A BEAN INTERSPECIFIC HYBRID

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AS part of a program to incorporate common bean blight resistance into *Phaseolus vulgaris* L., the common bean, hybridization of this species with *P. acutifolius* Gray, a tepary bean, was investigated.

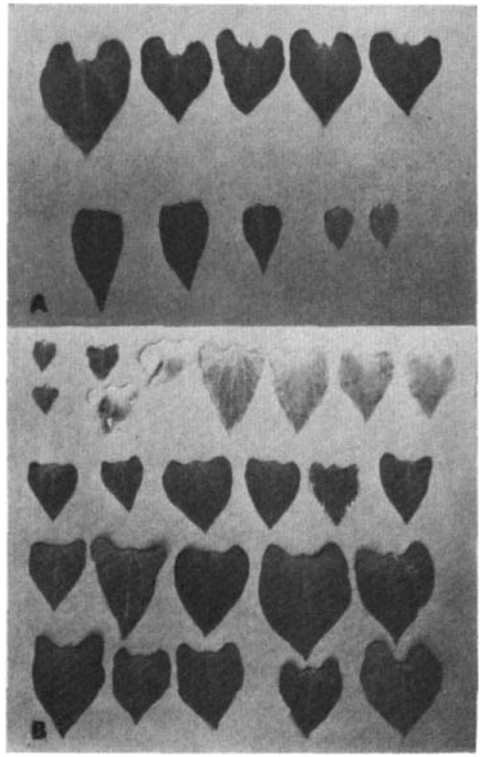
Interspecific hybridization in the genus *Phaseolus* is not new; Mendel[7] reported the crossing of *P. vulgaris* and *P. multiflorus* Lam., a runner bean. Lamprecht[5] has reviewed and described other studies of hybridization involving these two species. A cross between the species *P. vulgaris* and *P. mungo* L., the urd bean, has been reported by Strand[9]. Lorz[6] described a cross between *P. lunatus* L., the lima bean, and *P. polystachyus* (L.) B.S.P., a thicket bean. However, the cross *P. vulgaris* × *P. acutifolius* has not previously been reported to the writer's knowledge.

This paper summarizes the hybridization technique, the results of hybridization, and the characteristics in the F_3 generation.

Parental Description

The varieties Great Northern (*P. vulgaris*) and Tepary 4 (*P. acutifolius*) were used for the investigation. The tepary bean has been reported by Schuster[8] and other workers to be resistant to the common bean blight, *Xanthomonas phaseoli* (E.F.Sm.) Dows., while the Great Northern is considered to be susceptible.

Several distinctly different morphological characters of the two species are listed in Table I. The measurements of mature plant parts were taken from plants grown in the greenhouse. The flowers of the common bean are larger than those of tepary. The primary leaves of the two species are simple but the leaf base of *P. acutifolius* is truncate while that of *P. vulgaris* is cordate (Figure 10*A*). The secondary leaves of *P. acutifolius* have apices that are more acute than those of *P. vulgaris* (Figure 11). The parental differences recorded are similar to those described by Freeman[2] for these species.



PRIMARY LEAF BASE

Figure 10

A—The top row shows *Phaseolus vulgaris*, which is cordate. The bottom row shows the truncate type of *P. acutifolius*. *B*—Shows the primary leaf base segregation in the F_3 generation.

Hybridization Technique

The pollination method used was a modification of that described by Wade[10].

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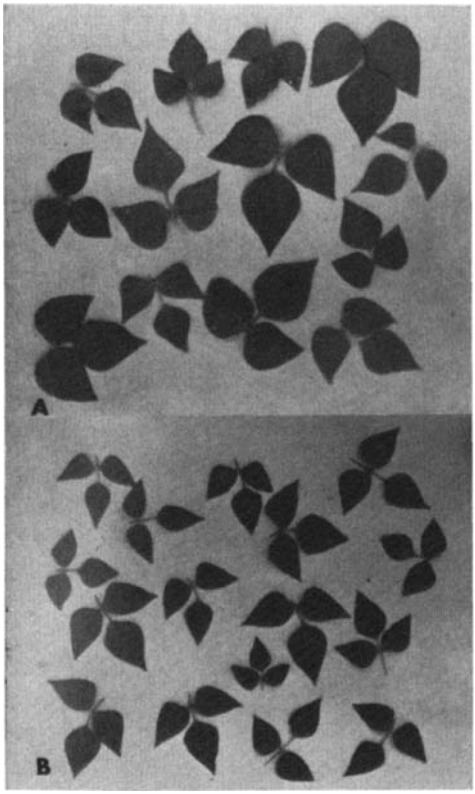
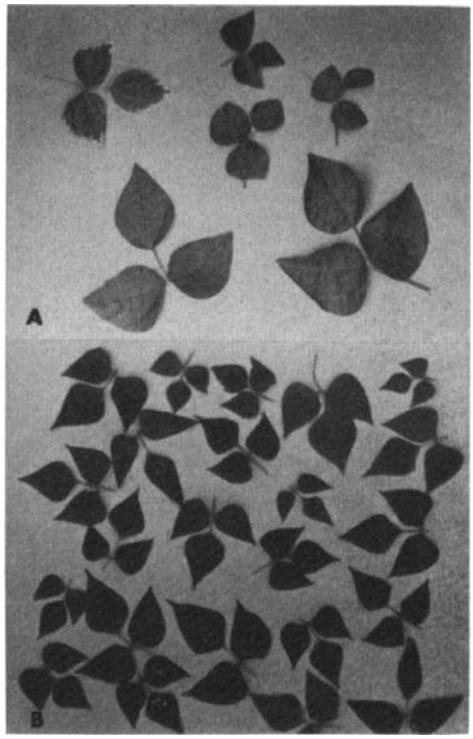


TYPICAL MATURE LEAVES

Figure 11

A—Phaseolus vulgaris. B—P. acutifolius. The secondary leaves of *P. acutifolius* have apices that are more acute than those of *P. vulgaris.*



MATURE HYBRID LEAVES

Figure 12

A—Shows the mature leaves of the interspecific hybrid. *B*—Mature leaf segregation in the F_2 generation.

Flowers of the female parent, *P. vulgaris,* were emasculated as follows: The standard was opened by scratching the suture with a sharp pointed tweezer. One of the wings was then clipped at the base to allow a better view of the keel. Small portions of the keel were then removed by pricking the bent portion near the apex, which minimized breakage and injury of the curled and brittle style. After removing the stamens, the stigmatic surface and style were examined with a 15× hand lens for pollen and possible injury.

Pollen-laden stigmas of the male parent, *P. acutifolius* were used as pollen bearers. These stigmas were obtained by bending the wings of the flower toward the pedicel, which forced the stigma to protrude from the aperture of the keel.

Pollen transfer was accomplished by brushing the "male" stigma carrying pollen on the stigmatic surface of the female flower. The "male" stigma was then left adhering to the newly pollinated stigma of the female parent. In order to maintain a high humidity about the stigma, the standard from the "male" flower was crushed in the palm of the hand with the thumb, moistened with saliva, and placed over the closed "female" standard. Excess flowers were removed to eliminate competition. During the crossing period, high humidity was maintained in the greenhouse.

Most of the flowers set pods, but all failed to reach maturity. Embryo abor-

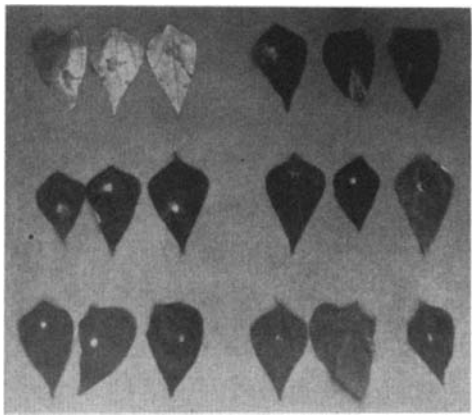
tion occurred within three to 24 days following pollination. Hormone applications suggested by Wester and Marth[11] to increase seed set did not increase the number of pods set nor did they delay the time of abortion. To obtain F_1 plants from the cross, embryos were excised and cultured *in vitro*[8]. From several hundred embryos, only four mature plants were obtained. The reciprocal cross using *P. acutifolius* as the female parent did not form pods or seeds. When intraspecific F_1 plants of *P. acutifolius* were used as female parents a few pods set but the embryos failed to attain sufficient size for culture *in vitro*.

Testing for Resistance

Inoculations were made in the greenhouse when the first set of secondary leaves were expanded. The underside of the terminal leaflet of the trifoilate of each plant was inoculated, using a modification of a method described by Schuster.[8] A shield with a six mm. hole placed against the leaflet and sprayed with the bacterial suspension was found to give a uniform inoculation.

Symptoms of blight infection were evident 10 to 14 days after inoculation. First, a thin border of water-soaked area appeared around the inoculated area. This was followed by chlorosis. The chlorotic area grew larger until the entire leaflet turned yellow and dropped off.

A classification of plant reaction was made at the time the entire leaflet of the susceptible lines and controls became yellow. Individual plants were classified as 0, 1, 2, 3, 4, and 5 reaction types. Type 0 showed no reaction around the tissue killed by inoculation; 1 included types with a narrow yellow ring around the inoculated area; 2 produced a slightly larger chlorotic band; 3 a larger band than 2; 4 being larger than 3 with irregular spreading; and in 5, yellowing of the entire leaflet. These reaction types are illustrated in Figure 13.



TYPES OF BLIGHT REACTION

Figure 13

Top row: left, type 5; right, type 4. Middle row: left, type 3; right, type 2. Bottom row: left, type 1; right, type 0.

Characteristics of the Hybrid

The interspecific hybrid plants were shorter than either parent. Foliar characteristics in the young plants resembled the tepary parent, but later stages of vegetative growth appeared more like *P. vulgaris* (Figure 12*A*). The inflorescence and pods were also similar to those of *P. vulgaris*. The flowers were self-fertile and produced pods with viable seeds. Seed number per pod were comparable to that of the *P. vulgaris* parent.

The diploid chromosome number of the two species has been reported as 22.[1] Root-tip preparations of the hybrid and parents did not suggest any marked morphological differences between the chromosome complements. A similar report on the chromosome morphology of these two species was made by Karpechenko.[4]

TABLE I. Parental averages and F_3 segregate averages and range for certain botanical characters used for comparison

Botanical Character	*Phaseolus acutifolius**	*Phaseolus vulgaris**	F_3 Segregation†		P‡
	avg.	avg.	avg.	range	
Seed size	.13 ± .01 gm.	.34 ± .02 gm.	.32 ± .05 gm.	.20 — .47 gm.	.001
Petiole length (primary leaves)	4.1 ± .2 mm.	43.9 ± 3.9 mm.	35.2 ± 5.1 mm.	9 — 53 mm.	.17
Length of primary leaves	46.3 ± 4.4 mm.	72.6 ± 5.2 mm.	75.7 ± 7.1 mm.	66 — 96 mm.	.73
Width of primary leaves	30.3 ± 3.7 mm.	52.8 ± 4.7 mm.	48.3 ± 5.5 mm.	41 — 63 mm.	.53
Ratio: length/width, primary leaves	1.52 ± .15	1.38 ± .09	1.57 ± .09	1.38 — 1.71	.11
Length of terminal leaves	51.5 ± 4.9 mm.	83.7 ± 6.2 mm.	87.2 ± 9.9 mm.	73 — 99 mm.	.76
Width of terminal leaves	32.0 ± 1.3 mm.	58.4 ± 3.4 mm.	52.9 ± 6.3	42 — 64 mm.	.44
Ratio: length/width, terminal leaves	1.62 ± .07	1.41 ± .04	1.65 ± .13	1.51 — 1.81	.07

* Measurements from 25 parental plants of each species.
† Measurements from 250 plants sampled from the F_3 segregating population.
‡ P is probability of true difference between the F_3 hybrids and *P. vulgaris*.

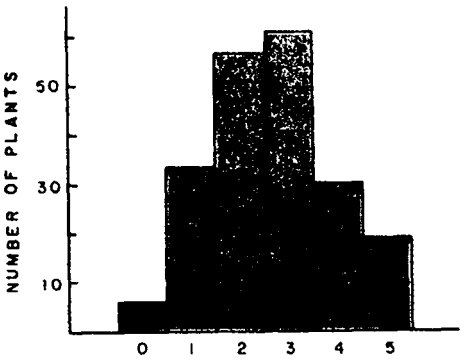


Figure 14

Distribution of 206 plants sampled from the F_3 population for blight reaction.

Characteristics of the Segregating Generation

Since only a limited number of seeds were obtained from the F_1 plants, blight reaction and botanical differences were studied in the F_2 generation. The parents and segregates were grown in the greenhouse.

Measurements of mature plant parts were made and the probability of true difference between the F_2 hybrids and *P. vulgaris* are shown in Table I. A definite difference in seed size was noted between the F_2 hybrids and the *P. vulgaris* parent. For the characters, petiole length and ratios of length/width of the primary and terminal leaves, the P values suggest a difference between the F_2 hybrids and *P. vulgaris*.

In general the segregates were larger than the *P. acutifolius* parent. The primary leaf bases ranged from truncate to cordate in shape (Figure 10*B*). Variations in mature leaf shapes were also present, (Figure 12*B*).

The plants of the F_2 population showed varying degrees of resistance to common blight. Distribution of reaction types sampled from the segregating population is shown in Figure 14.

Discussion

The data and figures demonstrate that a cross between the two species, *P. vulgaris* and *P. acutifolius*, was secured. The variation of characters in the F_2 generation suggest a quantitative type of inheritance. From the data as shown in Table I, there were measurements of the F_2 segregates which exceeded that of *P. vulgaris*, the larger parent, whereas none of the measurements of *P. acutifolius* were recovered. A probable explanation for the appearance of segregants larger than *P. vulgaris*, but none as small as *P. acutifolius*, may be the result of hybrid vigor.

Summary

Four plants were produced from the cross, *Phaseolus vulgaris* × *P. acutifolius*. The hybrids appeared more like the *P. vulgaris* parent. Chromosome smears of root tips showed no morphological differences between the complements of the hybrids and the parents. Common blight reaction and botanical characters which differed distinctly between the parents were found to be quantitatively inherited in the F_2 generation.

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