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, F1 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 (Szövényi 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. (Oskoueiyan 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 pojedinosti 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 tendril-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 F₁ hybrid progeny of field pea (Pisum sativum var. arvense) and Vavilovia (Vavilovia formosa): (left) ♀ vavilovia × ♂ field pea and (right) ♀ field pea × ♂ Vavilovia (Golubev, 1990)](image-url)
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*)

![Figure 3. Ideograms of field pea (Pisum sativum) and Vavilovia (Vavilovia formosa).](image)

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 (Šmýkal 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; Święcicki, 2019), such as:
- Long internodes, caused by the homozygous dominant genotype of LELE;
- Prominent basal branching, with either FRFR 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 homeologous 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 (Święcicki, 2019)

| Trait / Crossing generation | Parental gametes | $F_1$ |
|----------------------------|------------------|------|
| Internode length           | $LE$             | $LE$ LELE |
| Basal branching            | FR fru or frFRU ram; $rms$ | FR fru or frFRU ram; $RMS$ FRFR frufru or frfr FRUFRU ramram $rms$ |
| Lateral branching          | $st$             | $ST$ st_ |
| Stipule development        | $AF TL$          | $AF TL$ AFAF__ |
| Leaflet and tendril develop | $A$              | $A$ AA |
| Flower colour              | $A$              | $A$ AA |

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 (Święcicki, 2019)

| Trait / Crossing generation | Parental gametes | $F_1$ |
|----------------------------|------------------|------|
| Internode length           | $LE$             | $LE$ LELE |
| Basal branching            | FR fru or frFRU ram; $rms$ | FR fru or frFRU ram; $RMS$ FRFR frufru or frfr FRUFRU ramram $rms$ |
| Lateral branching          | $st$             | $ST$ st_ |
| Stipule development        | $AF TL$ or $AF_ $ | $AF TL$ AFAF__ |
| Leaflet and tendril develop | $A$              | $A$ AA |
| Flower colour              | $A$              | $A$ AA |
| Pod parchment              | $P V$            | $P V$ PP VV |
| Seed shape                 | $R$              | $R$ RR |
| Seed coat colour           | $FS^{ex}$        | $FS$ $FS^{ex}FS$ |
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, \textit{Medicago media} Pers. and \textit{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 \textit{Fabaeae}.

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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 Fabae, s ekonomski najznačajnijim vrstama poput sočiva (Lens spp.) ili graška (Pisum spp). Ovaj rad pokušava da dokuči genetičku pozadinu materijalno izgubljenog, ali i podrobnio 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 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, Fabae, homeologni hromozomi, međurodno ukrštanje, ortologni geni, grašak, Pisum sativum var. arvense, sintenija, Vavilovia formosa

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