Вавиловский журнал генетики и селекции. 2021;25(5):534-542
DOI 10.18699/VJ21.060

Original Russian text www.bionet.nsc.ru/vogis/

The implication of leaf anatomical structure for the selective breeding of lilacs

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Abstract. The cultivars of the common lilac (*Syringa vulgaris*) grown in the south of the Russian Far East are not always winter-hardy and are often damaged by fungal diseases due to a very humid climate. A promising trend in the selective breeding of lilacs in Russia is the creation of new breeding material based on the gene pool of the broadleaf lilac (*S. oblata*) and its hybrids in order to introduce valuable adaptive traits into cultivars. The present work aimed to identify the traits of leaf anatomy in species and cultivars of *Syringa* resistant and susceptible to *Pseudocercospora lilacis*, the causative agent of brown leaf spot disease. The study was carried out on the living collection of the Botanical Garden-Institute, Far Eastern Branch, Russian Academy of Sciences (Vladivostok). The leaf anatomical structure of two *Syringa* species showing different degrees of resistance to *P. lilacis* in the monsoon climate of the Far East (resistant *S. oblata* and weakly resistant *S. vulgaris*, and also their hybrid cultivars) has been analyzed. The differences between species, subspecies, and cultivars are quantitative: they differ in the number of spongy mesophyll layers, the cell height in the first layer of palisade mesophyll, the cell height in the upper and lower epidermises, and the thickness of both mesophylls. The interspecific hybrids resistant or weakly resistant to *P. lilacis* (brown leaf spot disease) mainly retain the leaf anatomy structure of the maternal plant. One of the traits determining the resistance of hybrid lilac cultivars is an increased number of spongy mesophyll layers in the leaf blade. The study of leaf anatomy has shown that the four-layered spongy mesophyll leaf parenchyma correlates with the resistance of lilacs from the subsection *Euvulgaris* to *P. lilacis*. In *S. oblata*, this trait is inherited down the maternal line. To establish lilac cultivars resistant to fungal diseases, it is advisable to cross the two species (*S. oblata* and *S. vulgaris*) or their cultivars using one of *S. oblata* subspecies as a maternal plant.
Key words: *Syringa oblata*; *Syringa vulgaris*; *Pseudocercospora lilacis*; leaf anatomical structure; adaptation; interspecific hybridization.

For citation: Pshennikova L.M. The implication of leaf anatomical structure for the selective breeding of lilacs. *Vavilovskii Zhurnal Genetiki i Selektsii = Vavilov Journal of Genetics and Breeding*. 2021;25(5):534-542. DOI 10.18699/VJ21.060

Значение анатомического строения листа в селекции сиреней

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Аннотация. Сорта сирени обыкновенной (*Syringa vulgaris*) на юге российского Дальнего Востока не всегда зимостойки, повреждаются грибными болезнями, что связано с избыточной влажностью климата. Перспективным направлением отечественной селекции сиреней является создание нового селекционного материала с использованием генофонда *Syringa oblata* и ее гибридов для введения в культурные сорта ценных адаптивных признаков. Целью работы было выявление анатомических особенностей листьев видов и сортов рода *Syringa*, устойчивых и восприимчивых к возбудителю бурой листовой пятнистости *Pseudocercospora lilacis*. Исследования проведены на коллекционном материале открытого грунта Ботанического сада-института ДВО РАН. Проанализировано анатомическое строение листа двух видов *Syringa*, различающихся по устойчивости к *P. lilacis* в муссонном климате Дальнего Востока: устойчивого *S. oblata* и слабоустойчивого *S. vulgaris*, а также гибридных сортов, полученных на их основе. Различия между видами, подвидами и сортами носят количественный характер: число рядов губчатой паренхимы, высота клеток первого ряда палисадной паренхимы, высота клеток верхней и нижней эпидермы, толщина палисадного и губчатого слоя. Устойчивые и малоустойчивые к *P. lilacis* межвидовые гибриды преимущественно сохраняют анатомическое строение листа материнского растения. Одним из определяющих признаков устойчивости гибридных сортов сиреней является повышенное количество рядов губчатой паренхимы листовой пластинки – признак, передающийся от *S. oblata* по материнской линии. Создание устойчивых к грибным заболеваниям сортов сиреней целесообразно проводить путем скрещивания видов *S. oblata* и *S. vulgaris* или их сортов, используя в качестве материнского растения один из подвидов *S. oblata*. В ходе исследования анатомии листа установлено, что губчатая ткань из четырех рядов коррелирует с устойчивостью сиреней из подсекции *Euvulgaris* Schneid. к *P. lilacis* на юге Приморского края. Этот признак листа *S. oblata* передается по наследству по материнской линии.
Ключевые слова: *Syringa oblata*; *Syringa vulgaris*; *Pseudocercospora lilacis*; анатомическое строение листа; адаптация; межвидовая гибридизация.

Introduction

The use of new cultivars that are resistant to pathogenic biota is a solution to problems of not only economic, but also environmental significance. Lilac species and cultivars have long been recognized as valuable ornamental plants. However, the habitat and climatic conditions of Vladivostok and its suburbs are very specific (Agroclimatic Resources…, 1973), which becomes a serious obstacle to the introduction of many exotic, alien trees and shrubs in southern Primorsky Krai, Russia, and their outdoor cultivation. The present study was conducted to extend our knowledge about the mechanisms of plant adaptation to the specific climate in the south of the Russian Far East such as, in particular, the mechanisms of protection against adverse biotic factors of the environment shown by some cultivars from the subsection *Euvulgaris* Schneid. of the genus *Syringa* L. There are a number of works published by various botanical institutions that elucidate the species composition of the pathogenic biota associated with the genus *Syringa* (Khomyakov, Tereschenko, 2000; Tomoshevich, Vorobjova, 2010; Chervyakova, Keldish, 2018; Pavlenkova, 2018; Polyakova, 2018, etc.). We could not find any studies that consider the factors of lilacs' resistance or susceptibility to fungal diseases, and there is also a lack of recent data on the mechanism of lilacs' resistance to fungal diseases.

According to our observations (Pshennikova, 2007, 2018), the most resistant cultivars in southern Primorsky Krai are those from the garden group *Hyacinthiflora*, which have been obtained through interspecific hybridization of *S. oblata* and *S. vulgaris*, freely interbreeding with each other. However, this group also includes cultivars that differ in the degree of susceptibility to pathogenic fungi.

The broadleaf lilac, *Syringa oblata* Lindl., is an introduced plant in Primorsky Krai, brought by S.I. Elovitsky from China in the early 20th century (Vasilyuk et al., 1987). In nature, it is found in the northern part of Northeast China (Saakov, 1960; Mei-chen et al., 1996). Currently it is often used for decorating the landscape of the city of Vladivostok and other populated areas of Primorsky Krai. This species is resistant not only to the winter conditions of the region, but also to pests and fungal diseases, and, apparently, has immunity acquired during the evolution in similar climatic conditions of China. Some interspecific hybrid cultivars possess this resistance (Pshennikova, 2007, 2018).

The common lilac, *Syringa vulgaris* L., a species close to *S. oblata*, is winter-hardy in the conditions of Primorsky Krai. It was introduced into Primorsky Krai in the mid-20th century, probably, from Chernigov Oblast, Ukraine (Vasilyuk et al., 1987). Under the continental climate of its natural habitats in highlands (cretaceous slopes on the Balkan Peninsula), this plant has developed a high drought resistance and tolerance to sudden temperature variations. However, for the same reason, it has not formed mechanisms of protection from high humidity characteristic of the climate in southern Primorsky Krai, which is one of the factors responsible for the high prevalence of pathogenic fungi damaging the species and its cultivars. The lilac brown leaf spot disease (Bunkina et al., 1971) causes especially serious damage to this lilac, resulting in loss of decorative appearance of bushes and premature leaf fall.

The features of leaf anatomical structure in pathogen-resistant species and cultivars are considered to be the primary barriers or passive immunity factors (Vavilov, 1964; Shkalikov et al., 2005; Plotnikova, 2007; Shestakova, 2010, 2013). Leaf traits (pubescence, the thick cuticular layer, the thick epidermis, and also the anatomical specifics of mesophyll) have a significant effect on plant immunity (Furst, 1968; Pautov et al., 2002; Sokolova, 2010; Motyleva, Dzhigadlo, 2012). The first data on specifics of the leaf apparatus structure in hybrid cultivars of ornamental woody plants in the literature date back to the 1970s–1980s (Eremin, Novikova, 1976; Novikova, 1976, 1982; Pham van Nang, 1976; Turovsky et al., 1978; Bykova, 1979).

The present study aimed to identify the features of leaf blade anatomical structure in the *Syringa* species and cultivars, bred on the basis of *S. oblata* and *S. vulgaris*, which differ in the degree of resistance to *Pseudocercospora lilacis* (Desm.) Deighton.

Materials and methods

A total of 22 representatives of subsection *Euvulgaris* Schneid. of the genus *Syringa* L. (Table 1) from the live collection grown on open-air plots of the Botanical Garden-Institute, Far Eastern Branch, Russian Academy of Sciences (BGI FEB RAS), located in the coastal zone of southern Primorsky Krai, were used as objects of the study. The material was collected from 2016 to 2019.

The degree of resistance of lilac species and cultivars to *P. lilacis* was scored on a 5-point scale for ornamental cultivated plants (Tamberg, Ulyanova, 1969), which we adapted for the genus *Syringa* (see Table 2): (1) no disease or up to 10 % of leaf surface of the plant damaged; (2) up to 25 % of leaf surface damaged; (3) up to 50 % of leaf surface damaged; (4) up to 75 % of leaf surface damaged; (5) over 75 % of leaf surface damaged.

To analyze mesophyll, five leaf blades were used. The third leaf from the base of a vegetative shoot, completely grown, was sampled from the southern aspect of crown. Leaves were fixed in 70 % ethyl alcohol. Cross-sections through the middle part of a leaf blade between the midrib and the leaf edge were cut on a freezing microtome, stained with a safranin solution, and embedded in glycerol/jelly. The sections were examined under a Zeiss Axioplan 2 Imaging microscope (Carl Zeiss, Germany) using the AxioVision 4 software. The data obtained were processed in the MS Excel package.

The following anatomical characters of a leaf were considered: leaf thickness, height of upper and lower epidermis, thickness of palisade mesophyll, thickness of spongy mesophyll, number of layers of spongy and palisade mesophylls, and size of cells in the 1st and 2nd layers of palisade mesophyll in hybrid cultivars (Fig. 1). The study was conducted at the Center for Collective Use "Microtechnical Laboratory", BGI FEB RAS, and "Bioresourse Collection".

Statistical analysis was carried out using the STATISTICA 6.0 software package. The data were tested for normality of distribution using the Shapiro–Wilk *W*-test (Shapiro, Wilk, 1965). To search for a statistical relationship between the variables, a correlation analysis was performed using

Table 1. Objects of study

No.	Species/subspecies/cultivar, Author	Cultivar's parents	Year of cultivar's creation
1	*S. vulgaris* L.	–	–
2	*S. oblata* subsp. *oblata* Lindl.	–	–
3	*S. oblata* subsp. *dilatata* (Nakai) P.S. Green et M.C. Chang	–	–
4	*S. oblata* var. *alba* Rehder	–	–
5	*S. oblata* 'Wan Hua Zi', Zang et Fan	*S. oblata* × ?	1984
6	'Xiang Xue', Zang et Fan	*S. oblata* × *S. vulgaris* 'Alba plena'	1984
7	'Luo Lan Zi', Zang et Fan	*S. oblata* × *S. vulgaris* 'Alba plena'	1962
8	'Maiden's Blush', Skinner	*S. oblata* subsp. *dilatata* × *S. vulgaris*	1966
9	'Olimpiada Kolesnikova', Kolesnikov	'Tamara Kolesnikova' × 'Berryer'	1941
10	'Vechernii Vladivostok' ('Evening Vladivostok'), Pshennikova	*S. oblata* subsp. *oblata* × *S. vulgaris*	2007
11	'Neizvestnyi Soldat' ('Unknown Soldier'), Pshennikova	*S. vulgaris* 'Bogdan Khmelnitsky' × *S. oblata* subsp. *oblata*	2017
12	'Neznakomka' ('Stranger Woman'), Pshennikova	*S.oblata* subsp. *oblata* × *S. vulgaris*	2008
13	'Bogdan Khmelnitsky', Rubtsov, Zhogoleva, Lyapunova	*S. vulgaris* × ?	1954
14	'Capitaine Baltet', Lemoine	*S. vulgaris* × ?	1919
15	'Charles Joly', Lemoine	*S. vulgaris* × ?	1896
16	'Mme Florent Stepman', Stepman-Demessemaeker	*S. vulgaris* × ?	1908
17	'Romance', Havemeyer et Eaton	*S. vulgaris* × ?	1954
18	'Buffon', Lemoine	*S.* × *hyacinthiflora*	1921
19	'Esther Staley', Clarke	*S.* × *hyacinthiflora*	1948
20	'Pocahontas', Skinner	*S.* × *hyacinthiflora*	1935
21	'Tom Tayler', Skinner	*S.* × *hyacinthiflora*	1962
22	'Dal'nevostochnitsa' ('Far Eastern Woman'), Pshennikova	'Olimpiada Kolesnikova' × ?	2018

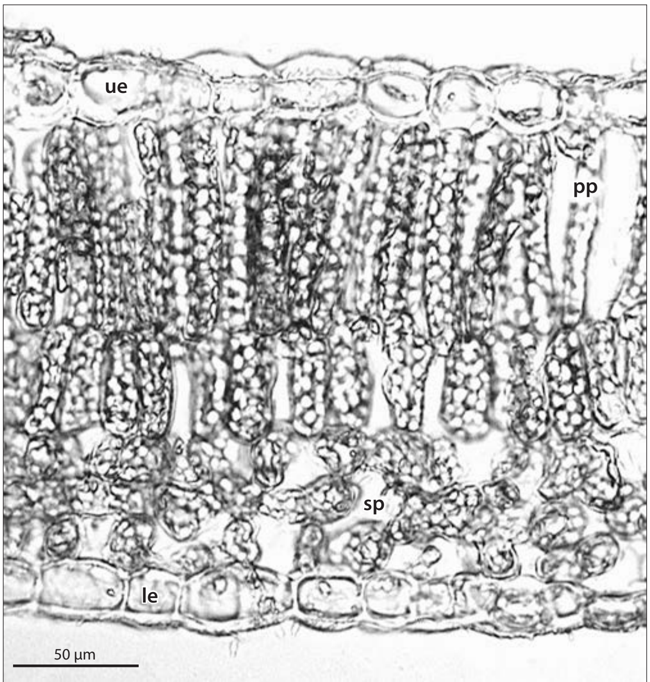

Fig. 1. A leaf cross-section from the lilac cultivar 'Neizvestnyi Soldat'.

The letter designations are as follows: ue, upper epidermis; pp, palisade mesophyll parenchyma; sp, spongy mesophyll parenchyma; le, lower epidermis.

the Spearman Rank Order Correlations (Fieller et al., 1957). Linear measurements of leaf blade tissues were assumed to be independent variables. Degree of resistance of a species/cultivar to *P. lilacis* was assumed to be a dependent variable. According to the *W*-test, the distribution of analyzed data differed from the normal one (p-value < 0.05). Measurements for each representative were made in 20 to 30 replicates; the total number of observations was 454.

Results and discussion

Long-term observations on the species and cultivars of the genus *Syringa* made it possible to arrange them in the order of increasing degree of their leaves' resistance to *P. lilacis* (Table 2).

The first group included two subspecies of *S. oblata* and their hybrid cultivars. Subspecies of *S. oblata* differ in the shape and size of leaf blade. The study of the leaf anatomy of *S. oblata* and *S. vulgaris* allowed identification of the distinguishing traits of these species (Fig. 2, Table 3).

According to the data obtained, the species *S. oblata* resistant to *P. lilacis* differs from the non-resistant species *S. vulgaris* by an increased number of spongy mesophyll layers. The subspecies of *S. oblata* differ from one another in the thickness of leaf blade and the height of palisade mesophyll cells. *S. oblata* subsp. *oblata* has cells of the upper layer of

Table 2. The degree of resistance of the *Syringa* varieties and species to the fungal diseases *Pseudocercospora lilacis*

Degree of stability	Taxa and cultivars
1 No disease or up to 10 % of leaf surface of the plant damaged	*S. oblata* subsp. *oblata*, *S. oblata* subsp. *dilatata*, *S. oblata* var. *alba*, 'Wan Hua Zi', 'Xiang Xue', 'Luo Lan Zi', 'Tom Tayler', 'Pocahontas', 'Maiden's Blush', 'Vechernii Vladivostok', 'Dal'nevostochnitsa', 'Neznakomka', 'Neizvestnyi Soldat'
2 Up to 25 % of leaf surface damaged	'Olimpiada Kolesnikova', 'Buffon'
3 Up to 50 % of leaf surface damaged	'Bogdan Khmelnitsky'
4 Up to 75 % of leaf surface damaged	'Romance'
5 Over 75 % of leaf surface damaged	*S. vulgaris*, 'Capitaine Baltet', 'Charles Joly', 'Mme Florent Stepman', 'Esther Staley'

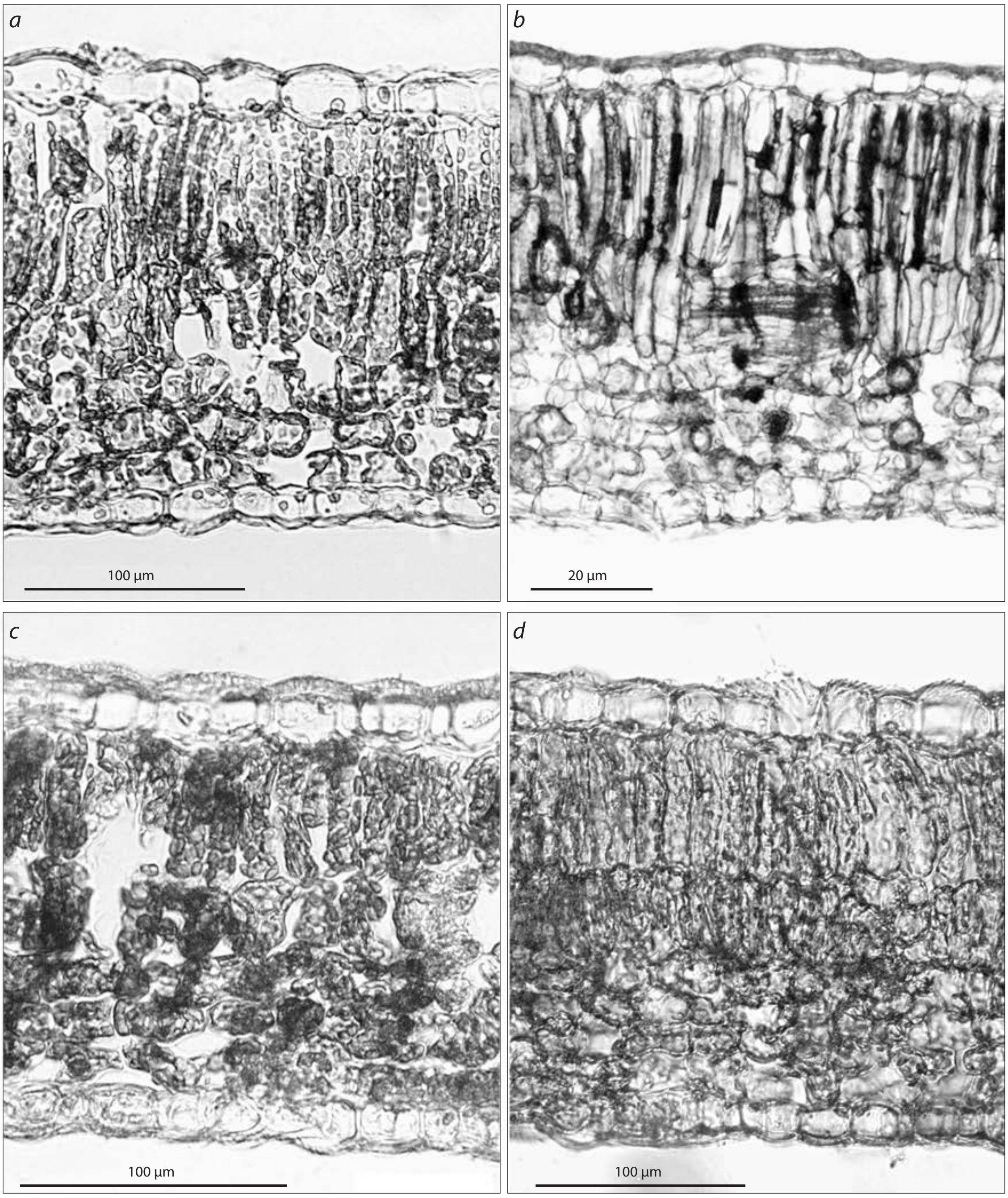

Fig. 2. Leaf cross-sections from the following lilacs:
a, *S. vulgaris*; *b*, *S. oblata* subsp. *oblata*; *c*, *S. oblata* subsp. *dilatata*; *d*, *S. oblata* var. *alba*.

Table 3. Characteristics of leaf cross-sections from species of the genus *Syringa*

Taxon	Epidermis cell length, µm		Palisade mesophyll				Spongy mesophyll		Leaf thickness, µm
	upper	lower	Thickness, µm	Number of layers	Cell length, µm		Number of layers	Thickness, µm	
					1st layer	2nd layer			
S. oblata subsp. *oblata*	24.9 ± 0.7	19.8 ± 0.6	209.9 ± 5.0	2 (3)	140.5 ± 1.0	64.7 ± 1.3	4 (5)	88.6 ± 2.4	351.8 ± 3.0
S. oblata subsp. *dilatata*	26.1 ± 0.6	17.9 ± 0.5	80.4 ± 2.2	2	46.6 ± 1.3	38.4 ± 1.5	3–4	50.9 ± 1.4	183.7 ± 2.8
S. oblata var. *alba*	26.3 ± 0.7	18.3 ± 0.5	103.6 ± 1.2	2	63.5 ± 0.8	41.7 ± 0.7	4 (5)	61.9 ±0.9	212.8 ± 1.3
S. vulgaris	26.8 ± 0.9	18.6 ± 0.7	110.1 ± 5.8	2 (3)	69.5 ± 1.9	44.3 ± 1.0	3	55.4 ± 1.5	219.6 ± 1.9

Table 4. Characteristics of leaf cross-sections from the *S. oblata* cultivars

Cultivar	Epidermis cell length, µm		Palisade mesophyll				Spongy mesophyll		Spongy mesophyll
	upper	lower	Thickness, µm	Number of layers	Cell length, µm		Number of layers	Thickness, µm	
					1st layer	2nd layer			
'Vechernii Vladivostok'	24.9 ± 0.9	18.2 ± 0.5	233.6 ± 3.3	2 (3)	142.6 ± 9.1	70.2 ± 2.4	4 (5)	102.2 ± 4.1	391.7 ± 3.9
'Neznakomka'	19.2 ± 0.6	16.7 ± 0.4	144.8 ± 2.5	2 (3)	92.7 ± 1.8	51.3 ± 1.4	4	79.6 ± 2.1	262.0 ± 3.5
‘Luo Lan Zi’	34.8 ± 1.0	20.7 ± 0.5	141.6 ±3.6	2	88.8 ± 4.8	44.9 ± 0.9	4 (3)	49.5 ± 1.6	245.9 ± 1.9
‘Maiden’s Blush’	29.6 ± 0.9	19.5 ± 0.5	120.3 ± 1.5	2	77.4 ± 1.1	42.8 ± 0.8	4 (3)	58.5 ± 1.1	225.8 ± 1.8
‘Xiang Xue’	22.9 ± 0.5	20.7 ± 0.6	88.8 ± 1.2	2	50.8 ± 0.9	39.4 ± 0.8	4 (3)	61.2 ± 1.3	192.4 ± 1.7
‘Wan Hua Zi’	23.9 ± 0.7	19.9 ± 0.7	188.8 ± 3.1	2 (3)	115.4 ± 2.0	55.1 ± 0.9	4	84.9 ± 1.9	312.8 ± 3.2

palisade mesophyll almost twice as large as the second layer cells. The revealed difference may be a systematic trait of *S. oblata* subsp. *oblata* on the anatomical level. In *S. oblata* subsp. *dilatata*, the heights of the layers of palisade mesophyll cells are either equivalent, or the first layer is slightly, almost 1.2-fold, larger than the second one (see Fig. 2, *c*). This subspecies has upper epidermis cells as large as those in *S. vulgaris*.

We found that the thickness of leaf epidermis and mesophyll in the species under study are not related to resistance against *P. lilacis* (see Table 3). Thus, the height of the upper epidermis cells in the weakly resistant species *S. vulgaris* is greater than that in the fungus-immune subspecies *S. oblata* subsp. *oblata*, or the ratio of the 1st and 2nd layers of palisade mesophyll in *S. vulgaris* is the same as in the subspecies *S. oblata* subsp. *dilatata* resistant to the fungal pathogen. The number of palisade mesophyll layers is not a constant trait in these species and can vary from 2 to 3. The subspecies *S. oblata* subsp. *dilatata* has the leaf anatomical traits close to those of *S. vulgaris* and *S. oblata* subsp. *oblata*.

The following group of lilacs combines cultivars obtained through hybridization of two species, *S. oblata* (maternal species) and *S. vulgaris*. The cultivars ‘Vechernii Vladivostok’, ‘Neznakomka’, ‘Luo Lan Zi’, and ‘Wan Hua Zi’ repeat the structural features of *S. oblata* subsp. *oblata*; they do not differ in the number of layers of palisade and spongy mesophylls (Table 4, Fig. 3, *a–d*); these cultivars are resistant to *P. lilacis*. It has been found that the *S. oblata* cultivars are distinguished by the height of the upper and lower epidermises, the thickness of palisade and spongy mesophyll parenchyma, and the leaf thickness. The resistant cultivars are characterized by the increased number of spongy mesophyll layers, 4 or more.

The cultivars ‘Xiang Xue’ and ‘Maiden’s Blush’ are also resistant to *P. lilacis*. Their leaf anatomical structure is characteristic of *S. oblata* subsp. *dilatata*. Their spongy mesophyll is mostly 4-layered, but 3 layers are also observed sometimes.

Another group under study combined the cultivars with both parents being *S. vulgaris*: ‘Capitaine Baltet’, ‘Charles Joly’, ‘Mme Florent Stepman’, ‘Romance’, and ‘Bogdan Khmelnitsky’, which proved to be non-resistant to *P. lilacis* to varying degrees. Their leaves are medium in thickness, the palisade mesophyll is 2-layered, and the spongy mesophyll is 3-layered. In ‘Bogdan Khmelnitsky’, the spongy mesophyll consists sometimes of 4 layers of cells (see Fig. 3, *f*). This can apparently be explained by the fact that the species *S. oblata* was used for breeding the parental cultivars of ‘Bogdan Khmelnitsky’ at some stage in the past. Compared to the other *S. vulgaris* cultivars, this one is more resistant to the fungal pathogen. The cultivars differ in the height of cells of upper and lower epidermises and in the thickness of palisade and spongy mesophylls. The leaf structure is typical of *S. vulgaris* (Table 5).

The following group of lilacs combined hybrid cultivars referred to as *Hyacinthiflora*, or hyacinth lilacs. These are the cultivars ‘Olimpiada Kolesnikova’, ‘Dal’nevostochnitsa’, ‘Neizvestnyi Soldat’, ‘Buffon’, ‘Esther Staley’, etc. (see Table 1). Plants from this group are distinguished both by

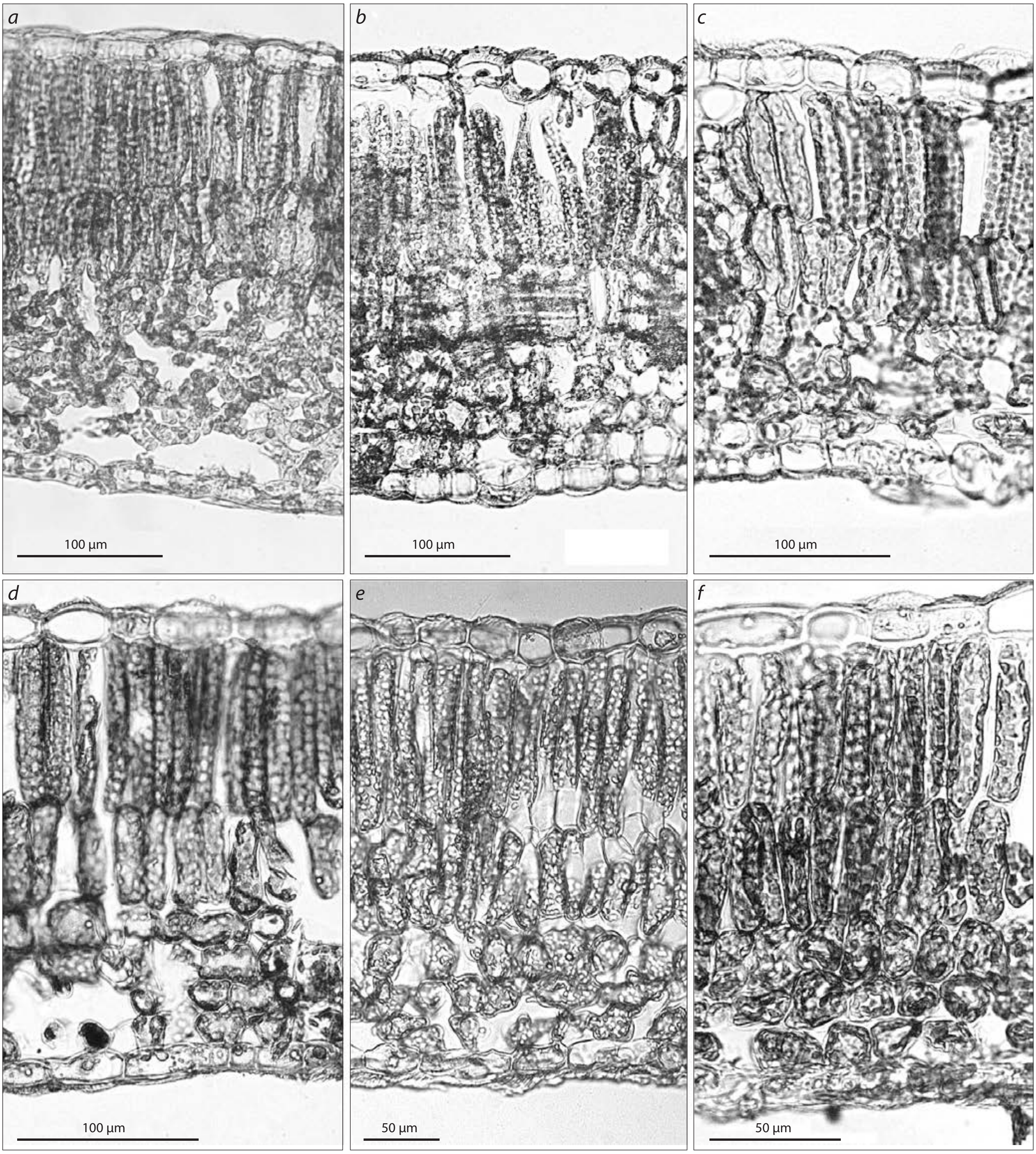

Fig. 3. Leaf cross-sections from lilacs of the following cultivars:
a, 'Vechernii Vladivostok'; *b*, 'Wan Hua Zi'; *c*, 'Maiden's Blush'; *d*, 'Neznakomka'; *e*, 'Olimpiada Kolesnikova'; *f*, 'Bogdan Khmelnitsky'.

leaf anatomical structure (Table 6) and by their resistance to *P. lilacis*. In Russian literature, the cultivar 'Olimpiada Kolesnikova' is attributed to the group of *S. vulgaris* cultivars (Rubtzov et al., 1980; Okuneva et al., 2008). According to the literature data, the cultivar 'Berryer' (*S. oblata* × *S. vulgaris*) was involved in breeding this cultivar. The mesophyll is 5-layered, consisting of 2 layers of palisade and 3(4) layers of spongy mesophylls. By its resistance to *P. lilacis*, the cultivar is placed in group 2 (see Table 2). The cultivar 'Esther Staley' bred by American lilac breeders (parents are unknown) is non-resistant to *P. lilacis*. The cultivar 'Ester Staley' was probably obtained on the basis of *S. vulgaris*. The mesophyll

Table 5. Characteristics of leaf cross-sections from the *S. vulgaris* cultivars

Cultivar	Epidermis cell length, μm		Palisade mesophyll		Spongy mesophyll		Leaf thickness, μm
	upper	lower	Thickness, μm	Number of layers	Thickness, μm	Number of layers	
'Capitaine Baltet'	29.4 ± 0.9	20.6 ± 0.5	150.4 ± 3.5	2	66.7 ± 2.7	3	257.9 ± 2.8
'Charles Joly'	25.0 ± 0.9	20.8 ± 0.9	133.7 ± 3.1	2	74.9 ± 1.8	3	255.3 ± 3.5
'Mme Florent Stepman'	28.9 ± 0.9	20.5 ± 0.5	108.5 ± 1.3	2	68.5 ± 0.9	3	221.9 ± 1.0
'Romance'	19.9 ± 0.6	13.1 ± 0.6	105.4 ± 5.9	2	52.6 ± 1.4	3	200.9 ± 4.1
'Bogdan Khmelnitsky'	21.1 ± 0.5	17.8 ± 0.5	113.6 ± 1.2	2	55.7 ± 1.6	3 (4)	204.7 ± 2.1

Table 6. Characteristics of leaf cross-sections from lilacs of the *Hyacinthiflora* group

Cultivar	Epidermis cell length, μm		Palisade mesophyll		Spongy mesophyll		Leaf thickness, μm
	upper	lower	Thickness, μm	Number of layers	Thickness, μm	Number of layers	
'Buffon'	21.8 ± 0.6	16.4 ± 0.6	126.5 ± 2.7	2 (3)	58.9 ± 2.13	3 (4)	226.5 ± 1.84
'Esther Staley'	25.5 ± 0.8	18.9 ± 0.7	148.3 ± 2.7	2 (3)	69.4 ± 1.5	3 (4)	248.9 ± 5.3
'Pocahontas'	25.3 ± 0.5	16.7 ± 0.4	89.8 ± 1.5	2	51.2 ± 1.1	3–4	192.02 ± 1.8
'Tom Tayler'	28.3 ± 0.8	22.2 ± 0.5	133.1 ± 1.9	2 (3)	57.8 ± 1.8	4 (3)	235.2 ± 1.7
'Olimpiada Kolesnikova'	31.4 ± 1.4	22.6 ± 0.6	163.2 ± 1.9	2	93.8 ± 2.5	3 (4)	301.2 ± 3.8
'Dal'nevostochnitsa'	24.9 ± 1.0	18.1 ± 0.8	154.1 ± 3.4	2	67.9 ±1.8	4	270.7 ±5.9
'Neizvestnii Soldat'	28.3 ± 0.8	22.2 ± 0.5	133.1 ± 1.9	2 (3)	57.8 ± 1.8	3 (4)	235.2 ± 1.7

Table 7. Spearman's coefficient of correlation between the leaf characters and the resistance to *Pseudocercospora lilacis*

Leaf character	*N*	*R*	*t*	*p*-value
Upper epidermis *vs*. Degree of resistance	454	0.013074	0.2780	0.781154
Lower epidermis *vs*. Degree of resistance	454	–0.019263	–0.4096	0.682290
Palisade mesophyll thickness *vs*. Degree of resistance	454	–0.091657	–1.9569	0.050975
Number of palisade mesophyll layers *vs*. Degree of resistance	454	–0.029622	–0.6301	0.528974
Number of spongy mesophyll layers *vs*. Degree of resistance	454	–0.555699	–14.2104	0.000001
Spongy mesophyll thickness *vs*. Degree of resistance	454	–0.028454	–0.6052	0.545361
Leaf thickness *vs*. Degree of resistance	454	–0.076463	–1.6304	0.103712

Note. *N*, number of observations; *R*, Spearman's correlation coefficient; *t*, value of the Student's *t*-test for the number of degrees of freedom of $n-2$; *p*-value, probability of error for the null hypothesis that there is no relationship between the characters.

structure in the cultivar 'Neizvestnyi Soldat' is similar to that described above, but it proved to be resistant to *P. lilacis*. Some cultivars of this group ('Dal'nevostochnitsa', 'Tom Tayler', and 'Pocahontas') are resistant to *P. lilacis* and have mainly 4-layered spongy mesophyll. It is likely that the resistance of the cultivars from the *Hyacinthiflora* group is related to the amount of genetic material obtained from parents. The 4-layered spongy mesophyll is not a trait of passive immunity, but rather serves as a marker of the presence of genetic material from *S. oblata* in the hybrid.

A statistical analysis (Table 7) showed the relationship between the number of spongy mesophyll layers and the degree of plants' resistance to *P. lilacis* (p-value < 0.001).

We have found that the more layers the spongy mesophyll includes, the higher the resistance to *P. lilacis* (see Table 2). We have not observed any correlation relationship between the other leaf characters and the degree of resistance to *P. lilacis*.

The cultivars that are non-resistant to *P. lilacis* have the leaf anatomical structure similar to that of *S. vulgaris*. Thus, the number of spongy mesophyll layers can be used to predict the resistance of new lilac cultivars in the climatic conditions of southern Primorsky Krai.

According to our data, a characteristic structural feature of *S. oblata* and all cultivars resistant to *P. lilacis* is the 4-layered spongy mesophyll parenchyma. The cultivars of *S. oblata* subsp. *oblata* are similar in the ratio of heights of the first and second layers of palisade mesophyll parenchyma. The increased number of spongy mesophyll layers (4 or more) correlates (see Table 7) with the resistance to fungal disease. On the other hand, hybrids *S. vulgaris* × *S. oblata*, in which 4-layered spongy mesophyll is sometimes found, can be both resistant and non-resistant to *P. lilacis*. Our conclusions drawn from the examination of the leaf blade anatomical structure in lilacs are consistent with those published by Chinese breeders (Zang et al., 1983; Shuying et al., 1995). These authors reported that the inheritance of maternal traits dominates in hybrid offspring of *S. oblata* × *S. vulgaris*.

We have also found that the examination of leaf anatomy allows identification of the parents of *Syringa* hybrid offspring on the species level, because lilac cultivars retain the structural plan of the maternal plant. In members of the genus *Cerasus* Mill. (Motyleva, Dzhigadlo, 2012; Shestakova, 2013), there is a clear relationship between the primary leaf barriers and resistance to another fungal disease, coccomycosis. Contradictory data were obtained by Turovsky with co-authors (Turovsky et al., 1978), who explain the resistance of cherry tree to a fungal pathogen by the functional features of the host plant. We have established that the structural elements of the leaf anatomical structure (such as the thickness of epidermis and mesophyll) in the studied taxa of the genus *Syringa* are not the primary defense against infection.

Conclusion

Certain traits of the leaf blade anatomical structure in lilacs from the subsection *Euvulgaris* Schneid. of the genus *Syringa*, such as, in particular, the spongy mesophyll parenchyma consisting of 4 layers, can be considered an indicator of the degree of their resistance to *Pseudocercospora lilacis* in southern Primorsky Krai. For creating lilac cultivars resistant to fungal diseases, it is expedient to cross two species (*S. oblata* and *S. vulgaris*) or their cultivars, using one of the subspecies of *S. oblata* as a maternal plant. With free pollination, only seeds from resistant cultivars should be taken. The difference in the mesophyll anatomical structure observed in *S. oblata* subsp. *oblata* and *S. oblata* subsp. *dilatata* can be used as an additional diagnostic trait.

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Acknowledgements. The study was conducted within the framework of the State assignment according to the research plan for the project No. AAAA-A20-120042090002-0 "Introduction and conservation of *ex situ* and *in vitro* of plant genetic resources in East Asia".

The author is grateful to the senior researcher, the head of the Microtechnical laboratory, Dr. M.N. Koldaeva, to the head of the Laboratory of introduction and selection, Dr. V.A. Kalinkina and Dr. E.V. Boltenkov for the help in preparing the manuscript, and to the junior researcher S.P. Tvorogov for the help with statistical analysis.

Conflict of interest. The author declares no conflict of interest.

Received October 14, 2020. Revised May 11, 2021. Accepted May 17, 2021.