

ONE CASUAL BUT PENSIVE LOOK AT THE GENETICS OF THE HYBRID PROGENIES OF *PISUM SATIVUM* AND *VAVILOVIA FORMOSA*

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Abstract

The only species of the legume genus *Vavilovia*, a paleoendemic to the Caucasus commonly known simply as *Vavilovia* (*V. formosa*) has an extraordinary significance for the evolution and phylogeny of the tribe *Fabeae*, with its economically important species, such as lentil (*Lens* spp.) or pea (*Pisum* spp.). This paper is attempting to deduce the genetic background of the materially perished, but thoroughly described, F_1 progeny between *Vavilovia* and field pea (*P. sativum* var. *arvense*) in both crossing combinations, obtained by the *N. I. Vavilov All-Russian Institute of Plant Genetic Resources* (VIR), in 1988. Although with only one plant in each direction, the resulting phenotypes are the only known successful intergeneric hybridisation in legumes with impressively interesting features, such as the trifoliolate leaves absent in both parents, as well as vastly inspiring and potentially useful for the conservation of *Vavilovia* and crop improvement of pea and other species of the tribe.

Key words: cytology incompatibility, *Fabeae*, homoeologous chromosomes, intergeneric hybridisation, ortholog genes, pea, *Pisum sativum* var. *arvense*, synteny, *Vavilovia formosa*

Introduction

The tribe *Fabeae* Rchb. is one of the economically most important genera of the family *Fabaceae* Lindl. on a global scale (Sinjushin and Belyakova, 2015). It comprises dozens of widely cultivated coolseason legume species, basically grouped in five genera, namely vetchling (*Lathyrus* L.), lentil (*Lens* Mill.), pea (*Pisum* L.), vetch (*Vicia* L.) and *Vavilovia* (*Vavilovia* An. Fed.) (Mikić et al., 2009). The last listed genus is monotypic, with 'beautiful' *Vavilovia* (*V. formosa* (Stev.) An. Fed.) as a relict and highly endangered species growing in mutually isolated populations on the island-like highlands, typical for paleoendemics (Szóvényi et al., 2009), between 2000 m to 3500 m above sea level and ranging from the West Taurus in Turkey to the Alborz mountains in Iran and from the Greater Caucasus of Dagestan in Russian Federation to the Lesser Caucasus in Armenia along with the Mount Lebanon on the border of Lebanon and Syria (Kenicer et al., 2009).

Vavilovia is a perennial plant of wild floras and rocky substrates, which morphology makes it most similar to pea and keeps alive its alternative name of *Pisum formosum* (Stev.) Alef. (Oskoueian et al., 2010). The most ancient herbarium specimens of *vavilovia* belong to the late 18th century, while it was described as a genuinely novel species around two centuries ago (Vishnyakova et al., 2016). During the last century, the *N. I. Vavilov All-Russian Institute of Plant Genetic Resources* (VIR) from St. Petersburg has been steadily remaining in the very forefront of the extraordinarily numerous aspects of the research relating to this puzzling plant. These praiseworthy efforts were mainly aimed at *Vavilovia* seed multiplication and transplantation in controlled environments, as well as at artificial *in situ* and *ex situ* intraspecific and intergeneric crossings of *Vavilovia* with its closest botanical relatives, such as grass pea (*Lathyrus sativus* L.), common pea (*P. sativum* L.) and common vetch (*V. sativa* L.), with a very limited success obtained exclusively with

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common pea. Unfortunately, all the material results, including hybrid plants, of this long-term investigation perished in the socially disastrous consequences of the dissolution

of USSR, leaving us purely a paper with two quite low-resolution printed black and white photographs (Golubev, 1990).



Figure 1. Some segments of the comparative morphology between (left) field pea (*Pisum sativum* var. *arvense*) and (right) Vavilovia (*Vavilovia formosa*): (from above to bottom) stems, stipules, leaflets, tendrils, flowers, pods and seeds

Slika 1. Neke pojednosti uporedne morfologije (levo) graška (*Pisum sativum*) i (desno) Vavilovije (*Vavilovia formosa*): (odozgo nadole) stabla, zalisci, liske, vitice, cvetovi, mahune i semena

After a period lasting nearly two decades, the interest in diverse aspects of and approaches to the Vavilovia research was revived by an informal international group of scientists (Mikić et al., 2009). This contemplation, as thorough contemplation as the available facts may allow, communicated merely in a printed form and relating to the progenies of pea and Vavilovia, hopes to enlighten at least a bit more the genetics of both Vavilovia itself and its hybrid progeny with pea, its closest botanical cousin.

Description

The Golubev's account contains abundant data on the continuous hybridisation endeavours between the pea cultivar Melkosemyannyi zelyonyi, belonging to an agronomic type of common pea labelled as field pea, which is identical to the taxon *Pisum sativum* L. subsp. *sativum* var. *arvense* (L.) Poir., and the Vavilovia population from the Gegham mountains in Armenia, carried within a remarkably complex procedure during summer 1988. There are two pivotal passages of this report that may be sufficient enough for further elaboration and drawing certain valuable conclusions (Golubev, 1990).

The first paragraph describes the outcome of a crossing with Vavilovia as a pollen recipient and field pea as a pollen donor. 'The hybrid plant had several branches, emerging

from the basal part of the stem, with elongated internodes and a small pair of inverted ovate leaflets. Instead of the impaired tendrils-acumen, present in Vavilovia in both natural and artificial conditions, in this plant, there was a leaf-like formation of a smaller size than the leaflets, which made the leaves similar to those of clover or lucerne. It also had lateral branches usual for Vavilovia (Fig. 2, left). We have conducted a test of causing generative development and the plant was moved in a room with decreased temperature, but with a small intensity of light, where, due to chlorosis, it withered and died out.'

Another excerpt depicts the result of the opposite crossing combination (Fig. 2, right). 'In the greenhouse conditions, there had been growing a hybrid plant with Vavilovia as the pollen donor. In this combination, the hybrid seeds had normal imbibition, sprout formation, plant growth and generative development. One heterotic plant, with a mixture of parental traits and significantly exceeding the height of Melkosemyannyi zelyonyi, the pollen recipient, was characterised by an abundant basal and lateral branching. The plant flowered well in the greenhouse. The first hybrid progeny was received - five pods, of which two were barren and with obviously unfertilised little seeds. In the other three pods, there were six seeds with defects in developing seed coat, which was green and hardly covered both cotyledons.'

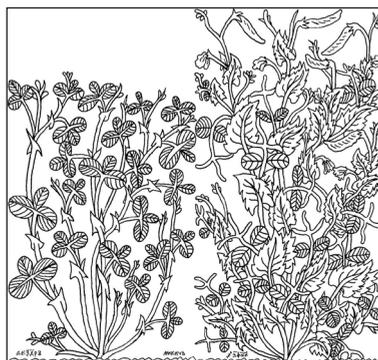


Figure 2. The phenotypes of the F_1 hybrid progeny of field pea (*Pisum sativum* var. *arvense*) and Vavilovia (*Vavilovia formosa*): (left) ♀ vavilovia × ♂ field pea and (right) ♀ field pea × ♂ Vavilovia (Golubev, 1990)
Slika 2. Fenotipovi F_1 potomstva poljskog graška (*Pisum sativum* var. *arvense*) i Vavilovije (*Vavilovia formosa*): (levo) ♀ vavilovija × ♂ poljski grašak i (desno) ♀ poljski grašak × ♂ vavilovija (Golubev, 1990)

Consideration

An almost entire lack of success in thoroughly designed and persistently carried out crossings between field pea and Vavilovia may be explained by a great number of factors of diverse nature. One of them has a molecular biology background and is responsible for establishing various biochemical and physiological barriers underlying the frequently monitored inability of the donor's pollen tube to reach the recipients' ovules or, in the cases where a zygote is nevertheless conceived, implicating the eventual embryo abortion at the different stages of its development, as often seen in the form of incompletely formed or unviable seeds (Ochatt et al., 2004; Ochatt et al., 2016). Among the other explanations of extremely rare positive aftermaths in the hybridisation among the taxa of unequal systematic order is the impact

of certain genetic factors, such as the allelic-induced nuclear-cytoplasmic incompatibility, not only between the species but also between subspecies, as seen in the examples of *Pisum sativum* L. subsp. *sativum* with *P. abyssinicum* A. Braun and *P. sativum* L. subsp. *elatius* (M. Bieb.) Asch. & Graebn., respectively (Bogdanova et al., 2009). Apart from these two unfavourable influences, it could easily be that the crucial role in the experiment has been played by cytological factors, since pea and Vavilovia, despite having the same number of chromosomes, $2n = 14$, have no full correspondence in their morphology (Abramova, 1971; Atlagić et al. 2010; Praça-Fontes et al., 2014; Fig. 3: the Arabic numerals denote the ordinal number of chromosomes, while the Roman ones correspond them to the consent linkage groups).

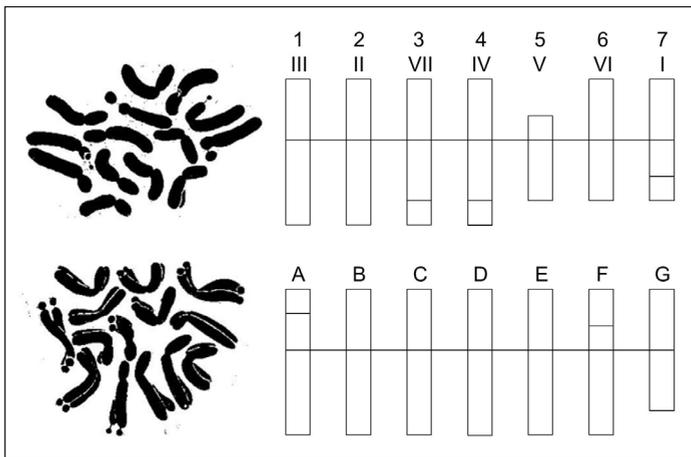


Figure 3. The ideograms of (above) field pea (*Pisum sativum*) and (bottom) Vavilovia (*Vavilovia formosa*)
 Slika 3. Ideogrami (gore) poljskog graška (*Pisum sativum*) i (dole) Vavilovije (*Vavilovia formosa*)

From the given description, it is doubtless that the physiological and biochemical obstacles were overcome and that the homoeologous chromosomes of two species paired in both crossing combinations. During these processes, it is most likely that their complete physical alignment was prevented because of the aforementioned nuclear-cytoplasmic obstructions, as well as due to the simple mutual morphological differences (Bogdanova et al., 2014). Thus, we may assume

that the chromosomes paired along with those segments that had preserved the prominent synteny among two species (Kosterin, 2016), especially since their very approximate phylogenetic relationships (Smýkal et al., 2017).

It is exceptionally ungrateful to examine the details of this most unique artificially carried out genetic trial, especially if it was done on solely one replication, that is, a single success in either crossing directions.

However, there were the traits in both F_1 plants that both parents had discernibly contributed with (Tables 1 and 2; Świącicki, 2019), such as:

- Long internodes, caused by the homozygous dominant genotype of *LELE*;
- Prominent basal branching, with either *FRFR frufru* or *frfr FRUFRU* genotypes;
- Prominent lateral stem branching, involving

the homozygous recessive *ramram* and a haploid *rms*, where its counterpart was either lost during the meiosis or because the pairing of the homoeologous chromosomes was incomplete;

- Flower colour, determined by the homozygous dominant genotype of *AA*.

Table 1. The supposed genotype of the only known hybrid plant (F_1) of a crossing with *Vavilovia* as a pollen recipient (♀) and field pea as a pollen donor (♂), carried out artificially by VIR in summer 1988 (Golubev, 1990); the gene symbols are given for both species based on the consensus labels (Świącicki, 2019)

Tabela 1. Pretpostavljeni genotip jedine poznate hibridne biljke (F_1) ukrštanja *Vavilovije* kao majke (♀) i poljskog graška kao oca (♂) veštački sprovedenog u VIR-u, leta 1988. godine (Golubev, 1990); oznake gena za obe vrste su date u skladu s dogovorom (Świącicki, 2019)

Trait / Crossing generation	Parental gametes		F_1
	♀	♂	
Internode length	<i>LE</i>	<i>LE</i>	<i>LELE</i>
Basal branching	<i>FR fru</i> or <i>frFRU</i>	<i>FR fru</i> or <i>frFRU</i>	<i>FRFR frufru</i> or <i>frfr FRUFRU</i>
Lateral branching	<i>ram; rms</i>	<i>ram; RMS</i>	<i>ramram rms_</i>
Stipule development	<i>st</i>	<i>ST</i>	<i>st_</i>
Leaflet and tendril development	<i>AF TL</i>	<i>AF TL</i>	<i>AFAF _</i>
Flower colour	<i>A</i>	<i>A</i>	<i>AA</i>

In the second crossing combination, there were normally parchment pods (*PP VV*), smooth seed shape (*RR*) and medium to strong violet spots on seed coat (*FS^{ex}FS*).

At the same time, there were assessed a few astonishingly interesting and phenotypic phenomena in both first hybrid generation plants (Fig. 2). In one of them, where *Vavilovia*

was a pollen recipient, the presence of only undeveloped stipules or the occurrence of trifoliolate leaves, maybe, cautiously though, explained by the absolute absence of the dominant field pea genes causing the development of large stipules and lamina, thus having a genotype of *st_ AFAF_* (Table 1).

Table 2. The supposed genotype of the only known hybrid plant (F_1) of a crossing with field pea as a pollen recipient (♀) and *Vavilovia* as a pollen donor (♂), carried out artificially by VIR in summer 1988 (Golubev, 1990); the gene symbols are given for both species based on the consensus labels (Świącicki, 2019)

Tabela 2. Pretpostavljeni genotip jedine poznate hibridne biljke (F_1) ukrštanja poljskog graška kao majke (♀) i *Vavilovije* kao oca (♂) veštački sprovedenog u VIR-u, leta 1988. godine (Golubev, 1990); oznake gena za obe vrste su date u skladu s dogovorom (Świącicki, 2019)

Trait / Crossing generation	Parental gametes		F_1
	♀	♂	
Internode length	<i>LE</i>	<i>LE</i>	<i>LELE</i>
Basal branching	<i>FR fru</i> or <i>frFRU</i>	<i>FR fru</i> or <i>frFRU</i>	<i>FRFR frufru</i> or <i>frfr FRUFRU</i>
Lateral branching	<i>ram; rms</i>	<i>ram; RMS</i>	<i>ramram rms_</i>
Stipule development	<i>st</i>	<i>ST</i>	<i>ST_</i>
Leaflet and tendril development	<i>AF TL</i> or <i>AF _</i>	<i>AF TL</i>	<i>AFAF TLTL</i> or <i>AFAF TL_</i>
Flower colour	<i>A</i>	<i>A</i>	<i>AA</i>
Pod parchment	<i>P V</i>	<i>P V</i>	<i>PP VV</i>
Seed shape	<i>R</i>	<i>R</i>	<i>RR</i>
Seed coat colour	<i>FS^{ex}</i>	<i>FS</i>	<i>FS^{ex}FS</i>

In another F_1 plant, with Vavilovia as a pollen donor, we may endlessly, in search for the proper meaning of the quoted cryptic expression 'with a mixture of parental traits', chiefly due to a too vague phenotypic portrayal. One of the hopefully balanced approaches could hopefully allow a possibility that this plant was characterised by a higher degree of the presence of the morphological traits typical for pea, in comparison to the formerly discussed hybrid plant, such as large stipules and at least few tendrils, thus having a genotype of *ST_AFAF TL* (Table 2).

Amidst the additional values of the Golubev's narrative is a documented testimony of, as far as is publicly known, the only attested successful intergeneric hybridisation in legumes, which, so far, have been limited to a single attempt between two forage species, *Medicago media* Pers. and *Trifolium repens* L. (Kazimierski and Kazimierska, 1985). With a powerfully renewed interest in and enhanced facilities for the Vavilovia research, such as the transfer and cultivation of a population from the Mount Gegham to the National Botanical Gardens of Armenia in Yerevan (Akopian et al., 2019), deciphering the genome of Vavilovia may cast essentially more light onto its relatedness to the recently accomplished sequencing of the pea genome (Kreplak et al., 2019) and the construction of its integrated and comparative genetic and cytological maps (Ellis and Poyser, 2002).

Conclusion

One of the farthest-reaching implications of this brief contemplation over a research finding, that has remained largely ignorant by the non-Russophone international scientific community could reflect in the form of a sufficient understanding and feasible management of the mechanisms underlying the intergeneric and interspecies hybridisation and ensuring thoroughly designed, precisely conducted and undisturbed repetition of the crossing combinations between Vavilovia, with a specific emphasis on practical breeding goals, such as introgressing additional tolerance to

cold intensity and duration and perenniality into common pea and other economically significant members of the tribe *Fabeae*.

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To Margarita, for the occasion of VIR's 125th birthday.

In memory of few rare good people, János Berényi, Đuro Gvozdenović, Dane Lukić, Tatyana Naumkina, Imre Pataki and Saša Strajner: God bless their souls.

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JEDAN USPUTNI ALI I PODROBNI POGLED NA GENETIKU HIBRIDNOG POTOMSTVA VRSTA *PISUM SATIVUM* I *VAVILOVIA FORMOSA*

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Sažetak

Jedina vrsta roda mahunarki *Vavilovia*, paleoendemska za oblast Kavkaza i uobičajeno poznata prosto kao Vavilovija (*V. formosa*) ima izuzetan značaj za evoluciju i filogeniju tribusa Fabeae, s ekonomski najznačajnijim vrstama poput sočiva (*Lens* spp.) ili graška (*Pisum* spp). Ovaj rad pokušava da dokuči genetičku pozadinu materijalno izgubljenog, ali i podrobno opisanog, F1 potomstva između Vavilovije i poljskog graška (*P. sativum* var. *arvense*) u obe kombinacije ukrštanja, ostvarene u Sveruskom institutu za biljne genetičke resurse N. I. Vavilov (VIR), 1988. godine. Mada, samo sa po jednom biljkom u oba slučaja, dobijeni fenotipovi su jedini poznati ishod međurodnog ukrštanja kod mahunarki, s upečatljivo zanimljivim odlikama poput listova s' tri liske nepostojećih ni kod jednog roditelja, koji nadahnjuje i može da ima koristi za očuvanje Vavilovije i poboljšanje graška i drugih vrsta tribusa.

Ključne reči: citologija, inkompatibilnost, *Fabeae*, homeologni hromozomi, međurodno ukrštanje, ortologni geni, grašak, *Pisum sativum* var. *arvense*, sintenija, *Vavilovia formosa*

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