



Achievements in Research on Vavilovia (*Vavilovia formosa* (Stev.) Fed.), a Legume Crop Wild Relative

Aleksandar Mikić · Petr Smýkal · Gregory Kenicer · Nune Sarukhanyan · Janna Akopian ·
Ivan Gabrielyan · Armen Vanyan · Andrey Sinjushin · Natalia Demidenko · Branko Čupina ·
Vojislav Mihailović · Margarita Vishnyakova · Mike Ambrose

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Summary: *Vavilovia* (*Vavilovia formosa* (Stev.) Fed.) belongs to the tribe *Fabeae* along with peas, vetchlings, vetches and lentils. It prefers high mountain areas in Armenia, Azerbaijan, Georgia, Iran, Iraq, Lebanon, Russia, Syria and Turkey. A true success in the *ex situ* conservation has recently been achieved, within the display plot Flora and Vegetation of Armenia in the Yerevan Botanic Garden. The hybridization between *vavilovia* and other *Fabeae* was done in the N. I. Vavilov Institute of Plant Industry with F_1 seeds and F_1 plants that did not produce the next generation. The recent molecular research showed that *vavilovia* belongs to a *Lathyrus-Pisum-Vavilovia* clade with a clearly distinct status.

Key words: conservation, crop wild relatives, *Fabeae*, genetic resources, molecular taxonomy, *Vavilovia formosa*

The Tribe *Fabeae*

The tribe *Fabeae* (syn. *Vicieae*) is among the richer ones within the family of legumes (*Fabaceae* Endl). It is estimated that it comprises more than 300 species, with a taxonomic structure that is still rather dynamic. Many of the *Fabeae* species have extreme economic importance and repre-

sent one of the most significant crops in the world. They also include one of the most ancient cultivated species in the world (Ljuština & Mikić 2010). Among them are common pea (*Pisum sativum* L.), faba bean (*Vicia faba* L.) and common lentil (*Lens culinaris* Medik.), as well as grass pea (*Lathyrus sativus* L.) and other vetchlings and common vetch (*Vicia sativa* L.) with other vetches. All these species may be regarded as multi-functional crops that are used both for human consumption and in animal feeding and numerous non-food purposes (Mikić et al 2006), in the forms such as green forage, forage dry matter, forage meal, pods with undeveloped grains, immature grain, mature grain, straw, green manure and aboveground biomass (Mihailović et al. 2010).

It is widely regarded that the tribe *Fabeae* consists of five genera. Two of them, vetchling (*Lathyrus* L.) and vetch (*Vicia* L.) comprise more than 100 species each. Another two, lentil (*Lens* Mill.) and pea (*Pisum* L.), are less numerous, with four species in the former and two species in the latter. So far, these four have had a well-established taxonomical status. Unlike them, the fifth monospecific genus *vavilovia* (*Vavilovia* Fed.) has been considered merely a species within nearly all the

A. Mikić (✉) · V. Mihailović
Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000
Novi Sad, Serbia
e-mail: aleksandar.mikic@ifvcns.ns.ac.rs

P. Smýkal
Agritec Plant Research Ltd., Šumperk, Czech Republic

G. Kenicer
Royal Botanical Garden Edinburgh, Edinburgh, UK

N. Sarukhanyan · A. Vanyan
Green Lane Agricultural Assistance NGO, Yerevan, Armenia

J. Akopian · I. Gabrielyan
National Academy of Sciences, Institute of Botany, Yerevan, Armenia

A. Sinjushin · N. Demidenko
M. V. Lomonosov Moscow State University, Moscow, Russia

B. Čupina
University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia

M. Vishnyakova
State Scientific Centre N.I. Vavilov All-Russian Research Institute of
Plant Industry of Russian Academy of Agricultural Science, St. Peter-
sburg, Russia (VIR)

M. Ambrose
John Innes Centre, Norwich, UK

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others. Although it was veiled into many uncertainties and despite its extreme rarity, resulting in the nicknames such as *Legume Fata Morgana* or *Legume Nessie*, vavilovia has recently been gaining a lot of new interest due to its potential to provide the legume research community with precious information on the past, the present and the future of the entire tribe *Fabeae* (Mikić et al 2009).

Status

The first written report ever on vavilovia was made nearly two centuries ago (Steven 1812). The newly discovered species was included in the genus *Orobus* L. and immediately, since its premier appearance before the scientific community, was acknowledged for the beauty of its flowers by being named *Orobus formosus* Stev.

The genus *Orobus* was subsequently disregarded and its species were included mostly to the genus *Lathyrus*. The species *O. formosus* was incorporated within numerous related genera. Most often, it was considered a pea species and was known as *Pisum formosum* (Stev.) Alef., symbolically 'beautiful' or perennial pea (Alefeld 1861, Alefeld 1866), as well as *P. aucheri* Jaub. et Spach., Aucher's pea, *P. formosum* Boiss. (Boissier 1872) and *P. frigidum* Alef. The results of a more contemporary research of certain floral features, such as diverse androecium and pistil characters, classified this species as a pea subgenus (Gunn & Kluge 1976). Vavilovia was also described as a vetch, named *V. aucheri* Boiss. and *V. variegata* var. *aucheri* (Jaub. & Spach) Bornm., as well as a vetchling species, denoted as *L. frigidus* Schott & Kotschy.

More recently, vavilovia was considered a species belonging to a novel genus within the tribe *Fabeae*. One of such classifications has become the most widely accepted, denoting it as *Vavilovia formosa*, 'beautiful' or simply vavilovia, belonging to the genus with the same name, *Vavilovia* Fed. (Fedorov 1939). Initially, this classification distinguished two species, *V. formosa* (Stev.) Fed. and *V. aucheri* Fed., with one coming from the Greater and one from the Lesser Caucasus. Later, obvious transitional plant forms in the Armenian material were found and these two species merged into one, under the name *V. formosa* (Fedorov 1952). The genus was named to honour N. I. Vavilov, who was a pioneer of the study of cultivated plants and remained as symbol of the importance of crop wild relatives.

Among the remaining classifications dealing with vavilovia, it is worthy to mention that it was placed in another novel genus, *Alophotropis* (Jaub & Spach) Grossh. as one or two species,

A. aucheri (Jaub. & Spach) Grossh. (Czerepanov 1981) and *A. formosa* (Stev.) Grossh. (Grossheim 1949, Lamprecht 1972).

There are few reports on the existence of certain variability within vavilovia as a species. This led to a suggestion of hypothetical subtaxa, such as *Orobus formosus* var. *microphyllus* Ser. or *Pisum formosum* var. *pubescens* C. C. Townsend (Townsend 1968).

Characterization

Vavilovia is a diploid species with $2n = 14$ chromosomes. The karyotype of vavilovia consists of three pairs of submetacentric chromosomes without satellites, one pair of submetacentric chromosomes with small-diameter satellites, one pair of submetacentric chromosomes with satellites of a diameter equal to that of a chromosome alone and two pairs of metacentric chromosomes (Abramova 1971).

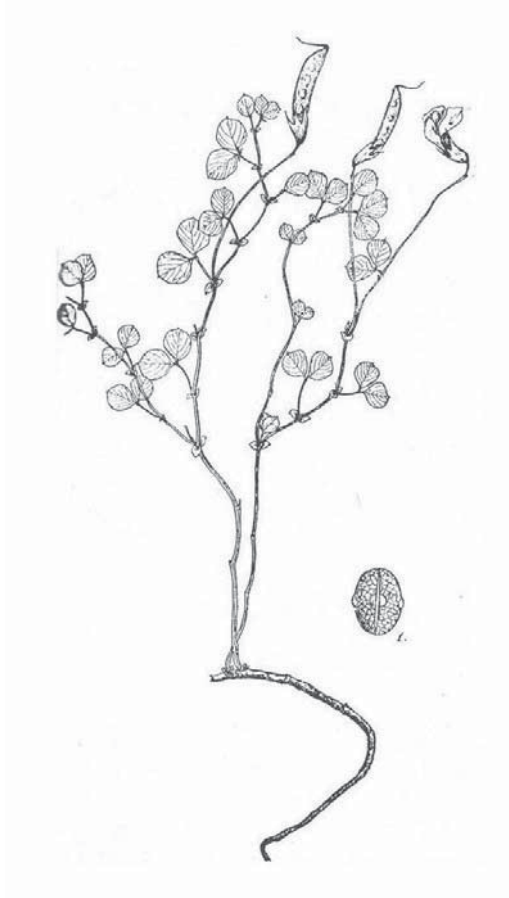


Figure 1. A plant of vavilovia, with rhizomes and aboveground organs (Gabrielyan 1962)

Slika 1. Biljka vavilovije, sa rizomima i nadzemnim organima

The results of rare anatomical analysis demonstrated a fragmented similarity between vavilovia on one side and Ethiopian pea (*P. sativum* subsp. *abyssinicum* Govorov) and grass pea on the other side (Makasheva et al. 1973).

As a perennial and herbaceous plant, vavilovia has a dwarf habit in comparison to other related genera such as pea. Its height, denoting a distance between the soil surface and the highest parts of a plant, varies from 5 cm to 15 cm. Vavilovia roots are long. The underground stem forms rhizomes that may be essentially responsible for its ability to survive grazing and stone deposition on easily movable screes. The aboveground stems are slender, sprawling or creeping, without wings and glabrous (Fig. 1).

Vavilovia leaves are compound, like in many other legume species. Its stipules are small, semi-sagittate, foliaceous and free from the petiole. The leaflets of a vavilovia leaf are broad, of a cuneate-obovate to suborbicular shape, thick, with glabrous surface and with entire margins. Certain populations have leaflets with keel-like basis, being a feature used to distinguish the two vavilovia species that were subsequently discarded in favour of only one (Sinjushin et al 2009). There is only one pair of leaflets in a vavilovia compound leaf. The petiole in vavilovia does not end with tendrils, like in many species of its closest genera, such as pea, grass pea or common vetch, but with one micro-licle formation, similar to that in faba bean.

The flowers of vavilovia are axillary, pedunculate and usually solitary. Bracts are small and/or inconspicuous and without bracteoles. Calyx is campanulate, while its teeth are subequal and narrowly triangular. Corolla in vavilovia flowers is pink or purple. Standard petal is usually oblong, with a short and broad claw. Wing petal is falcate or oblong. Keel is shorter than the wing, non-cristate and sometimes white. Stamens are monadelphous or diadelphous, while anthers are glabrous and smooth. Vavilovia is considered a cross-pollinating species, although without accurate data yet.

Vavilovia pods are linearly oblong, long from 20 mm to 35 mm and prone to dehiscence in full maturity. One pod bears between three and five seeds. Vavilovia seeds are globose or oval, smooth and often with dark blotches on the surface.

The seed protein composition of vavilovia is more similar to that of vetchlings in comparison to that of pea, with globulin, legumin and vicine rather partially identical. The largest portion of the vavilovia seed protein is a component not present in pea or vetchlings (Makasheva 1979).

Ecogeography and *in situ* Conservation

Vavilovia prefers mountain areas with altitudes from 1,500 m up to 3,500 m. It grows on shale or rocky ground, with loose limestone scree as a typical example. Although the main homeland of



Figure 2. Geographical distribution of vavilovia
Slika 2. Geografska rasprostranjenost vavilovije



Figure 3. A vavilovia population from Dagestan, Russia (photo A. Ivanov)
Slika 3. Populacija vavilovije u Dagestanu, Rusija (fotografija A. Ivanova)

vavilovia are Central and Eastern Caucasus (Grossheim 1952, Galushko 1980), its geographical area of distribution is rather disjunctive (Fig. 2). In the Russian Federation, isolated vavilovia populations may be found in Cabardino-Balkaria, Dagestan (Fig. 3), Karachevo-Cherkessia and Northern Ossetia (Dzuybenko & Dzuybenko 2009). Vavilovia is present in the wild floras of other ex-USSR countries, such as Armenia (Fedorov 1952), Azerbaijan (Carjagin 1954, Gadzhiev & Musaeu 1994) and Georgia (Kolakovskiy 1958, Arabuli 1981), as well as in the neighbouring regions in Iran (Rechinger 1979), Iraq (Townsend & Guest 1974), Lebanon and Syria (Maxted & Ambrose 2000) and Turkey (Davies 1970). In many of these countries vavilovia is considered an endangered species and is officially protected (Popov 1988, Gabrielyan 1990).

The N. I. Vavilov Institute of Plant Industry carried out several expeditions aimed at collecting vavilovia, between 1960 and 1989 and in the Caucasus region, mostly in Dagestan (Vishnyakova et al. 2007). In the early 21st century, several studies on vavilovia and other wild plants were completed in Turkey, such as those related to floras of a cedar forest near Antalya (Deniz & Sümbül 2004) and a wider region of the We-

stern Taurus Mountains (Eren et al 2004). Most recently, three expeditions have been made in July and August 2009 in Armenia, two in the locations of Mount Ughtasar and one in the vicinity of Lake Aknalitch, as a joint effort of the Green Lane NGO and the Institute of Botany towards an advance in vavilovia ecogeography and conservation (Sarukhanyan 2009).

In the light of recent observations, the most serious challenges of an *in situ* conservation of vavilovia are related to grazing by both domestic and wild animals, especially goats and sheep. Along with this, the area of vavilovia may diminish due to both global climate changes and its own physiology, causing a high susceptibility of vavilovia flowers to early frosts in late summer and an obvious inability of some populations to produce seeds each year.

***Ex situ* Conservation**

There have been several attempts aimed at the *ex situ* conservation of vavilovia. The first experiences made in the USSR were mostly unsuccessful due to inappropriate management of soil aeration and water flow (Zhukovskiy 1971, Makasheva 1973). Limited success was achieved in the



Figure 4. An example of the vavilovia *ex situ* conservation, Yerevan Botanic Garden, Armenia
Slika 4. Primer *ex situ* konzervacije vavilovije, Botanička bašta, Jerevan, Jermenija

Official Seed Testing Station in Edinburgh and at Southampton University, UK (Cooper & Cadger 1990) but these did not provide the production of new seeds or the plant multiplication. During 1974-1981, sound results were obtained in the N. I. Vavilov Institute of Plant Industry by combining growing the plants in the field and in climatic chambers, with some of the plants surviving for some years, blooming and even producing fruits with seeds (Golubev 1990).

According to historical data, vavilovia has been cultivation from time to time at the display plot Flora and Vegetation of Armenia in the Yerevan Botanic Garden since 1940 (Akhverdov & Mirzoeva 1949). A true success in the vailovia *ex situ* conservation has been achieved only recently, where several individuals of vavilovia from Lake Aknalitch and Mount Ughtasar were planted in the rock garden (Fig. 4), confirming that the conditions similar to natural habitat of this species enable its *ex situ* cultivation and propagation (Akopian & Gabrielyan 2008). Today, the plot Flora and Vegetation of Armenia contains more than 200 species belonging to 130 genera, with wild relatives of cereals, legumes, vegetables, condiments, fruit and nut crops (Akopian 2009). The artificial creation

of the alpine rock habitat suitable for the vavilovia *ex situ* conservation was based upon a period of long-term research on the biology and ecology of the alpine plants which enables their introduction from their native altitudes of 2,800-3,500 m into a relatively low-altitude location.

It is noteworthy that the N. I. Vavilov Institute of Plant Industry and the Royal Botanical Garden Edinburgh keep the vavilovia herbarium material of diverse geographical origin.

Interspecies Hybridization

The fact that vavilovia shares the same number of chromosomes with its close cultivated relatives, such as pea, common vetch or grass pea, opens a possibility for their hybridization. It has been often speculated a hypothetical gene for perenniality, present in vavilovia, could be introgressed into its cultivated relatives, especially pea, making vavilovia rather interesting for breeders.

The only known accounts of the hybridization between vavilovia and other *Fabeae* comes from the research undertaken by the N. I. Vavilov Institute of Plant Industry in its centre in St. Petersburg and the station in Dagestan (Golubev 1990).

The hybridization of ♀ *V. formosa* x ♂ *P. sativum* was successful, resulting in several normally developed F₁ seeds. However, only one of these was able to produce a true F₁ hybrid plant. This plant had several stems, or basal branches, with long internodes and no lateral branches that are typical for vavilovia. Its leaves were compound, with one pair of leaflets and, instead of the mucro-like organ present in vavilovia, a third and smaller leaflet, resembling the trifoliolate leaves of *Medicago* or *Trifolium* species. This, one and only ever received F₁ plant did not enter the generative stage and eventually withered due to chlorosis (Golubev 1990).

A reciprocal combination of ♀ *P. sativum* x ♂ *V. formosa* also resulted in only one F₁ hybrid plant. It had much greater height in comparison to both pea and vavilovia and numerous basal and lateral branches. Unlike the F₁ plant from another combination, this one produced flowers and five pods. In two of these six, all the F₂ seeds aborted, while in the other three the F₂ seeds remained immature (Golubev 1990).

According to unpublished data based upon personal communications from A. A. Golubev, the hybridization between vavilovia and its closest relatives, such as red-yellow pea (*Pisum fulvum* Sm.), was possible if vavilovia is used as the male

parent (Maxted & Ambrose 2000).

The evidence on the possibility to produce hybrids of vavilovia and pea, along with a susceptibility of vavilovia to pea-specific pathogens, such as *Uromyces pisi*, *Ascochyta pisi* and *Ascochyta pinodes*, has occasionally been used to demonstrate that the status of vavilovia as a distinct genus is doubtful (Yan'kov & Golubev 1999).

Molecular Taxonomy

Since the very beginning of its existence as its own genus, vavilovia has been considered rather important for the evolution of the whole tribe of *Fabeae*, being possibly the closest of all modern taxa to an extinct common ancestor. According to one of such early evolutionary schemes, it is the development of tendrils that accompanies the progress (Fedorov 1939). In that way, first a hypothetical primeval complex of oroboids produced the extinct genus *Orobis* and then it acted as a common ancestor of all modern *Fabeae*, with vavilovia and its mucro-like petiole tip, considered a kind of initial tendril, as its closest descendant. Further development of this theory retained vavilovia as being closest to the common ancestor (Makasheva 1975) and brought pea as close to it as vetchlings and vetches.

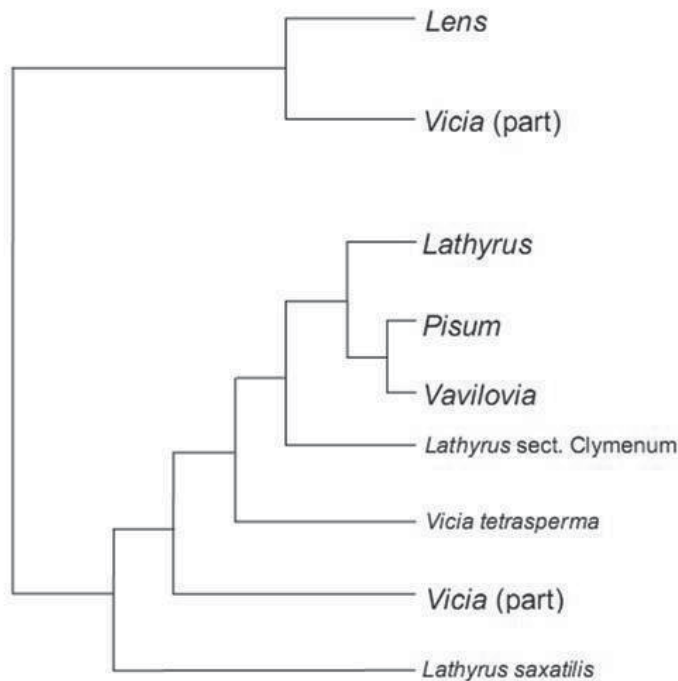


Figure 5. Hypothesised generic relationships in the tribe *Fabeae*, based on DNA sequence data (Kenicer et al 2009a)
Slika 5. Pretpostavljeni odnosi unutar tribusa *Fabeae* na osnovu sekvenci DNK

It has been expected that a molecular research on vavilovia could essentially contribute to defining its ultimate relationship to other *Fabaeae* genera, especially *Pisum* and *Lathyrus* (Kenicer et al 2009b). One of the first attempts in this direction included herbarium specimens of vavilovia from two different sources and the material of about 30 species of all other genera of the tribe *Fabaeae*. This investigation was aimed at the four phylogenetically informative regions, namely chloroplast maturase K, trn L-F and trn S-G fragments and the internal transcribed spacer (ITS) region of nuclear DNA. The results of both maximum parsimony and Bayesian analysis of combined sequence data have shown that vavilovia belongs to a *Lathyrus-Pisum-Vavilovia* clade with a clearly distinct status (Kenicer et al. 2008), as well as that there is a monophyly of *Pisum* and *Vavilovia* (Fig. 5), that the *Pisum - Vavilovia* clade is closest to most of *Lathyrus* and that all of them are nested within *Vicia* (Smýkal et al. 2009).

Other most recent molecular research on vavilovia provides more details for defining its true taxonomical position more precisely. There are suggestions that the similarity between vavilovia and pea is sufficient enough to treat vavilovia once again as *P. formosum*, although more distant from *P. sativum* than *P. fulvum* (Sinjushin & Demidenko 2009) and individual enough to be placed within its own monotypic section (Lock & Maxted 2005). Another study of the phylogenetic status of vavilovia, using nrDNA ITS and cpDNA trnL-F and trnS-G regions and revealing that Vavilovia is closely related to *Pisum*, strongly indicates that vavilovia should be subsumed under *Pisum* as *P. formosum*.

Integrated Approach

Recent achievements in the vavilovia molecular research and its *ex situ* conservation surely put even stronger emphasis upon more refined and accelerated approaches to a long-term and integrated preservation of this iconic legume species of a particular interest for taxonomists, geneticist and breeders.

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Dostignuća u istraživanju na vaviloviji (*Vavilovia formosa* (Stev.) Fed.), samoniklom srodniku gajenih mahunarki

Aleksandar Mikić¹, Petr Smikal², Gregory Keniser³, Nune Saruhanjan⁴, Jana Akopijan⁵, Ivan Gabrieljan⁵, Armen Vanjan⁴, Andrej Sinjušin⁶, Natalija Demidenko⁶, Branko Čupina⁷, Vojislav Mihailović¹, Margarita Višnjakova⁸, Majk Ambroz⁹

¹Institut za ratarstvo i povrtarstvo, Maksima Gorkog 30, 21000 Novi Sad, Srbija

²Istraživanja na biljkama Agritek, s.o.o, Šumperk, Češka

³Kraljevska botanička bašta Edinburg, Edinburg, Velika Britanija

⁴NVO Poljoprivredno savetodavstvo Grin Lejn, Jerevan, Jermenija

⁵Nacionalna akademija nauka, Institut za botaniku, Jerevan, Jermenija

⁶Moskovski državni univerzitet M. V. Lomonosov, Moskva, Rusija

⁷Univerzitet u Novom Sadu, Poljoprivredni fakultet, Novi Sad, Srbija

⁸Državni naučni centar i Sveruski istraživački institut biljne proizvodnje N. I. Vavilov Ruske akademije poljoprivrednih nauka (VIR), Petrograd, Rusija

⁹Centar Džon Ines, Norič, Velika Britanija

Izvod: Vavilovija (*Vavilovia formosa* (Stev.) Fed.) pripada tribusu *Fabeae*, zajedno sa graškovima, grahorima, grahoricama i sočivima. Vavilovija raste u visokoplaninskim oblastima Jermenije, Azerbejdžana, Gruzije, Iraka, Irana, Libana, Rusije, Sirije i Turske. Pravi uspeh u *ex situ* konzervaciji postignut je nedavno u okviru izložbenog polja "Flora i vegetacija Jermenije" u botaničkoj bašti u Jerevanu. Hibridizacija između vavilovije i graška izvršena je u institutu Vavilov, sa F₁ semenima i F₁ biljkama koje nisu uspele da daju sledeće pokoljenje. Skorašnja molekularna istraživanja pokazala su da vavilovija pripada grupi *Lathyrus* – *Pisum* - *Vavilovia* sa jasno izraženim posebnim statusom.

Ključne reči: *Fabeae*, genetički resursi, konzervacija, molekularna taksonomija, samonikli srodnici gajenih biljaka, *Vavilovia formosa*