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Hybridization between wild and domesticated types of *Phaseolus vulgaris* L. (Fabaceae) in Argentina

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Abstract

A polymorphic population of *Phaseolus vulgaris* L. var. *aborigineus* (Burkart) Baudet growing in northwestern Argentina was studied. In order to know the origin of this polymorphism, plants belonging to the var. *aborigineus*, others showing floral dimorphism and individuals with weedy type characters were collected. Their seeds, obtained after fieldwork treatments of autogamy and free pollination in order to know their reproductive system, were sown in a greenhouse. Information of their growth was recorded and several numerical analyses were performed. With the results obtained, we concluded that those individuals that showed floral dimorphism are probably a result of hybridization and/or introgression between the var. *aborigineus* and cultivated forms that grow in the area. This hypothesis is supported by the presence of divergent segregation in the offspring. Also, the offspring of the F1 was followed and allowed us to conclude the possible existence of degeneration of the hybrid progeny. This degeneration indicates that an unwanted gene flow in the area could lead to a decline in the wild bean population. The vigour and high reproductive success of the offspring belonging to the individuals corresponding to the var. *aborigineus*, whose progenitor was treated for outcrossing, and the existence of plants with weedy type characteristics, are indicative of the necessity to preserve this germplasm in order to evaluate its agronomic potential as brief as possible. The population here studied constitutes a wild-weed–crop complex growing in Argentina.

Introduction

Phaseolus vulgaris L. is one of the most important crops among the 'beans'. One of the related wild varieties of the common bean is *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet, which grows in northwestern Argentina and Bolivia (Burkart 1952; Baudet 1977).

Freyre et al. (1996) detected, as a result of molecular and exomorphological analyses, the existence of hybridization and introgression between *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet and commercial cultivars of *P. vulgaris* L. in Bolivia. Paredes and Gepts (1995) observed a high frequency of hybridization in the cultivated common bean growing in Chile. In Mesoamerica, Beebe et al. (1997) detected some hybrid complexes, pointing out that these do not exist in northwestern Argentina. Cattan-Toupance et al. (1998) reported the existence of polymorphism in some populations of *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet growing in NW Argentina, using RAPD markers, without any explanation about its origin.

During a study of the floral biology and the reproductive system of *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet at the locality of Quebrada del Toro (Dept. Rosario de Lerma, Prov. Salta, Argentina), it was detected floral polymorphism (Hoc and Amela García 1999). In addition, it was observed that near the studied area, cultivated forms of *P. vulgaris* L. were growing next to wild populations. Hoc and Amela García (*op.cit.*) determined that the effective pollinators of *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet are: *Bombus atrattus* Franklin, *Centris* spp. and *Megachile* spp. Also, it was observed that individuals of *Apis mellifera* L. were unable to act as pollinators or nectar robbers in this area.

Papa and Gepts (2003) pointed out that in spite of the self-breeding system of common bean, gene flow is sufficient to prevent genetic isolation between wild and cultivated forms, and selection appears to be a major evolutionary factor maintaining the identity of wild and domesticated populations in sympatric situations.

The objective of this work is to study the reproductive success and the offspring of each plant, in order to understand the origin of the variability found in Quebrada del Toro, Salta, Argentina.

Materials and methods

Field works

The population representative of *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet that was studied is located at Quebrada del Toro (24°54′S, 65°38′N, Salta, Argentina). Voucher specimens were deposited in the herbarium of Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires (BAFC) with the numbers Hoc 283, Hoc 346, Hoc 357 and Hoc 359. Each studied individual was identified with its accession number and a letter indicating the treatment performed in its flowers: A for autogamy (by bagging the buds) and F for free pollination (each bud was marked and the visits of pollinators were recorded). Accession Hoc 283 was studied for autogamy and free pollination, while Hoc 346 was only treated for autogamy, and the other two accessions acted as controls for free pollination.

The reproductive success was measured by calculating the RRS index (Dafni 1992), modified by Hoc and Amela (1999), defined as: RRS = number of produced seeds per fruit / number of ovules per flower.

The length and shape of the floral wings and the flag shape in each individual was recorded. After the fructification, each pod and its seeds were collected, and the following characters were measured: length, width and thickness of the pod and the seeds. Also, in each pod was recorded the length of the pod beak, the angle of divergence with respect to the axis, and the colour.

Greenhouse observations

In November of 1997, 112 seeds were set in Petri-dishes for germination, and the seedlings were transplanted to individual flowerpots at the greenhouse of the University of Buenos Aires. The isolation from possible pollinators was checked. The growing of each plant was followed, measuring the length and width of each simple leaf. From the beginning of flowering to harvest (3/4/1998–5/19/1998), the survival of each plant and those with faster growth were recorded. The fruits and seeds produced were characterized, also the shape of the flowers. In each pod, the number and position of abortive seeds was recorded from the tip to the bottom. The reproductive success of each plant was analysed using the RRS index as indicated above. This analysis was performed in parental plants and in the progeny obtained from self-pollination and free pollination tests.

During November 1998 and December 1999 the seeds produced by the greenhouse plants were sown as indicated above, and their growth was analysed.

Numerical analysis

The mean values and the standard deviations of the characters length, width and thickness of seeds were estimated in parental plants and also in their progeny. We also estimated those parameters for length and width of the primary leaf of accessions Hoc 283, 357 and 359. A one-way ANOVA test was performed, and contrasts within parents, progeny and between each other were calculated.

In addition, a discriminant analysis was used in order to study the possible relationships between individuals from each progeny. The characters analysed were: length, width and thickness of seeds and pods; length of the pod beak and divergence angle with respect to the axis for all the pods; and length of the floral wings. For the analyses, the Statistica 5.1 (1998) program package was used.

Results

Field observation of the polymorphism

Individuals collected in Quebrada del Toro, Salta, Argentina, showed differences in the floral and fruit morphology. It was observed the existence of:
(1) Plants that presented characters of *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet, i.e. flowers with resupinated flag and orbicular wings shorter than 10 mm long (Nf: normal flowers), one-coloured (light yellowish brown) pods (Hoc 283A and F) or the same colour with violet spots (Hoc 346A).
(2) Plants with similar morphology to cultivated forms of *P. vulgaris* L. The flowers of these plants had a cucullate flag and elliptic wings greater than 12 mm (Bf: big flowers), their pods had violet spots (Hoc 359F).
(3) Plants that exhibited floral dimorphism, with normal and big flowers in the same plant, and one-coloured (light yellowish brown) pods (Hoc 357F).

Analysis of the F1 generation

All the forms observed in Quebrada del Toro were found in the plants growing in the greenhouse. Numerical analysis of the first simple leaf showed that the offspring of accession Hoc 359F were significatively different ($p < 0.05$) from the progeny of Hoc 283A and F (Figure 1).

All the pods harvested from the progeny of each accession were single coloured, except for two plants belonging to the offspring of accessions Hoc 283F and Hoc 346A, which presented violet spots, and one plant of the offspring of Hoc 283F and two of Hoc 357F that had single coloured and spotted pods in the same plant.

The seeds collected in the field were compared to those that were obtained in the greenhouse

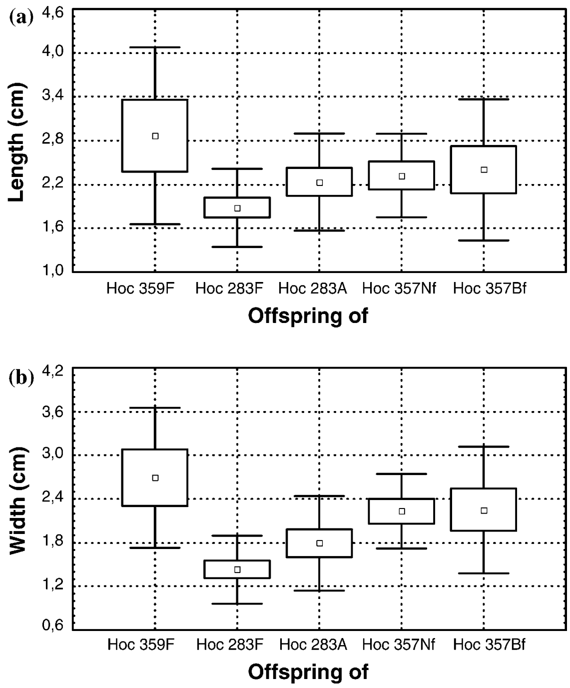

Figure 1. The illustration shows the length (a) and width (b) mean values of the first simple leaf in the seedlings of the F1.

(Figure 2). Significant differences ($p < 0.05$) were detected in the three parameters between the accessions Hoc 283 and their progeny, obtained from both autogamy and free pollination treatments (Figure 2a–c). A great variability within the progeny of Hoc 283F and Hoc 283A can be observed, some of the plants had greater values than the parents, and other (Plant 2F, Figure 2a–c) which had significantly lower values ($p < 0.05$).

In the progeny of Hoc 346A, segregation for the three characters was observed: some individuals kept the parents' dimensions, while most of the offspring had greater mean values (Figure 2d–f).

The offspring of plant Hoc 357Bf did not exhibit significant differences with respect to the parental plant (Figure 2g–i). On the other hand, significant differences ($p < 0.05$) between accession Hoc 357Nf and its progeny for the three parameters were found. Progeny of Hoc 357F showed bipolar segregation, especially for the character thickness.

Finally, the progeny of accession Hoc 359 is differentiated from the parents. The width and thickness of the seeds are significantly different ($p < 0.05$) (Figure 2j–l).

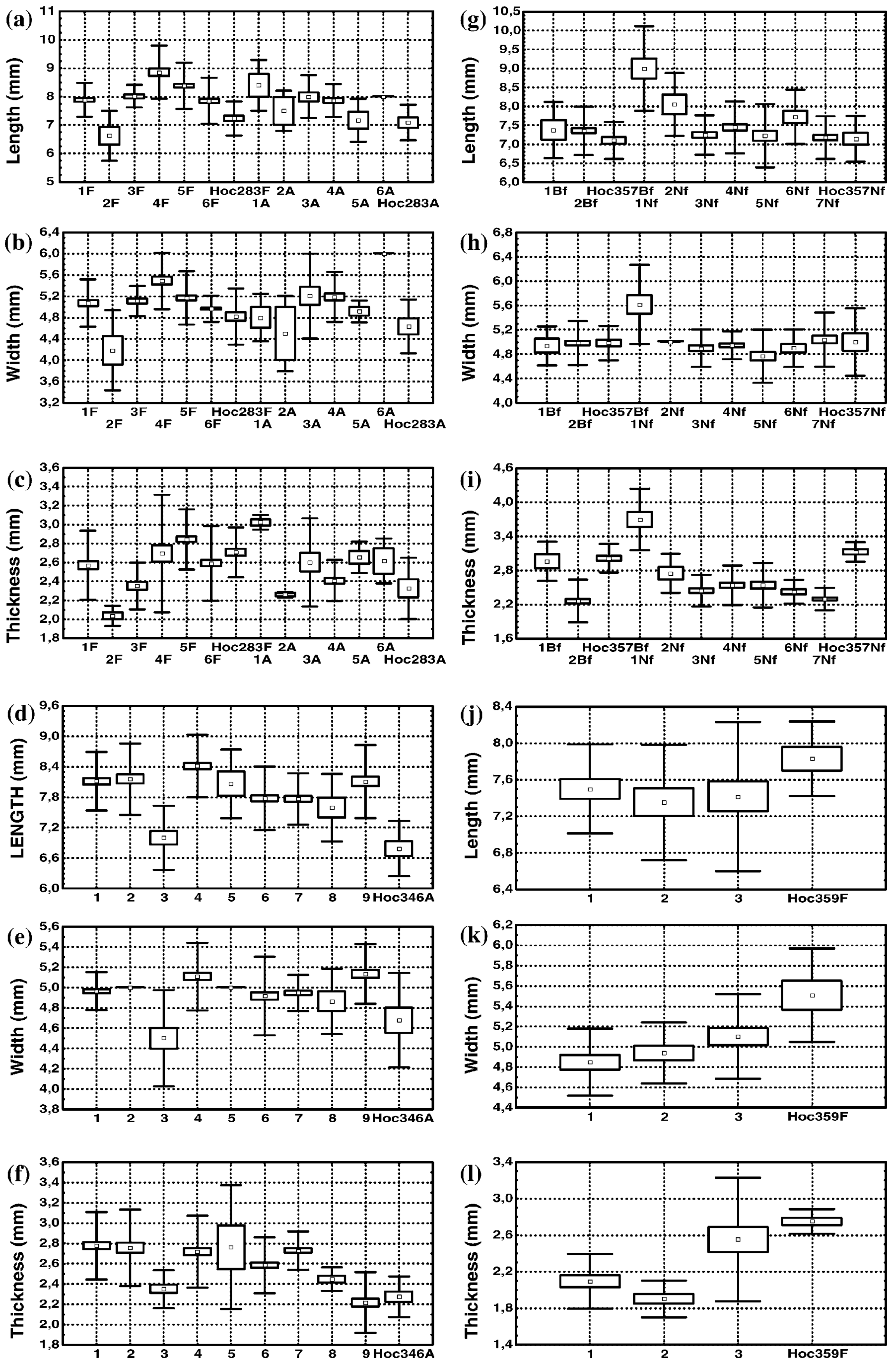

Figure 2. Length, width and thickness media values of the accessions' seeds (Hoc 283, 346, 357 and 359), and their offspring.

Discriminant analysis

The distribution of the individuals in the space delimited by the two first canonical variables performed by this analysis is shown (Figure 3). The progeny of Hoc 359F is grouped separately from the rest of the individuals. The offspring of Hoc 283 has a great dispersion on the horizontal

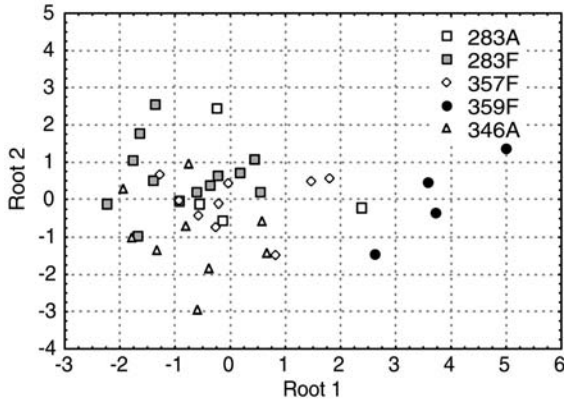

Figure 3. F1 individuals distributed in the space defined by the first two canonical variables of the discriminant analysis.

axis, where the characters that most contribute are seed measures and length of the floral wing. One individual of the progeny of Hoc 346A, which presented spotted pods, is separated from the rest of the offspring with respect to the vertical axis, where the characters that most contribute are the length of the rostrum and width of fruits. Some individuals of the offspring of Hoc 357F (which presented flower dimorphism) exhibited similar morphology to accession Hoc 359F.

Reproductive success

The RRS mean values transformed into percentage, obtained from the progeny of each parental plant studied are exhibited in Table 1 and Figure 4. As can be seen in the table, those plants belonging to accession Hoc 283F exhibited the highest reproductive success, although a high dispersion between them could be observed (Figure 4). In all the pods, the highest number of abortive seeds was observed at the base.

The offspring of accession Hoc 346A (with similar morphology to the wild variety) and Hoc 359F (similar to cultivated forms), exhibited the most vigorous growth: they grew more than 3 m high. The other plants reached only 2 m or less.

Analysis of the F2 generation

(a) November 1998 harvest. F2 of Hoc 283A: Nineteen seeds were set in Petri-dishes and only three germinated and grew. They did not flower.

F2 of Hoc 283F: Ten plants were obtained, and in January 1999, they presented secondary branches. They did not flower.

F2 of Hoc 346A: Fifteen seeds were put in Petri-dishes. Only one germinated and grew until possessing secondary branches. They did not flower.

(b) January 1999 harvest. F2 of Hoc 357F: Eleven seeds were put in Petri-dishes and five of them developed a green primary root, secondary roots and emergent cotyledons, but the plumule developed partially. Three seeds had primary and secondary roots, neither the cotyledons nor the plumule emerged and developed. The rest of the seeds germinated, but two died because of hypocotyl strangulation; the third grew and had secondary branches, although it did not flower.

(c) November–December 1999 harvests. F2 of Hoc 346A: Seven of eight seeds produced by one of the plants germinated. They grew, but after the 1st week, some of them suffered strangulation at the hypocotyl and died soon after. In the other group the stem apex died and buds placed at the axil of each cotyledon produced branches. The seedlings grew and had secondary branches, but did not flower.

F2 of Hoc 283A: None of the seeds germinated.

F2 of Hoc 357F: Plants that grew after germination of the seeds obtained from one of the plants had an equivalent development to the F2 of Hoc 346A.

F2 of Hoc 359F: All the seeds germinated, but in one of them the radicle developed abnormally (it was circinated) and the plants died soon after. In the rest of the seedlings, the same abnormal

Table 1. Reproductive success of the offspring of each accession.

Offspring of:	Hoc 283F	Hoc 283A	Hoc 346A	Hoc 357F	Hoc 359F
Reproductive success	81.19%	69.25%	70.04%	62.32%	77.01%

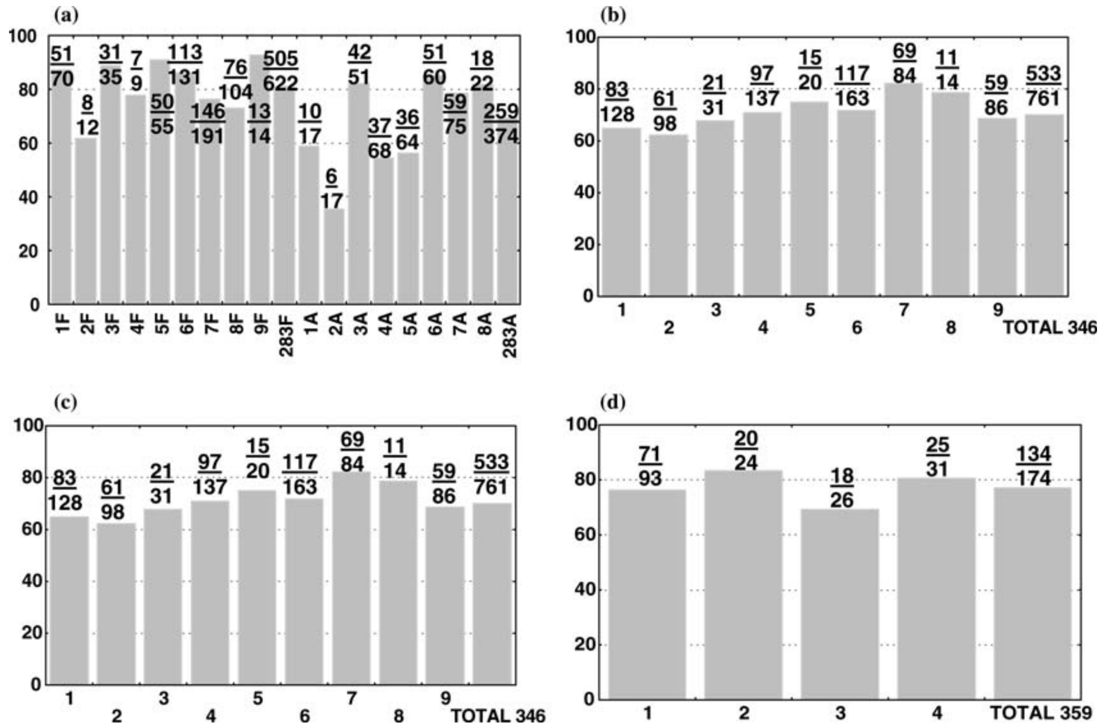

Figure 4. Reproductive success of the offspring (expressed in percentage in the vertical axis) of each one of the analysed accessions: (a) accession Hoc 283; (b) accession Hoc 346; (c) accession Hoc 357, and (d) accession Hoc 359. The seeds/ovule fraction is indicated above each bar.

growth found in F2 plants of Hoc 346A was observed. In addition, they never flowered.

Discussion and conclusions

It was observed that the offspring of plant Hoc 283 showed a high variability if they were the product of free pollination. On the other hand, if the flowers were bagged, a tendency toward the maintenance of the parental characters (length and width of the seeds) was observed. The individuals of the progeny obtained by self-breeding are represented in the discriminant analysis (Figure 3) as a group of points with lower dispersion that those obtained by free pollination. This is expected in outbreeding species, in which outcrossing increases variability of the progenies. In addition, results shown in Table 1 allow concluding that self-pollination decreases the reproductive success.

The offspring of plant Hoc 357F exhibit a bipolar segregation never reported before in *Phaseolus vulgaris* L. var. *aborigineus* (Burkart) Baudet. This study allowed inferring that the parental plant should be a product of hybridization between the wild variety and other varieties cultivated in the area.

The offspring of plant Hoc 346A exhibit a divergent segregation (increase or maintenance of seed dimensions, Figure 2). This could be an evidence of genetic flow from the cultivated forms to the wild ones. Further studies will allow testing this hypothesis.

The progeny of the plant with weedy type characters (Hoc 359) showed significant differences compared to the wild plants, taking into account the variables here analysed; this is also shown by the discriminant analysis. The group formed by the progeny of accession Hoc 359 is clearly separated from the other ones in the sense of the first canonical variable (influenced by dimension of the seeds). Maréchal et al. (1978) and Beebe et al. (2001) pointed out that the co-existence of the wild variety and the cultivated forms could originate intermediate lines. So it can be inferred that accession Hoc 359 would belong to a weedy form, as the ones described by Beebe et al. (1997).

The possible gene flow between wild and cultivated forms, might conduct to a reduction or an increase in the genetic diversity of the wild relatives, extinction of some populations, and the development of new and more aggressive weeds (Papa and Gepts 2003). Present results allowed the authors to conclude that xenogamy contributes to increase the variability of the gene pool of the wild entity, and that the successive autogamy results in the accumulation of mutations that inhibit the growth of the offspring. This degeneration indicates that an unwanted gene flow in the area could lead to a decline in the wild bean population. The results of this work support the hypothesis of an allogamic reproductive system for *P. vulgaris* L. var. *aborigineus* (Burkart) Baudet, as were reported by Hoc and Amela García (1999).

Although Beebe et al. (2001) pointed out that wild-weed–crop complexes of *Phaseolus vulgaris* L., do not exist in northwestern Argentina, the population from Quebrada del Toro (Salta, Argentina) here studied constitutes one of those complexes, since those individuals that exhibited floral dimorphism, bipolar segregation and weedy characters behaved as products of hybridization.

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