD- AND ISSR-MARKERS

Spiridovich E.V., Vlasova A.B., Yukhimuk A.N., Reshetnikov V.N.

Central Botanical Gardens of the National Academy of Sciences of Belarus, Minsk, Belarus

Introduction

Creation of lilac collection in Central Botanical Garden of NAS of Belarus (CBG) joins the work of several generations of botanists-introducers and breeders: N.V. Smolski, V.F. Bibikova, E.A. Buraya, G.I. Matusevich, N.V. Makedonskaya [13]. For lilac culture employees of Central Botanical Garden in 70-80s widely used a variety of breeding and genetic techniques, such as selection of seedlings obtained after random pollination and hybridization. For hybrid seedlings lilac cultivars were crossed as well as species. By the intercrosses attempts to improve some economical and decorative features in the introduced lilac species were made. On the base of experimental studies Veronica Feodorovna Bibikova obtained lilac cultivars with large simple and double flowers of pure colours, abundant and long blooming 'Lebedushka', 'Nesterka', 'Pavlinka', 'Minchanka', 'Zashchitnikam Bresta', 'Vera Khoruzhaya' 'Pamyati A.T. Smolskoy', 'Uspeh', 'Konstantin Zaslonov', 'Lunnyi Svet', 'Zorka Venera', 'Partisanka', 'Khoroshee Nastroenie', 'Marat Kazei', 'Svityazanka', 'Belaruskie Zori', 'Polesskaya Legenda' [1]. Based on the huge experimental work with the original cultivars of French breeding, with limited contact with breeders of lilac in other countries, minimum access to the main sources of information on lilac collection of Belarusian cultivars was created, and, like the collection of cultivars by Leonid Kolesnikov, it is an unique line of lilac evolution [13].

Along with traditional methods of plant genetic diversity conservation *ex situ*, application of plant cell biotechnology that ensure preservation of valuable breeding samples of previous years, accelerated acquisition and propagation of new forms and lines of ornamental crops with improved traits of stress-resistance and increased productivity is becoming increasingly important in living botanical collections [17]. Regenerative potential of primary meristems most fully realized in micropropagation. This is especially important for propagation of plant genotypes and decorative forms with valuable characteristics that are difficult to maintain during the seed reproduction. In Central Botanical Garden of NAS of Belarus regenerated lilac plants of Belarusian breeding were obtained by methods of biotechnology [15], great assistance was provided by the employees of N.V. Tsitsin`s Botanical Garden RAS [3]. At present, *in vitro* collection of the genus *Syringa* in CBG NASB is presented by 67 varieties; two more species, several cultivars of L.A. Kolesnikov selection and new varieties of American selection are at the stage of sterile culture preparing. An important objective of this work is *in vitro* introduction of all cultivars bred in CBG as the most valuable objects of genetic diversity and national heritage.

A necessary step in creating, preserving and maintaining *in vitro* collections, as well as in the exchange of material between the institutions is harmonization of the rules for keeping collections and development of screening techniques on the base of molecular genetic markers. The last is of great practical importance for collections` certification, assess of their genetic diversity, forming core collections, identification and selection of the most valuable taxa for further breeding. [6]

Systematization and documentation of lilac collection in CBG NASB started in 2002 during the creation of an integrated database of lilac collection, including morphological characteristics of genotypes and the first data on their genetic certification [7, 8]. Nowadays information retrieval database of Belarusian *in vitro* collections is supplemented with molecular genetics passports of the objects providing efficient storage and processing of information, research, enhance cooperation and information exchange in order to preserve biodiversity. [2]

An integrated approach multilocus DNA markers, when some marker systems are used at the same time, is widely used for plant genotyping and it gives the opportunity to differentiate both interspecific and intraspecific genotypes, resolve the problems of cultivars origin and affinity, provide valuable information for further breeding with minimum time and costs [14, 16]. Literature presents a small number of studies on tmolecular marking of *S. vulgaris* cultivars basically involving marker systems and genotypes other than studied in this work [2, 10].

This article presents the results of molecular genotyping of lilac cultivars of Belarusian breeding in the collection of CBG NASB based on polymorphism RAPD- and ISSR-loci identification. This study is aimed for development of effective systems of genetic markers (RAPD and ISSR) for the genus *Syringa*, followed by differentiation and certification of lilac cultivars and creation of a genetic passport for each genotype. Such passports give the possibility not only to distinguish samples from each other, but also to determine the degree of genetic similarity between them, identify the most unique genotypes [5, 11, 12], select the original material for breeding, monitor genetic purity and uniformity of varieties in creating *in vitro* collections and multiplication of plant material.

Materials and methods

The study included 13 lilac varieties of Belarusian selection from the collection of CBG NASB. Data of studied varieties origin are shown below (Table. 1).

Table 1

Lilac varieties of Belarusian selection from the collection of CBG NASB

	Name (Transliteration; variants)	Parent forms	Species identification
	Lebedushka	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Pavlinka	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Zashchitnikam Bresta (For Defenders of Brest)	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Minchanka	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Vera Khoruzhaya	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Khoroshee Nastroenie (Good Mood)	Mme Abel Chatenay	*Syringa vulgaris* L.
		Reaumur	*Syringa vulgaris* L.
	Lunnyi Svet (Moonlight)	Mme Abel Chatenay	*Syringa vulgaris* L.

		Reaumur	*Syringa vulgaris* L.
	Polesskaya Legenda (Woodland Legend)	Ludwig Shpaeth	*Syringa vulgaris* L.
		Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
	Pamyati A.T. Smolskoy	Ludwig Shpaeth	*Syringa vulgaris* L.
		Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
0	Partisanka	Ludwig Shpaeth	*Syringa vulgaris* L.
		Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
1	Konstantin Zaslonov	Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
		Reaumur	*Syringa vulgaris* L.
2	Zorka Venera	Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
		Reaumur	*Syringa vulgaris* L.
3	Svityazanka	Hyacinthiflora	*Syringa* × *hyacinthiflora* Rehder (*Syringa vulgaris* L. × *Syringa oblata* Lindl.)
		Reaumur	*Syringa vulgaris* L.

Genomic DNA preparations were prepared from silica dehydrated leaf tissue using CTAB-method with modifications [5, 9]. Quality and concentration of the DNA preparations were measured spectrophotometrically. Multilocus DNA fingerprinting was performed using RAPD- and ISSR-PCR techniques. After a preliminary screening for PCR it was selected five of the most informative primers, i.e. effectively identify genetic variability at intraspecific level among all genotypes: three RAPD (OPA-18, OPE-02, OPP-09) and two ISSR (UBC-808, UBC-862). PCR was performed in 25μl reaction mixture SureCycler device 8800 (Agilent). Fragment analysis and visualization of the amplification products was performed by capillary electrophoresis instrument Bioalalyzer 2100 (Agilent) (Fig. 1). Calculation of genetic distances, UPGMA clustering and construction of phylogenetic trees were performed using the software Treecon ©.

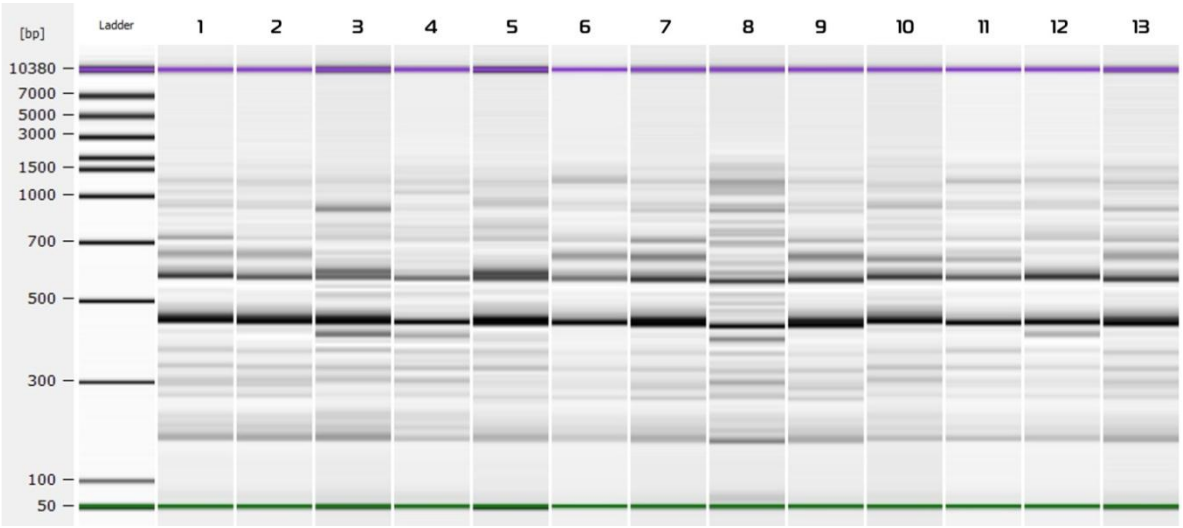


Fig. 1 Representative electrophoretic separation of the amplification products of genomic DNA of 13 lilac varieties with primer UBC-862

1 - 'Lebedushka' 2 - 'Zashchitnikam Bresta' 3 - 'Pavlinka' 4 - 'Minchanka', 5 - 'Zorka Venera', 6 - 'Partisanka' 7 - "Pamyati A.T. Smolskoy" 8 - 'Polesskaya Legenda' 9 -'Vera Khoruzhaya' 10 - 'Lunnyi Svet'11 - 'Konstantin Zaslonov' 12 - 'Svityazanka' 13 -'Khoroshee Nastroenie"; Ladder - molecular weight marker.

Results and discussion

To investigate genetic differentiation of lilac cultivars of Belarusian selection complex method of multilocus DNA fingerprinting based on two PCR techniques: RAPD and ISSR was used. Pre-selected primers (RAPD: OPA-18, OPE-02, OPP-09; and ISSR: UBC-808, UBC-862) generated distinct and reproducible amplicons, which set is unique for each of the investigated cultivars. Table 2 shows the spectra of the amplicons obtained using RAPD and ISSR primers. Informativeness of the primers used varied. Thus, maximum number of loci - 23 (including 15 polymorphic) was identified using primer UBC-808 min - 11 (including four polymorphic) generated with primer OPP-09 (see Table 2). In total, 76 loci markers were identified - 40 RAPD- and 36 ISSR-markers, respectively. In this pool 44 markers were polymorphic. Both PCR techniques revealed high level of polymorphism in the studied lilac cultivars - an average of 57.89%. Maximum polymorphism was detected using primer OPA-18 (66.67%), the lowest - 36.36% with the amplification primer OPP-09.

Table 2

Characteristics of Syringa spp. Amplicons spectra, generated by RAPD- and ISSR-primers

Primer	Nucleotide sequence, 5′→3′	Number of amplicorns		Polymorphism degree, %
		Total	Polymorphic	
RAPD-primers				
OPA-18	AGGTGACCGT	12	8	66.67
OPE-02	GGTGCGGGAA	17	10	58.82
OPP-09	GTGGTCCGCA	11	4	36.36
ISSR-primers				
UBC-808	AGAGAGAGAGAGAGAGC	23	15	65.22
UBC-862	AGCAGCAGCAGCAGCAG C	13	7	53.85
Total number:		76	44	—
An average meaning:		15.2	8.8	57.89

Multilocus DNA fingerprinting for 13 *Syringa* varieties of Belarusian selection using 3 RAPD- and 2 ISSR-primers gave us possibility to differentiate all studied genotypes, to

develop markers, including cultivar-specific ones, create unique profiles for each of them and calculate genetic distances relationship/distance between genotypes. Thus, used RAPD + ISSR approach was adapted for genetic certification of *Syringa* spp. genotypes. Based on RAPD and ISSR-markers for 13 lilac cultivars Multilocus genetic passports were made. Table 3 shows an example of a genetic passport for variety Polesskaya Legenda (Woodland Legend). All data of DNA typing for samples of Belarusian selection lilac were included in a separate section "Molecular genetic passport" of information retrieval system Hortus Botanicus Centralis - Info (№ GR 20053449 from 14.11.2005). This system serves as a source of data for sites "Botanical Collections of Belarus» (http://hbc.bas-net.by/bcb/) and sections of the portal of the Council of Botanical Gardens of Russia, Belarus and Kazakhstan (http://hortusbotanicus.ru), that provides a base for enhanced cooperation and information exchange in order to preserve biodiversity.

Table 3

Representative multilocus genetic passport of Belarusian selection lilac cultivar Polesskaya Legenda (Woodland Legend)

Variety Polesskaya Legenda
Markers
OPA18255, OPA18355, OPA18370, OPA18395, OPA18430, OPA18590, OPA18930, OPA181030, OPA181225
OPE02260, OPE02270, OPE02355, OPE02415, OPE02440, OPE02480, OPE02495, OPE02530, OPE02570, OPE02650, OPE02775, OPE02890, OPE02945, OPE021380
OPP09260, OPP09365, OPP09485, OPP09535, OPP09610, OPP09645, OPP09665, OPP09780, OPP09875, OPP09980
UBC808210, UBC808250, UBC808265, UBC808330, UBC808355, UBC808420, UBC808440, UBC808455, UBC808480, UBC808525, UBC808550, UBC808595, UBC808665, UBC808700, UBC808810, UBC808885, UBC808950
UBC862185, UBC862270, UBC862335, UBC862375, UBC862450, UBC862580, UBC862605, UBC862725, UBC862960

Developed methodical scheme of lilac cultivars genotyping on the basis of complex RAPD- and ISSR-markers was used for molecular genetic documentation of lilac living collections, including for verification samples of *in vitro* collection. It is known that somaclonal variations in plants regenerated *in vitro*, can be very high, so monitoring and maintaining of the genotype stability is important for keeping obtained microshoots. This work was initiated by comparing microclones from *in vitro* collections of CBG NASB and GBS RAS that are supported in the collections of various botanical gardens, in particular varieties Partisanka and Svityazanka [4].

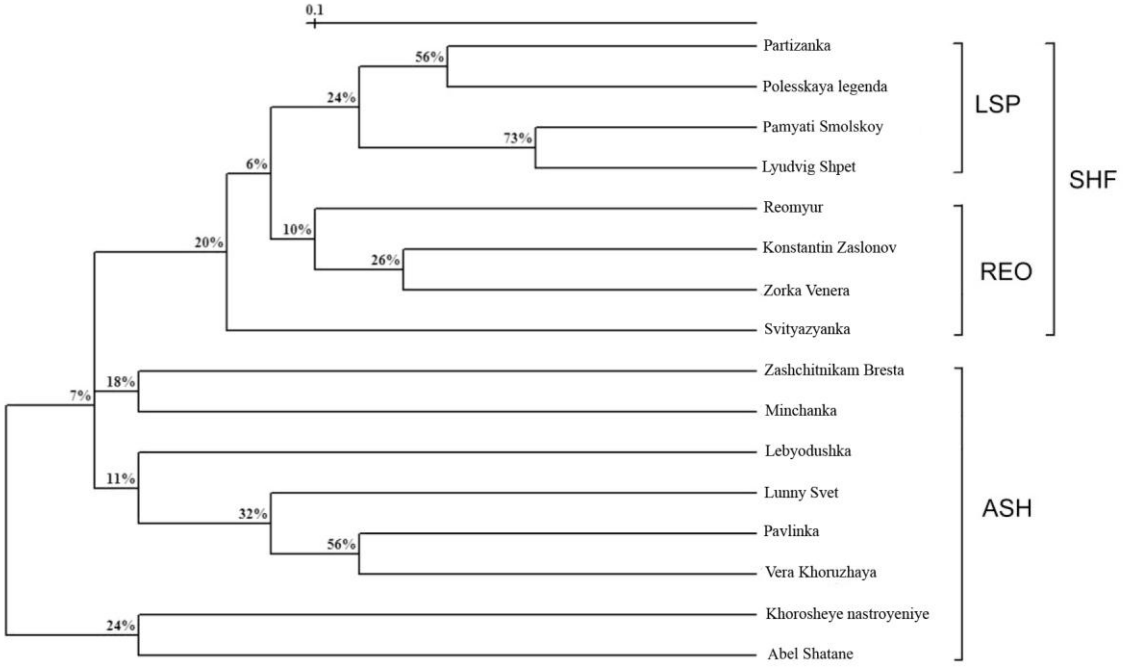
In this work we assessed varietal conformity for genotypes of Belarusian selection 'Lunnyi Svet' (Moonlight), 'Pavlinka', 'Lebedushka', 'Zashchitnikam Bresta' (For Defenders of Brest) under *in vitro* propagation. With the help of developed RAPD- and ISSR-markers conformity of material propagated *in vitro* and collections of the open air was affirmed. In a number of cases variability of genetic homogeneity parameters (profiles of amplicons, genetic distances; data are not shown) was detected. It may be due to the emergence of acceptable changes resulted different responses of genotypes for cultivation under *in vitro* conditions, as well as due to the length of passages.

To study the degree of genetic divergence for Belarusian selection lilac cultivars and to determine their phylogenetic relationships parental lilac cultivars 'Mme Abel Chatenay', 'Ludwig Shpaeth' and 'Reaumur' were included in the study. Based on the identified DNA

markers Nei genetic distances between the studied lilac cultivars were calculated, their clustering by method UPGMA was made. These data were used in the construction of phylogenetic trees for each primer (RAPD and ISSR) (data are not shown), and consensus (RAPD + ISSR) dendrogram shown in Figure 2. These RAPD and ISSR cultivars` genotyping showed similar degree of relationship for studied cultivars. Rod clustering in generated consensus (RAPD + ISSR), as well as RAPD- and ISSR-dendrograms generally preserved; denrograms detect small differences in subclusterization in some varieties.

Fig. 2 Consensus RAPD + ISSR dendrogram, which demonstrates the degree of genetic similarity between lilac cultivars of Belarusian selection based on 40 RAPD and 36 ISSR markers generated with primers OPA-18, OPE-02 and OPP-09, UBC-808 and UBC-862. Magnitude bootstrap (100 replicas) pointed about the corresponding node (%)

Designations clusters: SHF - Hyacinthiflora; REO - Hyacinthiflora × Reaumur; LSP - Hyacinthiflora × Ludwig Shpaeth; ASH - Mme Abel Chatenay × Reaumur



Conclusions

Application of the developed methods of complex RAPD + ISSR-genotyping of *Syringa vulgaris* L. on intraspecific level allowed to differentiate and certify all studied lilac genotypes of Belarusian selection in CBG NASB collection, develop genotypic certificates, verify genealogy of cultivars, clarify phylogenetic relationships between them. Developed method is effective for sampling, monitoring of varietal purity in propagated cultivars, formation and genetic verification of the samples in *in vitro* collections. Developed markers are the basis for further breeding on valuable traits.

References

1. *Bibikova V.F.* et al. Derevia i kustarniki, rosy i siren. Kratkie itogi introduktsii: monografia / red. N/V/ Smoloskii; Akademia nauk BSSR, Tsentralnyi botaniheskii sad. – Minsk: Nauka i tehnika, 1968. – 384 s.

2. *Melnikova N.V., Borhert E.V., Martynov S.P., Okuneva I.B., Molkanova O.I., Upelniek V.P., Kudriavtsev A.M.* Ispolzovanie molekuliarno-geneticheskih markerov dlia

verifikatsii kollektsii *in vitro* sireni obyknovennoi (*Syringa vulgaris* L.) // Genetika. – T. 45, № 1. – 2009. – S. 97–103.

3. M*olkanova O.I., Zinina Yu.M., Makedonskaia N.V., Brel N.G., Fomenko T.I., Spiridovich E.V.* Razrabotka biotehnologicheskih priemov razmnozhenia sireni obyknovennoi // Fiziol. i biohim. kultur. rastenii. – 2010. – T. 42., №2. – S. 117–124.

4. *Molkanova O.I., Spiridovich E.V., Konovalova L.N., Brel N.G., Zinina Yu.M., Reshetnikov V.N.* Kompleksnoe izuchenie vidov i sortov roda *Syringa* L. v GBS RAN i CBS NAN Belarusi // Vestnik Udmurtskogo universiteta. – Vypusk 2. – S. 66–75.

5. *Reshetnikov V.N., Spiridovich E.V., Makedonskaia N.V., Chizhik O.V., Antipova T.V., Brel N.G., Baranov O.Yu.* Sohranenie I izuchenie genofonda sireni v CBS NAN Belarusi // Problemy lesovedenia i lesovodstva: sbornik nauchnyh trudov. – Gomel, 2007. – Vyp. 67. – S. 238–245.

6. *Reshetnikov V.N., Spiridovich E.V., Chizhik O.V., Antipova T.V., Zubarev A.V., Garanovich I.M., Makedonskaia N.V.* Obogashchenie, sohranenie i izuchenie genofonda sireni v CBS NAN Belarusi // Sovremennye napravlenia deiatelnosti botanicheskih sadov I derzhatelei botanicheskih kollektsii po sohraneniu bioraznoobrazia rastitelnogo mira: materialy Mezhdunarodnoi nauchnoi konferetsii, posviashchennoi 100-letiu so dnia rozhdenia akademika N.V. Smolskogo (Minsk, 27-29 sentiabria 2005). – Minsk, 2005. – S. 250–254.

7. *Spiridovich E.V., Vlasova A.B., Shabunia P.S.* Kollektsia sireni Centralnogo botanicheskogo sada NAN Belarusi i selektsia nekotoryh sortov na osnove belkovyh markerov // Genetika i selektsia v XXI veke: mater. VIII sezda BOGIS (Minsk, 23-25 iulia 2002). – Minsk, 2002. – S. 151–152.

8. *Spiridovich E.V., Goncharova L.V., Chizhik O.V., Vlasova A.B., Zubarev A.V.* Razrabotka molekuliarno-biologicheskih I biohimicheskih podhodov pasportizatsii unikalnyh kollektsii rastenii Centralnogo botanicheskogo sada NAN Belarusi // Materialy II Resp. nauch.-prakt. konf. (Minsk, 20-22 noiabria, 2003). – Minsk, 2003. – S. 120–126.

9. *Doyle J.J., Doyle J.L.* Isolation of plant DNA from plant tissue / Focus. – V. 12. – 1990. – P. 13–15.

10. *Juntheikki-Palovaara I., Antonius K., Lindén L., Korpelainen H.* Microsatellite markers for common lilac (Syringa vulgaris L.) // Plant Genetic Resources. – 2013, 11(03). – P. 279–282.

11. *Kim K.J., Jansen R.K.* A chloroplast DNA phylogeny of lilacs (Syringa, Oleacae): plastom groups show a strong correlation with crossing groups // Am. Bot. – 1998. – V. 85, № 9. – P. 1338–1351.

12. *Li J., Alexander J.H., Zhang D.* Paraphylitic Syringa (Oleaceae): evidence from sequences of nuclear ribosomal DNA ITS and ITS regions // System. Botany J. – 2002. – V. 27, № 3. – P. 592–597.

13. Lilacs: a gardener's encyclopedia. By John L. Fiala, Freek Vrugtman // Timber Press, Jul 18, 2008. – Gardening. 416 p.

14. *Moose S.P., Mumm R.H.*. Molecular plant breeding as the foundation for 21st century crop improvement // Plant physiology. – 2008, 147(3). – P. 969–977.

15. *Popowich E.A., Filipenya V.L.* Features of micropropagation of Syringa vulgaris L. // Scientific works of the Lithuanian Institute of horticulture and Lithuanian university of agriculture. Horticulture and vegetable growing. Horticulture. – 2000. – V. 19(3). – P. 434–440.

16. *Rao V.R., Hodgkin T.* Genetic diversity and conservation and utilization of plant genetic resources // Plant cell, tissue and organ culture. – 2002, 68(1). – P. 1–19.

17. *Rao N.K.* Plant conservation biotechnology // Plant genetic resources: advancing conservation and use through biotechnology. African Journal of biotechnology. – 2004, 3(2). – P. 136–145.

Spiridovich E.V., Vlasova A.B., Yukhimuk A.N., Reshetnikov V.N. Assessment of genetic divergence of lilac (Syringa L.) cultivars of Belarusian selection based on integrated application of RAPD- and ISSR-markers // Works of the State Nikit. Botan. Gard. – 2014. – V. 139 – P. 191– 198.

An integrated approach of RAPD+ISSR molecular certification of lilacs cultivars of Belarusian selection (CBG NASB) was developed and applied aimed at verification of genotypes identity at propagation, collections maintenance and unique genotypes conservation. Generated in total 93 RAPD and 67 ISSR markers (including cultivar-specific) allowed to differentiate 13 studied genotypes, create genetic certificates for each of them, calculate the degree of genetic relationship and clarify the phylogenetic relationships between cultivars. Proposed method of DNA-passportization of Syringa vulgaris cultivars is an effective tool to study the genetic diversity and molecular certification of cultivated lilacs forms, verification of collection banks when depositing in vitro.

Key words: Syringa vulgaris L., lilac cultivars, certification molecular markers, RAPD-, ISSR-loci, genetic distance, in vitro collection, genetic diversity, conservation