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MOLECULAR GENETICS

The Genus *Syringa*: Molecular Markers of Species and Cultivars

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Abstract—RAPD analysis was carried out with 22 accessions of the genus *Syringa*, including six species, one interspecific hybrid, and 15 cultivars. In total, 512 polymorphic fragments were detected; species-specific and cultivar-specific markers were identified. For the first time, genetic polymorphism and genome similarity coefficients were estimated and phylogenetic relationships were established for the genus *Syringa.*

INTRODUCTION

Lilac is among the most popular ornamental bushes and is cultivated in the middle latitudes of Eurasia and North America. The genus *Syringa* L. belongs to the family Oleaceae Lindl. and includes 22–30 species according to different classifications. The major range of lilac includes highlands of East Asia, and only two species, common and Hungarian lilac, have ranges in the Balkan–Carpathian region of Europe. The genus *Syringa* includes two subgenera, *Ligustrina* and *Syringa* (syn. *Euvulgaris*), the latter comprising most lilac species. On the basis of morphophysiological data and analysis of flavonoid composition, the subgenus *Syringa* has been divided into four series: *Syringa*, *Villosae*, *Pubescentes*, and *Pinnatifoliae* [1, 2]. Some authors combine species of the series *Syringa* and *Pinnatifoliae* into one group. This classification is generally supported by molecular analysis of the chloroplast genome and sequence analysis of the transcribed spacers of the ribosomal genes [3, 4].

Lilac species are relatively easy to hybridize, yet interspecific hybridization is successful only when the two species belong to one series. An exception are hybrids resulting from crossing species of the series *Syringa* with *S. pinnatifolia*, the only species representing the series *Pinnatifoliae.* There are now approximately 2000 known lilac cultivars. Most of them were obtained from hybridization and breeding of common lilac *S. vulgaris*; however, breeding programs involves virtually all known species of the genus *Syringa.* Russian breeders, L.A. Kolesnikov, in particular, have made a substantial contribution to development of new lilac cultivars.

Notwithstanding the broad use of lilac as an ornamental culture, no systematic genome studies were carried out with wild or cultivated lilac species until recently, when Kim and Jansen [3] performed a detailed analysis of the chloroplast genome in lilac species and interspecific hybrids and established the phylogenetic relationships in the genus *Syringa.* Four major chloroplast DNA types were revealed and shown to correspond to the crossability groups of lilac and to agree with the taxonomic division of the genus into series. One plastome type proved to be characteristic of species of the species *Syringa* and *Pinnatifoliae.* In addition, the phylogenetic relationships in the genus *Syringa* were inferred from nucleotide sequence comparisons of the internal and external transcribed spacers of the ribosomal genes [3, 4]. The results support the isolation of the two subgenera and three series in the genus, with *S. pinnatifolia* belonging to the series *Syringa.*

Since only the specific plastome sequences and ribosomal gene regions were a subject of earlier molecular studies, it was of interest to generally characterize the nuclear genome of lilac with appropriate methods. The objectives of this work were to use RAPD analysis in searching for markers of lilac species and cultivars from the collection of the Tsitsin Main Botanical Garden and to estimate among-species and among-cultivar polymorphism of the genus *Syringa.*

MATERIALS AND METHODS

For molecular analysis of the lilac genome, 22 accessions of the genus *Syringa* were selected from the collection of the Tsitsin Main Botanical Garden. We examined *S. amurensis* (syn. *S. reticulata* ssp. *amurensis*) of the subgenus *Ligustrina* and several accessions of the subgenus *Syringa*, including *S. vulgaris, S. oblata* (series *Syringa*), *S. josikaea*, *S. villosa* (series *Villosae*), *S. velutina* (syn. *S. pubescenc* ssp. *patula*) (series *Pubescentes*), hybrid species *S.* × *chinensis* (*S. persica*

var. *laciniata* (or *S. protolaciniata*) × *S. vulgaris*). In *S. vulgaris*, we used cultivars Flora, Lucy Balte, Madam Floren Stepman, Mrs Edward Harding, Ami Shott, Primrose, Fantasy, Mulatka, Aleksei Mares'ev, Valentina Grizodubova, Ogni Donbassa, Krasnaya Moskva, Russkaya Pesnya, and Olimpiada Kolesnikova. In addition, we used cultivar Buffon of hybrid species *S. hyacinthiflora* (*S. vulgaris* × *S. oblata*).

Plant DNA was isolated from buds according to a standard protocol [5]. PCR was run on a PCT-100 thermal cycler (MJResearch, United States). The amplification products were electrophoretically separated in 1.7% agarose gel in 1× TBE buffer. Gels were stained with ethidium bromide and photographed. We used standard oligonucleotide primers of the OPA, OPD, and OPN series. After preliminary testing, 11 primers were selected for RAPD analysis of the lilac genome. A matrix of genetic distances was constructed using Nei's coefficient (PHYLIP software package). Dendrograms were constructed by means of hierarchic cluster analysis (UPGMA).

RESULTS AND DISCUSSION

RAPD analysis of 22 species and cultivars of the genus *Syringa* revealed 512 fragments in total. In the case of species, 384 fragments were obtained. Of these, 372 were polymorphic and were used to analyze genome variation in lilac species. The maximum and minimum numbers of fragments per primer were respectively 42 (primer OPD6) and 32 (primer OPD16). Fragments ranged from 0.2 to 3.2 kb in size. A unique RAPD pattern with species-specific fragments was obtained for each lilac species. Twelve monomorphic fragments were observed in the patterns of all species and might be considered as markers of the genus *Syringa* (Fig. 1).

On the basis of the RAPD data, we estimated the genetic distances between *Syringa* species by means of UPGMA cluster analysis and constructed a dendrogram (Fig. 2). As evident from the dendrogram, relatively high interspecific polymorphism is characteristic of the genus *Syringa*. The coefficient of similarity between species varied from 0.2 to 0.42, being comparable with the genome similarity estimated for the genus *Olea*, which belongs to the same family. However, wild *Olea* species showed higher genome variation, with the similarity coefficient ranging from 0.15 to 0.25 [6].

As expected, *S. vulgaris* cultivars clustered together with a wild *S. vulgaris* accession on the dendrogram. Hybrid species *S.* × *chinensis* was located between *S. vulgaris* and *S. oblata*. According to the RAPD data, the genome of *S.* × *chinensis* is more similar to that of *S. vulgaris*. The coefficient of similarity between *S. vulgaris* and *S.* × *chinensis* was 0.2, while that between *S.* × *chinensis* and *S. oblata* was 0.35. The similarity established for the *S.* × *chinensis* and *S. vulgaris* genomes by means of RAPD analysis agreed with mor-

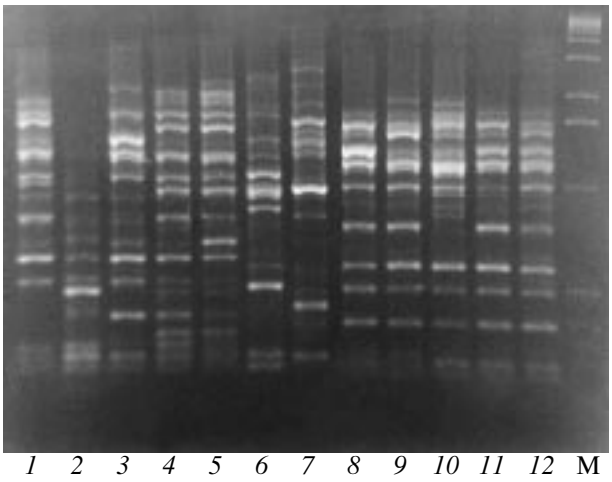

Fig. 1. RAPD patterns obtained with primer OPD6 for 12 accessions of six species of the genus *Syringa*, including (*1*) *S. vulgaris*; (*2*) *S. oblata*; (*3*) *S.* × *chinensis*; (*4*) *S. josikaea*; (*5*) *S. villosa*; (*6*) *S. velutina*; (*7*) *S. amurensis*; and (*8–12*) *S. vulgaris* cultivars: (*8*) Krasnaya Moskva, (*9*) Lucy Balte, (*10*) Ami Shott, (*11*) Flora, and (*12*) Aleksei Mares'ev. M, I-kb molecular weight marker (Ladder, Gibco BRL, United States).

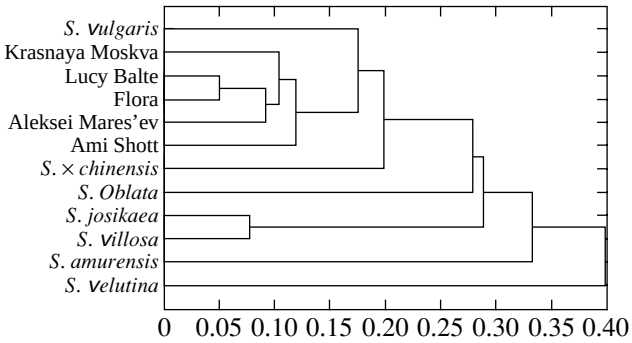

Fig. 2. UPGMA dendrogram of genetic similarity estimated from the RAPD data for lilac species and cultivars.

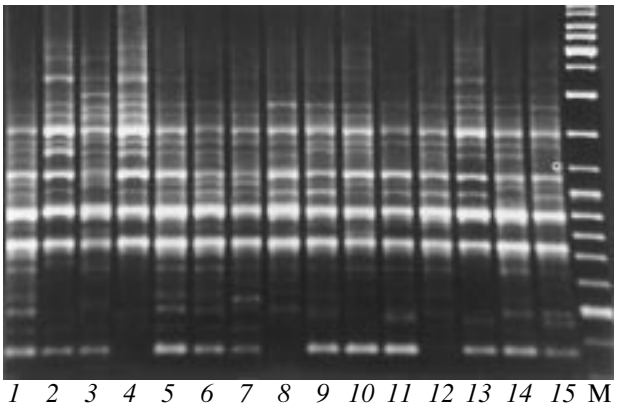

Fig. 3. RAPD patterns obtained with primer OPD5 for 15 lilac cultivars, including (*1*) Krasnaya Moskva, (*2*) Lucy Balte, (*3*) Ami Shott, (*4*) Flora, (*5*) Mulatka, (*6*) Valentina Grizodubova, (*7*) Aleksei Mares'ev, (*8*) Olimpiada Kolesnikova, (*9*) Buffon, (*10*) Primrose, (*11*) Fantasy, (*12*) Madam Floren Stepman, (*13*) Mrs Edward Harding, (*14*) Ogni Donbassa, and (*15*) Russkaya Pesnya. M, molecular weight marker (Ladder Mix, Fermentas GenRuler).

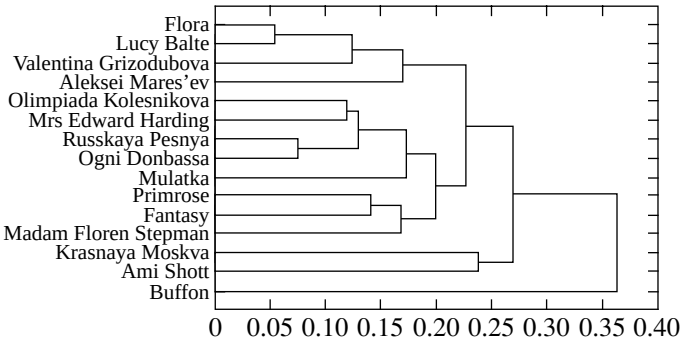

Fig. 4. UPGMA dendrogram of genetic similarity estimated from the RAPD data for 15 lilac cultivars.

phological [2] and molecular [3, 4] data. Separate branches were formed by *S. velutina* and *S. amurensis*, which represent the series *Pubescentes* and *Ligustrina*, respectively. Two species, *S. villosa* and *S. josikaea*, which belong to one series *Villosae*, clustered together. Of note, their genomes proved to be highly similar, having a similarity coefficient of 0.14, which corresponds rather to among-cultivar variation. According to the results of chloroplast DNA and ribosomal sequence analyses and the morphophysiological data, species of the subgenus *Ligustrina* are isolated from other series of the genus. However, as is clearly evident from the dendrogram, *S. amurensis*, the only representative of the subgenus *Ligustrina* in our set, is quite similar in RAPD pattern to the other lilac species (Fig. 2). The greatest difference from the other species of the genus was observed for *S. velutina* (series *Pubescentes*).

In addition to assessing interspecific polymorphism of the genus *Syringa*, we subjected several widespread lilac cultivars to molecular analysis. Unfortunately, for many cultivars, pedigrees were unknown. All but one cultivar represented *S. vulgaris* in our sample. The only exception, cultivar Buffon, has been selected from a population of the hybrid species *S.* × *hyacinthiflora*. RAPD analysis of the lilac cultivars revealed 128 polymorphic DNA fragments. For each cultivar, we obtained a unique RAPD pattern and established cultivar-specific RAPD fragments, which may be employed in cultivar identification (Fig. 3). Genome polymorphism proved to be relatively high in lilac cultivars, and the similarity coefficient reached 0.25 in some cases. This could be explained by the high genome variation characteristic of *S. vulgaris* or by interspecific hybridization, which might be employed in cultivar breeding. As the dendrogram shows, the cultivars clustered into several groups (Fig. 4). However, we could not associate the clustering pattern with any common phenotypic features of cultivars. An exception were cultivars Mrs Edward Harding, Ogni Donbassa, Russkaya Pesnya, and Olimpiada Kolesnikova, which clustered together and were similar in truss structure. As expected, cultivar Buffon was most distant from the other cultivars on the dendrogram, which was attributed to its hybrid origin.

Thus, RAPD analysis of lilac species and cultivars allowed us to estimate for the first time the genetic polymorphism of the genus, to calculate coefficients of genome similarity, to establish phylogenetic relationships among *Syringa* accessions, and to identify species-specific and cultivar-specific markers.

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