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Polymorphism in *Syringa* rDNA regions assessed by PCR technique

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Abstract: The *Syringa* genus is characterized by a multiplicity of forms. Its chief asset is the ornamental value of thousands of accessions, species or hybrids. From a phylogenetic point of view the genus is difficult in an explicit classification due to its frequently complex genome. The aim of this study was to determine the possibility for the identification of genotypic diversity and genetic relationships in the nrDNA sequence of some selected *Syringa* accessions – part of a collection of the Dendrological Garden in Przelewice (Poland). For this purpose, the PCR technique together with a combination of various 'universal' primers designed for the nrDNA sequence analysis were employed. Fourteen *Syringa* accessions: *Syringa* × *chinensis* Willd., *S.* × prestoniae Mc Kelv., *S.* × *prestoniae* 'Telimena', *S.* × *prestoniae* 'Jaga', *S.* × *prestoniae* 'Basia', *S. meyeri* 'Palibin', *S. vulgaris* 'Miss Ellen Willmott', *S. vulgaris*, *S. vulgaris* 'Jules Simon', *S. vulgaris* 'Katherine Havemeyer', *S. vulgaris* 'Krasawica Moskvy', *S. vulgaris* 'Mirabeau', *S. vulgaris* 'Madame Lemoine' and *S. vulgaris* 'Niebo Moskvy' made up the research material. In the conducted amplifications, genetic profiles were obtained for 14 combinations among the 25 combinations of different pairs of primers used. The nrDNA templates coding the small subunit (SSU), 5.8S subunit and ITS1, ITS2 and IGS sequences were amplified. In PCR reactions a total of 33 PCR products were generated, of which 21 (64%) products were polymorphic, 6 (18%) monomorphic and 6 (18%) were genotype-specific. For the lilac accessions examined 246 amplicons were generated from ~230 to ~1100 bp in length. The analysis of both the dendrogram and the genetic similarity matrix revealed low diversity between the examined accessions. For most they ranged from 70 to 80%, and the greatest diversity (87%) was found between the *S.* × *prestoniae*: 'Basia' and 'Telimena' accessions, while the lowest (57%) was observed between *S. vulgaris* 'Katherine Havermeyer' and *S.* × *chinensis*.

Additional key words: *Syringa* accessions, rDNA polymorphism, IGS variability, additional amplicons

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Introduction

Lilac (*Syringa vulgaris*) is an ornamental plant from the *Syringa* genus. The *Syringa* L. genus belongs to the family of *Oleaceae* Lindl. and according to different classifications, includes 22–30 species (Fiala 2002, Kochieva et al. 2004). It originated in the Balkan Peninsula and is one of the most common ornamental bushes cultivated at Eurasian and North American latitudes (Wallander et al. 2000, Kochieva et al. 2004).

At present, about 2,000 lilac accessions are known. Victor Lemoine, the author of many interspecific hy-

brids and numeorous accessions, carried out cultivation work particularly on the species *Syringa vulgaris* and Kolesnikov in the 19th century in France and significantly contributed to lilac origination (Kochieva et al. 2004).

The common lilac (*Syringa vulgaris*) is the most frequently cultivated; less often cultivated accessions include ornamental accessions and hybrids originating from Far Eastern lilacs: Chinese lilac (*Syringa* × *chinensis*) or Preston lilac (*Syringa* × *prestoniae)*, as examples. The lilac species cross relatively easily, however, interspecific crossing is only possible when two species are of the same series (Sax 1945, Pringle 1977). Hybrids obtained from crossing of a species from the *Syringa* series with the *S. pinnatifolia* species (the only species of the *Pinnatifoliae* series) are an exception (Anderson and Rehder 1935).

There are many diverse forms (genotypes) among the *Syringa* genus, originating from variants of crossed genotypes. Many interesting lilac genotypes are located in different collections, among others the Dendrological Garden in Przelewice (Poland). This institution conducts diversified research on lilac morphology and phenology with the aim to systematizing their collected genotypes, of which many are probably hybrids (Chylarecki et al. 2008).

One of the many methods of determination or *de novo* identification of phylogenetic relationships between various plant genotypes is the analysis of variability in gene coding rRNA subunits (nrDNA), including the ITS (**i**nternal **t**ranscribed **s**pacers), IGS (**i**nter**g**enic **s**parcer) sequences and the sequences of rRNA genes: SSU (**s**mall **su**b**u**nit), 5.8S and LSU (large subunit) (Appels and Dvorak 1982, Baldwin 1995, Hershkovitz and Lewis 1996, Pillay and Kenny 1996, Álvarez and Wendel 2003, Shneyer 2009, Poczai and Hyvönen 2010).

The gene coding of ribosomal RNA (rDNA), if taking into account the number of studies, are an increasingly analysed region of DNA research. In contrast to ITS or IGS sequences, characterized by relatively high diversity and which alternate with RNA have low diversity. Usually the evolutionary stability of the rDNA genes (SSU, 5.8S or LSU) is used in the identification of given research subjects as a target of hybridization of specifically designed 'universal' primers, employed in the amplification of shorter or longer sequences containing the sequence of the subunit rRNA gene or a respective sequence module – the most frequently, ITS (ITS1+5.8S+ITS2). Thus with the use of the information on the sequence of one species, other species which are related to it may be examined (Gerbi 1985, McManus and Bowles 1996).

According to Liston et al. (1996), the elements coding the angiosperm rRNA have little difference with respect to length. The ITS1 region is characterized by a length from 565 to 700 bp, while the region 5.8S RNA-ITS2 is 375–450 bp long. However, in the gymnosperms the lengths of these sequences are significantly different (in the *Pinaceae* the ITS1 sequences are 1550–3125 bp long, in the *Cupressaceae*, *Taxodiaceae*, *Cephalotaxaceae*, and *Taxaceae* – from 975 to 1125 bp, while in the *Coniferales*, *Cycadales*, *Ginkgoales*, and *Gnetales* – 975–3125 bp). Research on the rRNA subunits has been also conducted on a protozoan (*Giardia lamblia*), whose ITS regions were 41 bp and 55 bp long respectively; in mice the ITS1 and ITS2 were 999 bp and 1089 bp long, respectively (Hauser and Wang 2005). One of the longest ITS regions can be found in beetles (*Coleoptera*): ITS1 – 791 bp, ITS2 – 2572 bp (Schulenburg et al. 2001).

The aim of the present study was to determine the diversity among the multiple repeat nrDNA sequences in the selected *Syringa* accessions using the PCR technique with a combination of various primers, described in relevant literature as 'universal'. In addition, we aimed to examine the identification (amplification) of polymorphic or genotype-specific amplicons, generated on the nrDNA templates of fourteen different *Syringa* accessions collected in the Dendrological Garden in Przelewice.

Material and methods

Fourteen *Syringa* accessions: *Syringa* × *chinensis* Willd., *S.* × *prestoniae* Mc Kelv., *S.* × *prestoniae* 'Telimena', *S.* × *prestoniae* 'Jaga', *S.* × *prestoniae* 'Basia', *S. meyeri* 'Palibin', *S. vulgaris* 'Miss Ellen Willmott', *S. vulgaris*, *S. vulgaris* 'Jules Simon', *S. vulgaris* 'Katherine Havemeyer', *S. vulgaris* 'Krasawica Moskvy', *S. vulgaris* 'Mirabeau', *S. vulgaris* 'Madame Lemoine' and *S. vulgaris* 'Niebo Moskvy' north-west part of the collection of the Dendrological Garden in Przelewice (Poland) constituted the research material.

DNA isolation. Replicate DNA extraction from young leaves of fourteen *Syringa* accessions was used to assess the reproducibility of band patterns (Qiagen DNeasy Plant Mini Kit). RNA contamination was removed by digestion with RNaseA (100 mg · ml^{-1}). Genomic DNA was quantified (GeneQuant DNA/RNA Calculator – Pharmacia LKB) to obtain 50 ng DNA pro 1μl of DNA template.

Primers for PCR amplification. The analysis encompassed nuclear rDNA, including the following sequences: SSU, ITS1; 5.8S; ITS2 and IGS. The general structure and the arrangement of the analyzed sequences encoding the formation of rRNA genes was presented in Figure 1.

The figure also includes names, approximate locations and directions of the hybridization of 'universal' primers used for PCR reactions. The origin of primers, sequences and their authors are presented in Table 1.

Amplification of nrDNA regions. PCR mixtures (25 μl) contained: 2.5μl 10× PCR buffer with $(NH_4)_2SO_4$

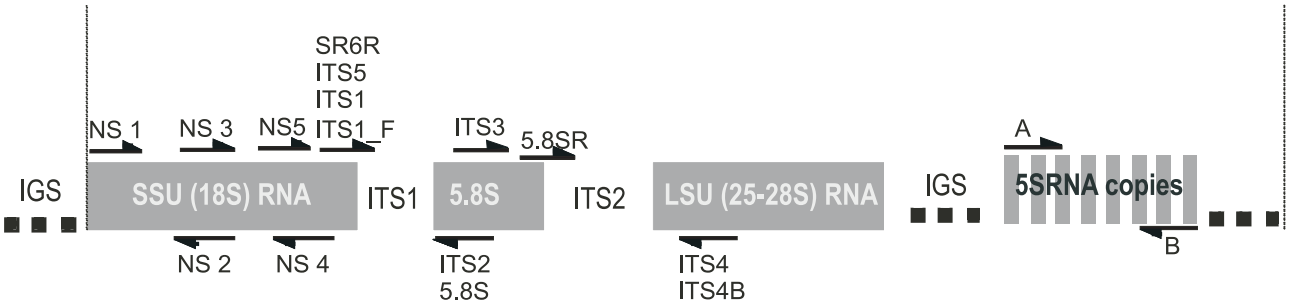

Fig. 1. nrDNA unit. Arrows indicate aproximate positions of primers used to generate amplicons in different nrDNA regions. Information about primer used were listed in Table 1

[750 mM Tris-HCl pH 8.8, 200 mM $(NH_4)_2SO_4$, 0.1% Tween20] (Fermentas MBI), 2 mM dNTPs (Fermentas MBI), 25 mM $MgCl_2$ (Fermentas MBI), 2.5 μM of each primer, 1.0 unit of *Taq* DNA polymerase (Fermentas MBI) and 100 ng of template DNA. DNA was amplified using a Mastercycler 5333 (Eppendorf) thermocycler using the following program: initial denaturation at 94°C for 7 min, 40 cycles of 30s at 94°C, annealing temperature, 2 min at 72°C and 7 min at 72°C for a final extension. The annealing temperature was usually adjusted according to the T_m of the primers being used in the reaction (Table 1). Amplification reactions were performed according to the *touchdown* PCR protocol by raising the temperature of the first singles cycles by 6°C above the annealing temperature, adopted from the preliminary experiments for both primers.

Electrophoresis and data analysis. PCR products were mixed with 6' Orange Loading Dye Solution and were analysed by electrophoresis. O'RangeRuler 200bp DNA Ladder and O'RangeRuler™ 100+500bp DNA Ladder (Fermentas MBI) were used as a size marker (3000–200 bp and 100–500 bp). PCR products were analyzed on 2% agarose gels (Basica LE GQT – Prona) in 1X TBE buffer, then stained with ethidium bromide, visualized (MiniBIS Pro – DNT Bio-Imagining System – USA) and scored for band present or absence. The rDNA fingerprints generated by each primer pairs used (Table 1), were used to compare relatedness of *Syringa* accessions. Presence (1) or absence (0) of each amplicons was scored for each genotype. Only stable amplification products were scored. The similarity level between accessions compared was determined by Jaccard's coefficient (S_J) according to the formula $S_J = a(n–d)$ where a is the number of matched 1's, n is the total sample size, and d is the total number of matched 0's. Dendrograms were produced by cluster analysis of the similarity coefficients using UPGMA (Nei and Li 1979). The strength of the internal branches from the resulting tree were tested by TREECON bootstrap analysis application using 2,000 replications (Felsenstein 1985, Van de Peer and De Wachter 1994).

Table 1. Primer pairs used in the experiment

nrDNA fragment amplified	Primer's name	Author(s)
SSU	NS1, NS2, NS4, NS3	White et al. (1990)
ITS1	NS5, ITS2, NS6, ITS5, ITS1	White et al. (1990)
	5.8S	Vilgalys and Hester (1990)
	SR6R	Vilgalys*
	ITS1-F	Gardes and Bruns (1993)
ITS2	ITS3, ITS4	White et al. (1990)
	ITS4-B	Gardes and Bruns (1993)
	5.8SR	Vilgalys and Hester (1990)
ITS1–5.8S-ITS2		as above
5SRNA	A, B	Ko and Henry 1990

*Vilgalys unpublished [http://www.botany.duke.edu/fungi/mycolab]

Results

A set of 'universal' primers was used for the analysis of genetic diversity among 14 lilac accessions (Table 1, Fig. 1), which were combined into 25 pairs different in respect to the length and its site of hybridization to the nrDNA cistron templates.

In the reactions with 14 primers pairs, amplification products visible on agarose gels were obtained. Out of 33 products amplified, 21 (66%) were polymorphic, 6 (18%) monomorphic and 6 (18%) genotype-specific, identified for the accessions: *S.* × *prestoniae* 'Jaga', *S. meyeri* 'Palibin' and *S. vulgaris* 'Mirabeau', respectively. Information about the origin of amplicons generated for the investigated lilac accessions was presented in Table 2.

The widest range of diversity among the examined lilac accessions, resulting from polymorphic products profiles visualized on electrophoregrams, was found in the reactions with the pair of primers A + B (4) (Fig. 2a). The range of length of amplified products were from ~800 to ~230 bp (Table 2).

Genotype-specific products were generated in the reactions with the following pairs of primers: ITS1+ITS2, ITS1_F+ITS4, ITS5+ITS4, 5.8SR+ITS4,

Table 2. Characteristics of fragments amplified. Number of monomorphic, polymorphic and accession-specific amplicons generated in the reaction with 'universal' primers on the nrDNA templates for selected *Syringa* accessions

Primer pairs	Fragment size range (bp)	*S. vulgaris* 'Krasavitsa Moskv'	*S. vulgaris* 'Niebo Moskvy'	*S. vulgaris*	*S.* × *prestaniae*	*S.* × *prestaniae* 'Jaga'	*S. meyeri* 'Palibin'	*S. vulgaris* 'Mme Lemoine'	*S. vulgaris* 'Katherine Havermeyer'	*S. vulgaris* 'Jules Simon'	*S. vulgaris* 'Mirabeall'	*S. vulgaris* 'Mrs Ellen Wilmott'	*S.* × *chinensis* Wiild.	*S.* × *prestoniae* 'Basia'	*S.* × *prestoniae* 'Telimena'	Total	Monomorphic	Polymorphic	Accessions- specific
SSU																			
NS1+NS4	1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1	0	0
NS3+NS4	580–400	2	2	2	2	1	2	2	1	1	2	2	2	1	2	24	1	1	0
NS5+5.8S	940–880	1	1	2	0	1	1	1	1	1	1	1	2	1	1	15	0	2	0
ITS1																			
ITS1+ITS2	370–230	1	1	2	2	1	3	1	1	1	1	1	1	1	1	18	0	2	1
ITS1+5.8S	300–240	1	2	2	2	2	2	2	2	2	2	3	2	1	2	27	0	3	0
ITS5+5.8S	300	1	1	1	0	1	1	1	1	1	1	1	0	1	1	12	0	1	0
SR6R+5.8 S	380–240	2	2	2	1	1	2	1	0	3	2	3	3	1	2	25	0	3	0
ITS1-5.8S-ITS2 (ITS)																			
ITS1_F+ITS4	860–460	2	1	2	3	2	2	2	5	3	2	2	2	2	2	32	1	3	1
ITS5+ITS4	760–440	1	0	1	2	2	1	0	2	2	1	1	0	1	0	14	0	2	1
ITS2																			
5.8SR+ITS4	560–350	1	1	1	1	2	1	1	1	1	2	1	1	1	1	16	1	0	2
5.8SR+ITS4B	530	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1	0	0
ITS3+ITS4B	520	1	1	1	1	1	1	1	1	1	1	1	1	1	1	14	1	0	0
5SRNA																			
A+B	800–230	2	1	1	3	1	1	1	1	3	2	2	1	1	1	21	0	4	1
Total	1100–230	17	15	19	19	17	19	15	18	21	19	20	17	14	16	246	6	21	6

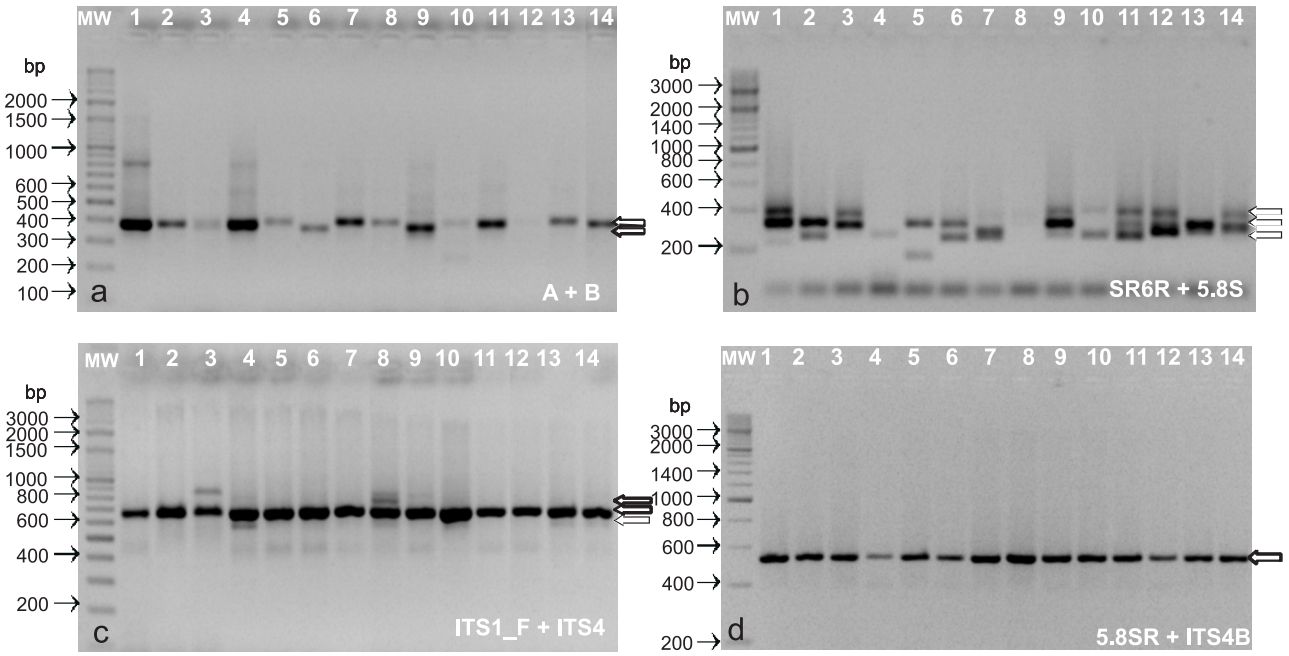

Fig. 2. Profiles of the PCR products in selected nrDNA regions of investigated *Syringa* accessions
MW – Molecular Weight, 1 – *S. vulgaris* 'Krasavitsa Moskvy', 2 – *S. vulgaris* 'Niebo Moskvy', 3 – *S. vulgaris*, 4 – *S.* × *prestaniae*, 5 – *S.* × *prestaniae* 'Jaga', 6 – *S. meyeri* 'Palibin', 7 – *S. vulgaris* 'Mme Lemoine', 8 – *S. vulgaris* 'Katherine Havermeyer', 9 – *S. vulgaris* 'Jules Simon', 10 – *S. vulgaris* 'Mirabeall', 11 – *S. vulgaris* 'Mrs Ellen Wilmott', 12 – *S.* × *chinensis* Wild., 13 – *S.* × *prestoniae* 'Basia', 14 – *S.* × *prestoniae* 'Telimena'

and A+B, designed to analyse the sequence variability of the subunits: SSU-ITS1-5.8S, 5.8S-ITS2-LSU and the IGS sequence (Fig. 1). In the present work 6 monomorphic products were amplified, obtained in the experiment by the amplification of the genes region coding the small subunit SSU, ITS and ITS2 sequences (Table 2).

SSU. Three pairs of primers (NS1+NS4, NS3+NS4 and partially NS5+5.8S) were used for the amplification of the genes coding the SSU. In the amplification performed with the primers NS1+NS4 and NS3+NS4, monomorphic products (~1100 and ~580 bp) were generated (Table 2), whereas in the reaction with the primers NS5+5.8S and NS3+NS4 polymorphic products of ~880, ~940 and ~400 bp were generated, respectively (Table 2).

ITS1. Four pairs of universal primers were used for the amplification of the ITS1 sequence. Pair of primers: ITS1+ITS2, ITS1+5.8S, ITS5+5.8S and SR6R+5.8S, hybridized in the opposite directions on the DNA templates of the SSU and 5.8S subunit sequences (Fig. 1). In the reactions with primers ITS1+ITS2, genotype-specific product (~230 bp) for *S. meyeri* 'Palibin' was amplified. In amplifications with the primers ITS1+5.8S, ITS5+5.8S and SR6R+5.8S, polymorphic products of the lengths from ~240 to ~300 bp (ITS1+5.8S), ~300 (ITS5+5.8S) and from ~240 to ~380bp (SR6R+5.8S) were found on electrophoregrams, respectively (Table 2, Fig. 2b).

ITS (ITS1+5.8S+ITS2). The ITS sequences were amplified using two pairs of primers: ITS1_F+ITS4 and ITS5+ITS4 (Table 2, Fig. 2c). In performed amplification with primers ITS1_F+ITS4, one genotype-specific products for *S. vulgaris* 'Katherine Havermeyer' (~780 bp) and three other polymorphic products were amplified discriminating the examined *Syringa* genotypes. Similar results were obtained after using the ITS5+ITS4 pair of primers in the amplification. The genotype-specific products was amplified for *S. vulgaris* 'Jules Simon' (~550 bp) (Table 2).

ITS2. Three pairs of primers: 5.8SR+ITS4, 5.8SR+ITS4B and ITS3+ITS4B were used in the amplification of the ITS2 sequence (Fig. 2d). In the reactions with the primer combinations: 5.8SR+ITS4B and ITS3+ITS4B, monomorphic products with lengths of ~530 and ~520 bp were amplified (Fig. 2d), whereas in the reaction with primers 5.8SR+ITS4, one monomorphic (~400 bp), and two genotype-specific products, specific for *S. vulgaris* 'Mirabeau' (~350 bp) and *S.* × *prestoniae* 'Jaga' (~560 bp), were generated.

IGS. One pair of A+B primers was used for the amplification of the IGS sequence. In performed amplification, 4 polymorphic products were generated with lengths from ~230 to ~800 bp. Additionally, one genotype-specific product of the length of ~230 bp was obtained for *S. vulgaris* 'Mirabeau' (Fig. 2a).

Phylogenetic analysis. A dendrogram of phylogenetic similarity was constructed with the use of the UPGMA cluster analysis in the assessment of diversity of the nrDNA sequence revealed on electrophoregrams as a set of genetic profiles obtained in the reactions with different pairs of primers for the fourteen lilac accessions (Fig. 3).

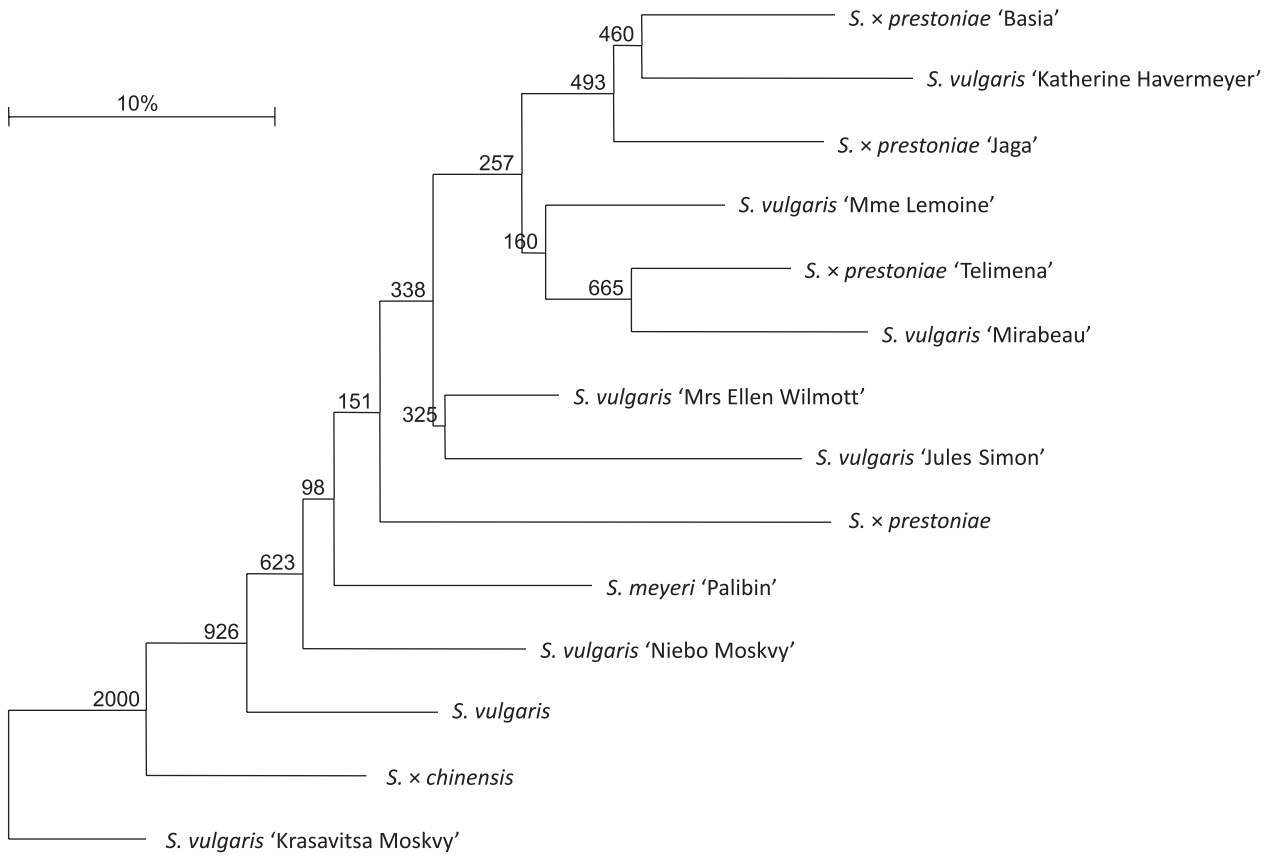

Fig. 3. UPGMA dendrogram representing genetic relationship among fourteen accession of *Syringa*. The number at the branches indicate 2,000 bootstrap replications expressed in percent

The analysis of genetic relationship matrices between the examined accessions showed that the genetic similarity calculated for the examined *Syringa* genotypes amounted from 57 to 87%. The greatest genetic similarity (87%) was found between the accessions *S.* × *prestoniae*: 'Basia' and 'Telimena', and the smallest between the *S. vulgaris* 'Katherine Havermeyer' species and the hybrid *S.* × *chinensis* (57%). Between the other examined genotypes, the similarity ranged from 70 to 80% (data not shown).

Discussion

The genus *Syringa*, due to the ornamental importance of the range of species, accessions and hybrids and to its complex genotype, has been the subject of much research on the phylogenetic relationships between its accessions with the use of the various aforementioned methods.

In scientific literature there are many reports on various methods for the analysis of phylogenetic relatioships between plant genotypes, enormously useful in the creation and verification their taxonomy. Among others, the research focuses on the analysis of morphological (plant habit, leaf shape and pubescence, inflorescence colour and structure), chemical and molecular traits (Damtoft et al. 1995, Chen et al. 2008, Archak et al. 2003, Kochieva et al. 2004, Pfosser et al. 2000, Cabrita et al. 2001, Crowford et al. 2001, Mondal 2002, Rzepka-Plevneš et al. 2006).

In the examination of the rDNA sequences it is important to take into consideration that a frequently a complex genome may seem to be the source of a wide range of diversity resulting from its origin, for example.

The experiment presented here on the 14 selected lilac accessions, consisted of a part of the lilac collection of the Dendrological Garden in Przelewice (Poland), and proved the possibility to identify some additional (polymorphic) sites within the nrDNA cistron with the use of amplifications of a combination of various pairs of 'universal' primers without the sequence or restriction analysis of the amplicons obtained.

The decision for the selection of primer and primer combinations was based on scientific literature data (Table 1). After preliminary research and verifications of its usefulness some primer or pairs of primers that in the preliminary amplifications generated clear visible amplicons were used for the amplification of the target nrDNA comprising of SSU, 5.8S, ITS1, ITS2 and IGS sequences.

In order to minimize the possibility of generating artefacts (non-specific amplicons), actually in accor-

dance to opinions expressed by Cronn et al. (2002), each amplification was performed with a touchdown thermal profile by increasing the temperature of primers annealing in the first reactions cycle by 6°C above the annealing temperature.

In the preliminary research, using the same reaction conditions, polyploid maples, amphidiploid apples and one sample of human DNA, as well as lilacs were included. The amplification of the human DNA sample with selected primer pairs provided monomorphic reaction products, while the amplification in the same conditions for maples, lilacs or apples generated some additional products (Smolik et al. in reviews).

In the presented experiment, 25 combinations of various pairs of primers were used in the amplification of the nrDNA sequences (18S, 5.8S and 28S) of selected lilac accessions. Clear amplification products were obtained in the reactions with 14 pairs. The specific amplicons for the SSU and 5.8S subunit genes were amplified in PCR reactions. Variability within the ITS1, ITS2 and IGS sequences was assessed in the form of additional amplified products. The amplicons lengths obtained did not differ from those amplified for other plant genotypes.

The assessment of variability in the SSU and IGS regions revealed that depending on the used pairs of primers, the PCR products obtained in the present experiment were in most cases polymorphic (SSU: 400, 880 and 940 bp; IGS: 800, 500, 390 and 230 bp).

The amplification of the ITS sequences (ITS1-5.8S-ITS2) revealed that the respective lengths of the amplicons visible on electrophoregrams amounted to: ITS1 (~230 – ~380 bp) and ITS2 (~350 – ~560 bp), while the ITS fragment, amplified in the reactions with the pairs of its flanking primers (ITS5 + ITS4 and ITS1_F + ITS4), was from 860 to 440 bp long.

Some range of diversity in the nrDNA sequence, including diversity in the rDNA cistron sequence, may result from not completely effective mechanisms of 'homogenisation', genome rDNA repair by concerted evolution (Hillis and Dixon 1991). This phenomenon has been precisely described for many plant groups with findings that the presence of multiple divergent ITS paralogs within individual several plant groups showed that they (intra-genomic ITS paralogs) are often considerably divergent (Harpke et al. 2006; Grimm et al. 2007; Ochieng et al. 2007).

Contrary to the classical concept of concerted evolution, there is increasing evidence for the non-concerted evolution of ITS in specific lineages of plants (e.g. Harpke 2005; Bayly and Ladiges 2007). Non-concerted evolution is characterized by the presence of a large number of rDNA copies, which are highly polymorphic within one organism (Venkateswarlu and Nazar 1991, Baldwin et al. 1995).

In the case of polyploid genomes, the presented methodological approach may seem quite reasonable. The above-mentioned plants including, among others, the examined genus *Syringa,* have great genetic potential, whose diversity often hinders correct determination of mutual phylogenetic relationships, for example due to the hybrid nature of their many genotypes.

The genus *Syringa* includes polyploid species (3×, 4× often 5×). They are developed in a natural way or by colchicination (especially cultivars). And thus for example in the karyotype of the species *S. vulgaris*, 46, 47 or 48 chromosomes were found. In *S.* × *prestoniae* (hybrid of *S. reflexa* and *S. villosa*) or *S.* × *chinensis* (hybrid of *S. vulgaris* × *S. laciniata*) the number of chromosomes was not counted (Fiala 2002). Probably, we can only speculate, there is also some deviation from the 'normal' number of chromosomes.

Kim and Jansen (1998) determined the phylogenetic relationships among the *Syringa* genus with the use of the PCR reactions amplified the sequences of chloroplast DNA and the ITS of rDNA. The obtained results allowed the division of the examined accessions into four so-called plastome groups (I, II, III, IV). Group I included the subgenus *Ligustrina,* group II included the series *Syringa* and *Pinnatifoliae*, group III comprised three well-defined groups within the series *Pubescentes*, and group IV comprised the series *Villosae* and had the lowest level of the cpDNA interspecific diversity. Different lengths of nuclear rDNA, inherited from both parents, and cpDNA, inherited from the mother, enabled the identification of origin of several lilac hybrids.

Li et al. (2002) determined the phylogenetic similarity of *Syringa* by comparing the ITS and ETS sequences. On the basis of the obtained results, two subgenera (*Ligustrina* and *Syringa*) and four series were distinguished and within *Syringa*. The usefulness of the ETS and ITS fragments in this type of study was earlier confirmed in a study by Baldwin and Markos (1998).

Within the genus *Syringa* and *Ligustrum*, the ETS sequences are significantly shorter than the ITS sequences (the ETS sequences are about 384 bp long, and the ITS sequences are about 635 bp long). The sequences of these two regions correspond markedly and gathering data on them has enabled phylogenetic analysis to be conducted. The research on both the ITS and ETS regions proves they are very useful in the analysis of phylogenetic relationships on all taxonomic levels (Li et al. 2002).

Youlong et al. (1998) presented results of the research on idioplasm *Beta* similar to the results obtained in the present study. The authors used 13 primers, designed by Baldwin et al. (1992) and generated (additional) products of the length from ~470 to ~690 bp. With the comparison of electrophoregrams, they found a high diversity between the accessions from the *Beta* genus.

Bernardi et al. (2002) in their study amplified the region of ITS1-5.8S-ITS2 of the olive tree (*Olea europaea)*. Two primers complementary to the genes of the subunit 18S and 25S rDNA were used in the amplification. The obtained products were ~700 bp long. Next, the regions ITS1 (250 bp), ITS2 (200 bp) and 5.8S were entirely sequenced, but no polymorphism was found in the length of the analysed sequences between the examined accessions.

In the present study, in the nrDNA amplification with the ITS1 and ITS2 primers, the products of a similar length of 370 – 230 bp were amplified, and the sequence region flanked by the pair of primers ITS5 and ITS4 had a length of ~780 bp.

In scientific literature there are many reports where authors use nrDNA 'universal' primers, designed in leading scientific institutions, in phylogenetic research or research on the diversity of various taxons from bacteria, fungi to plants, hence confirming their 'universatility' and the possibility of combining them easily into pairs in order to determine genetically diverse "new regions".

The research presented here on the 14 selected *Syringa* accessions, part of a very interesting and valuable collection of lilacs in Poland, demonstrated the possibility of identifying several polymorphic sites in the nrDNA cistron with the use of a combination of various pairs of 'universal' primers complementary to its selected sequences, presented on Fig. 1, without restriction analysis and sequencing of the obtained amplicons.

Ko and Henry (1996) searched for an effective method of distinguishing cereal grain species, and for this purpose used a pair of primers called A and B in the amplification of the non-transcribed spacer sequences (IGS). The A and B primer sequences come from this study and were used in the assessment of diversity in the IGS sequences of the selected *Syringa* genotypes.

According to Singh and Singh (2001), the number of the 5S rRNA genes in plants is frequently much greater than the number of the 18S, 5.8S and 25S genes. In their study on tea (*Camellia sinensis)*, the authors also used primers A and B (called in the study M27 and M28) in differentiating 10 various tea accessions.

The non-transcribed spacer between the 5S rRNA genes is also rapidly evolving and has also been used successfully to produce phylogenies in a number of species (Udovicic et al. 1995, Persson 2000). However, in some plant species the variation between the repeated units of an individual array makes the 5S rRNA gene spacer difficult to use in phylogenetic studies. According to Cronn et al. (2002) this variation may be due to the lack of interlocus concerted ecolution for 5S rDNA arrays in these plants (Ran et al. 2001).

The research on the fourteen lilac accessions confirms the usefulness of the application of both the A and B primers and 'universal' primers in the identification of small differences in rDNA sequences. The relatively high level of similarity obtained between the examined accessions proves the fact that rDNA sequences are characterized by a high level of similarity or, in other words, evolutionary conservativeness, and the differences observed may be informative, indicating the genotypic distinctness of the examined accessions.

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