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# Protein Characters and Relationship between *Phaseolus vulgaris* ssp. *aborigineus* BURK. and Related Taxons of the Genus *Phaseolus*

JOSEF KLOZ, EVA KLOZOVÁ and VĚRA TURKOVÁ

Institute of Experimental Botany, Czechoslovak Academy of Sciences, Praha*

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**Abstract.** Both quantitative and qualitative immunochemical methods were used for studying the mutual relationships of several species and the subspecies of the genus *Phaseolus: Ph. vulgaris* L. ssp. *vulgaris, Ph. vulgaris* L. ssp. *aborigineus* BURK., *Ph. coccineus* L., *Ph. acutifolius* A. GRAY, *Ph. lunatus* L. (American endemites) and *Ph. aureus* L. (a typical Asian bean). Protein characters of cotyledons (i.e. „storage" proteins) of the above species were compared with the aid of antisera prepared against seed (cotyledon) proteins of *Ph. vulgaris* L. ssp. *vulgaris*, cv. Veltruská Saxa, using

(a) the whole complex of cotyledon protein,
(b) the albumin fraction of this complex,
(c) the globulin fraction,
(d) crystaline phaseolin.

Our results are in agreement with the morphological and genetic data of BURKART and BRÜCHER on the close relationship between *Ph. vulgaris* L. ssp. *vulgaris* and *Ph. vulgaris* L. ssp. *aborigineus* (both contain a character designated as *Ph. vulgaris* protein 1 which is lacking in the others, both contain an identical phaseolin and exhibit only negligible differences in the specificity of proteins). The closest to these two species is *Ph. coccineus* (almost identical phaseolin, small differences in the albumin and globulin fractions, a greater quantitative difference in protein specificity). *Ph. acutifolius* contains a somewhat different phaseolin, exhibits greater deviations in the albumin and globulin fractions and a greater quantitative difference in protein specificity. *Ph. lunatus* and *Ph. aureus* are quite different in all the above respects. These data are in good agreement with genetic data (crossability).

At present there is no doubt that the most important cultivated species of the genus *Phaseolus* originated in the present Central American countries and in Southern and South-East Asia (IVANOV 1937, 1961). Among the more important American species one should mention above all *Phaseolus vulgaris* L., *Ph. coccineus* L., *Ph. acutifolius* A. GRAY, *Ph. lunatus* L., whereas the more important Asian species comprise *Ph. aureus* L. With the possible exception of *Ph. acutifolius*, these species have been cultivated for several thousands of years.

PIPER (1926) classifies *Ph. vulgaris* in the section *Euphaseolus* PIP., while

* *Address:* Flemingovo nám. 2, Praha 6 — Dejvice.

*Ph. coccineus*, *Ph. lunatus* and *Ph. acutifolius* to *Leptospron* PIP. At that time he still did not know *Ph. aborigineus.*

The American species *Ph. coccineus*, *Ph. acutifolius*, *Ph. lunatus* still have wild-growing counterparts in the overgrown higher levels of Central America (PIPER 1926, IVANOV 1937, 1961). The wild original species of *Ph. vulgaris* was not known, similarly as in the case of the maize *Zea mays* L. PIPER (1926) considered *Ph. macrolepis* PIP. from Guatemala as very close to the species *Ph. vulgaris* but did not claim that it was the original species for *Ph. vulgaris*. Only in 1941 and 1943 some reports appeared on the wild forms of *Ph. vulgaris* in northern Argentine and these findings were described by the Argentinian botanist Art. BURKART in 1952 as *Ph. aborigineus*, nov. spec. vel subspecies BURKART (BURKART 1952). In further work carried out in cooperation with another independent discoverer of the new plant (BURKART, BRÜCHER 1953) some habitual and genetic evidence was supplied for the view that *Ph. aborigineus* and *Ph. vulgaris* are subspecies of a single species (crossability with *Ph. vulgaris*). It appeared that *Ph. aborigineus* is the long-sought ancestor of *Ph. vulgaris*. *Ph. aborigineus* and *Ph. vulgaris* were described by BURKART and BRÜCHER (1953) as *Ph. vulgaris* L. sensu amplissimo, subsp. *aborigineus* BURK. and subsp. *cultigenus* BURK.*)

Facts on the crossability between *Ph. aborigineus* and *Ph. vulgaris* were confirmed by RUDORF (1958) and we also obtained normal fertile hybrids between *Ph. vulgaris* L. ssp. *vulgaris* and *Ph. vulgaris* L. ssp. *aborigineus* BURK.

*Ph. vulgaris* L. ssp. *aborigineus* grows in the primeval forests and stands of the eastern slopes of the Andes and was found in a number of localities beginning with northern Argentine up to Honduras in Central America. It grows in rich moist soil, and as a vine may be as much as 3 m high. Seeds are relatively small (about $8 \times 6$ mm from our experience), of various dark shades, ranging to black. Ripe pods crack when dry, their halves roll up rapidly and spread the seeds around. It is a late, relatively short-day plant, relatively cold-resistant when mature. According to BURKART and BRÜCHER it is a clearly wild-growing plant and local inhabitants occasionally gather its seeds for food. Thus, the properties of wild-growing *Ph. vulgaris* ssp. *aborigineus* are still attractive and show how the plant became cultivated in the past.

In the present work we concentrated our attention on the investigation of protein characters of *Ph. vulgaris*, ssp. *aborigineus* in relation to *Ph. vulgaris* ssp. *vulgaris*, *Ph. coccineus*, *Ph. acutifolius*, *Ph. lunatus* and *Ph. aureus*. Immunochemical methods were used to express qualitatively as well as quantitatively the degree of compatibility between the seed proteins of the species mentioned.

## Materials and Methods

Starting plant material

(a) *Ph. vulgaris* L. ssp. *vulgaris*, cv. Veltruská Saxa, (b) *Ph. vulgaris* L. ssp. *aborigineus* BURK. (black seeds, All-Union Institute for Plant Production, U.S.S.R.), (c) *Ph. vulgaris* L. ssp. *aborigineus* BURK. (dark-brown to black

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* According to the new rules of nomenclature (DOSTÁL 1958), the subspecies should be designated as follows: *Ph. vulgaris* L., ssp. *aborigineus* BURK. and ssp. *vulgaris*. This nomenclature is used here throughout.

seeds with light-brown streaks, School of Agriculture, Cambridge), (d) *Ph. vulgaris* L. ssp. *aborigineus* BURK. (grey-brown seeds with dark streaks, Institut für Kulturpflanzenforschung, Gatersleben), (e) *Ph. coccineus* L. (flore rubro, semine nigro), (f) *Ph. acutifolius* A. GRAY (No. 9, brown seeds, Service de la recherche agronomique et de l'expérimentation agricole, Rabat, Maroc), (g) *Ph. lunatus* L. (dark-red seeds, Institut für Kulturpflanzenforschung, Gatersleben, G.D.R.), (h) *Ph. aureus* L. (Academia Sinica, Peking, China).

Seeds of all these plants were reproduced here.

Immunochemical methods

Of immunochemical methods we used the objective determination of the intensity of the overlaying precipitation reaction (quantitative overlaying precipitation, KLOZ 1960, 1961) and immunoelectrophoresis (GRABAR, WILLIAMS 1953) in ŠKVAŘIL's micromodification (1961) with some smaller adaptations.

Antisera. For testing proteins by the above methods we used antisera prepared by immunizing rabbits with proteins of the seeds (cotyledons) of *Ph. vulgaris* L. ssp. *vulgaris* cv. Veltruská Saxa:

(I) Antiserum against the whole complex of proteins extractable from ground seeds (cotyledons) with physiological saline;

(II) antiserum against the albumin fraction isolated from cotyledons; the albumin fraction was prepared in the cold by eight-day dialysis of the extract from ground cotyledons against distilled and deionized water and by centrifuging the precipitated proteins; the supernatant was freeze-dried and used for immunization;

(III) antiserum against the globulin fraction isolated from cotyledons; this fraction was obtained from the whole extract during the separation of albumin by dialysis (see above); the globulin precipitate was centrifuged, the supernatant decanted and the sediment washed twice with deionized water;

(IV) antiserum against crystalline phaseolin isolated from the seeds of *Ph. vulgaris* L. ssp. *vulgaris* according to BOURDILLON (1949).

Rabbits were immunized intravenously with 3—7 mg doses of the antigen every other day for 4 to 9 weeks, with occasional interruption and test of titre.

The antisera were obtained by a conventional method and kept frozen-dried (e.g. the antiphaseolin serum from several rabbits is thus stored unchanged for 6 years). The lyophilizates were used as required for making solutions of antisera by simple dissolution in distilled water.

Preparation of proteins (antigens). Seeds of the above-named species of beans without testa were finely ground and defatted with cold toluene (twice 10 min.) and petroleum ether (once, 5 min.) in a cold bath (below 0° C) and centrifuged. After the last centrifugation the ground material was rapidly dried in air. The fat-free preparations of seeds of all the bean species were also stored dry and frozen. The defatting procedure may in some cases remove the persistent turbidity and reduce the non-specific influence on specific precipitation reactions. But for routine work with proteins of *Phaseolus* seeds no preliminary defatting is required, in particular not for immunoelectrophoresis.

Quantitative overlaying precipitation. The fat-free flour was extracted with physiological saline overnight and centrifuged in the cold at 35,000 g for about

30 min. until the supernatant was perfectly clear. After precipitation with trichloroacetic acid the protein content of the supernatants was determined by the biuret reaction in the more sensitive modification according to LEVIN and BRAUER (1951) on a spectrophotometer. The solution was diluted with physiological saline to 2 mg protein/ml and after a second estimation (control and reduction of methodical error) diluted accurately to 1 mg protein/ml. This concentration produces sufficiently high specific reactions with antisera and no more non-specific reactions due to phytohaemagglutinins (present in plant seeds, in particular of the *Viciaceae*).

These accurately diluted extracts from seeds of eight bean samples and gradually all four prepared antisera were subjected to the quantitative overlaying precipitation reaction. In using this method the antiserum is overlaid with the antigen in a suitable cuvette and the specific reaction taking place at the boundary between antigen and antibody is assayed objectively with the aid of LIBBY's "photronreflectometer". Since exactly equal amounts of protein are used both in the homologous reactions, the possibly lower values of heterologous reactions are at the expense of qualitative differences between the proteins tested (or rather between their immunochemically active determinant groups). The advantage of this arrangement lies in the fact that, unlike in common precipitation, no complete precipitation curve must be constructed and the zone of optimal proportions sought so that material is not wasted, time saved and reproducibility guaranteed. In contrast with the previous papers (KLOZ 1960, 1961), round annular cuvettes 10 mm in diameter and 2 mm high are used so that the need for reactants was reduced to a minimum (0·05 ml antiserum is overlaid with 0·2 ml antigen in each reaction).

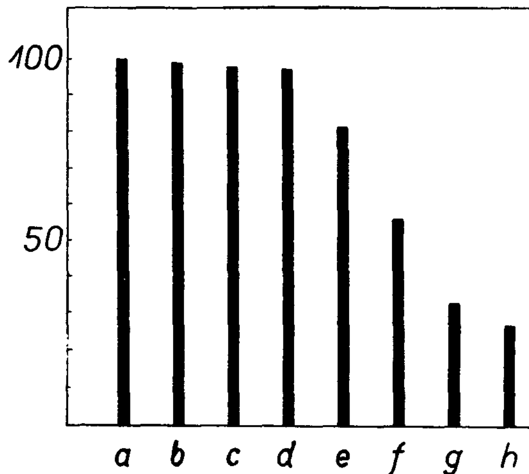


Fig. 1. Results of determining the intensity of serological reactions between antisera against cotyledon proteins of *Phaseolus vulgaris* L. ssp. *vulgaris* and cotyledon proteins of: a *Ph. vulgaris* L. ssp. *vulgaris* (homologous reaction = 100%), b *Ph. vulgaris* L. ssp. *aborigineus* BURK. (= 99%), c *Ph. vulgaris* L. ssp. *aborigineus* BURK. (= 98%), d *Ph. vulgaris* L. ssp. *aborigineus* BURK. (97%), e *Ph. coccineus* L. (81%), f *Ph. acutifolius* A. GRAY (= 56%), g *Ph. lunatus* L. (= 33%), h *Ph. aureus* L. (= 27%). Designation corresponds to the text.

Determination with the aid of quantitative overlaying precipitation was carried out in two parallel samples and, moreover, was always repeated with freshly prepared solutions of proteins and antisera. The relationship between protein characters was determined with four different antisera, each directed against a different protein fraction of the same material (species specificity or taxonic breadth of the individual fractions of proteins will not be dealt with here, in order not to unduly complicate matters). By taking an average of all these determinations we could reduce the methodical error to a minimum. The results are shown in Fig. 1.

Immunoelectrophoresis. In addition to the above-mentioned objective expression of the intensity of immunochemical reactions, a qualitative analysis of protein characters was carried out with antisera against the albumin and globulin fractions of cotyledons of *Ph. vulgaris* (their antiserum characteristics are shown above sub II and III) using immunoelectrophoresis. Seven to eight % solutions of proteins obtained by extracting the fat-free flour of the various seeds were placed into the start pits. Agarose was used as immunoelectrophoretic carrier (HJERTÉN 1961, GHETIE, MOTET-GRIGORAS 1962). Electrophoresis took place under standard conditions for 55 min. at 15 mA/one $5 \times 5$ cm plate and 4 V/cm in a veronal-citrate buffer of pH 8·6, at $\mu$ 0·05. After separation antisera were placed in the longitudinal grooves; they then diffused in the moist chamber at room temperature for about 18 hours. At the end of diffusion the plates were washed, dried and stained in the usual way. The results are shown in Figs. 2a—h and 3a—h.

## Results and Discussion

The results are shown in Figs. 1—3. They hardly require a commentary. It should be emphasized that there is certainly less subjective element involved here than in evaluating the morphological features. The degree of objectivity in this case is not determined simply by excluding the subjective evaluation of the significance of individual characters. On the other hand, it depends also on the selection of protein characters which possess different degrees of specificity, i.e. inequal taxonic breadth. In this case we do not examine all the protein characters defining a given organism but only their small part which is technically most convenient (in this case the storage proteins of the cotyledons). The degree of objectivity is partly determined by the interaction with the animal which can produce antibodies against the various proteins involved with different intensities, depending on its individuality. For this reason it was necessary to immunize always a greater number of animals and to select the highest-quality antisera. Therefore, antisera against individual fractions were used. Every method lacks perfection but the more methods used in investigation the greater the probability of finding the right answer.

It is known that boundaries between taxons and species are often only relative. In some cases there exist transitions between taxons which persist throughout the phylogenetic development.

In spite of these objections it may be noted that immunochemical differences between seed proteins of *Ph. vulgaris* ssp. *vulgaris* and *Ph. vulgaris* ssp. *aborigineus* are only minute (see Figs. 1—3) and that they lie probably within

a single species. This is best evidenced by the results showing the degree of compatibility between protein characters (Fig. 1). The protein character of the albumin fraction which occurs in *Ph. vulgaris* ssp. *vulgaris* and which may be designated as *Ph. vulgaris* protein 1 is present with the same physico-chemical and immunochemical characteristics, thus practically identical also in *Ph. vulgaris* ssp. *aborigineus.* It is lacking in the other species examined here (see Fig. 2).

The analysis of the globulin fraction yields a different picture. Phaseolin is the most pronounced protein character here (OSBORNE 1894, BOURDILLON 1949, Fig. 3), and is present in both subspecies of *Ph. vulgaris* and in *Ph. coccineus* in practically the same form (see also KLOZ 1962). This indicates again that both the subspecies are very closely related and it follows, moreover, that *Ph. coccineus* is not very distant. Phaseolin of *Ph. acutifolius* is not identical with the preceding ones but is similar. This fact will be taken up elsewhere. Phaseolin-resembling proteins are practically absent in *Ph. lunatus* and *Ph. aureus.*

The relatively close relationship between *Ph. coccineus* and *Ph. vulgaris* ssp. *vulgaris* and *Ph. vulgaris* ssp. *aborigineus* follows not only from Fig. 3 (globulin fraction) but also from Fig. 1. The close relationship is supported by other facts, such as fairly good crossability (e.g. LAMPRECHT 1941, 1948), graft affinity (KLOZ, TURKOVÁ 1963) and some morphological-ecological data (IVANOV 1937, p. 60).

On the basis of the above evidence it is our view that Piper's classification of *Ph. vulgaris* and *Ph. coccineus* (1926) into two sections (*Euphaseolus* and *Leptospron*) requires revision. The same, with even more urgency, holds for Taubert's classification (in ENGLER, PRANTL 1891, see also IVANOV 1928) who places *Ph. vulgaris* and *Ph. lunatus* into *Euphaseolus* and *Ph. multiflorus* (= *Ph. coccineus*) and *Ph. acutifolius* into *Drepanospron* (= *Leptospron* PIP.), while the Asian species belong to *Strophostyles* (= *Ceratotropis* PIP.).

*Ph. lunatus* and *Ph. aureus* cannot be differentiated by antisera against seed proteins of *Ph. vulgaris* as would correspond to differences between their genomes and "protenomes" (cf. KLOZ 1962, KLOZ, TURKOVÁ 1963, KLOZ, KLOZOVÁ, TURKOVÁ, in press).

Our experimental arrangement offers at least a partial solution of the relationships of *Ph. acutifolius.* This species resembles in its protein characters more *Ph. vulgaris* ssp. *vulgaris* and *Ph. vulgaris* ssp. *aborigineus* as well as *Ph. coccineus* than it does *Ph. lunatus* (cf. Figs. 1—3).

Whereas *Ph. coccineus* belongs thus clearly to the closest relatives of *Ph. vulgaris* ssp. *vulgaris* and *Ph. vulgaris* ssp. *aborigineus*, *Ph. acutifolius* stands somewhat apart and *Ph. lunatus* is still more distant.

Beside the quantitative evaluation (see Fig. 1) expressing the quantitative relationship in protein specificity, another semi-quantitative criterion may be offered, viz. the number of protein characters in immunoelectropherograms in Figs. 2 and 3, even if the counting of less pronounced characters cannot always be precise and merely expresses the fact whether they are "present or absent" but not whether "present but only more or less similar".

BURKART and BRÜCHER (1953) assumed the close relationship between *Ph. vulgaris* ssp. *vulgaris* and *Ph. vulgaris* ssp. *aborigineus* not only on the basis of habit features but also of their mutual crossability. It is known that cross-

Table 1.

| | Number of protein characters detected by immunoelectrophoresis using | | |
|---|---|---|---|
| | albumin antiserum about | globulin antiserum about | total |
| *Ph. vulgaris* ssp. *vulgaris* | 13 | 8 | 21 |
| *Ph. vulgaris* ssp. *aborigineus* | 13 | 8 | 21 |
| *Ph. coccineus* | 12 | 6 | 18 |
| *Ph. acutifolius* | 6 | 6 | 12 |
| *Ph. lunatus* | 7 | 5 | 12 |
| *Ph. aureus* | 6 | 4 | 10 |

ability can be considered to a certain extent as one of the criteria (even if with certain limitations) for defining species boundaries (SCHIEMANN 1932, DAVIS, HEYWOOD 1963). Thus, according to phylogenic relationship (which should be fixed in taxonomy) mutual crossability could be partly assessed and vice versa. There is a number of exceptions known where non-crossability is due to other factors than lack of relation between genotypes.

If the group of American beans is considered from this aspect, the following table can be compiled from our and other authors' experiences.

All the crosses carried out were brought to at least the $F_2$ generation, even if the fertility of hybrids of *Ph. coccineus, Ph. acutifolius* and *Ph. lunatus* is definitely reduced. Data are found in the literature on crosses between *Ph. vulgaris* and *Ph. mungo* (an Asian species nearest *Ph. aureus* (STRAND 1942), but this observation should be investigated in greater detail since the species are rather wide apart.

Thus, it is believed that in this case even the degree of crossability confirms our results on the mutual relationship between the species examined here.

## Conclusions

(1) According to quantitative and qualitative analysis of protein characters, *Ph. aborigineus* BURK. is practically identical with *Ph. vulgaris*. This supports

Table 2.

| Parents | Crossability | Authors |
|---|---|---|
| *Ph. vulgaris* ssp. *vulgaris* × *Ph. vulgaris* ssp. *aborigineus* | Very good | BURKART and BRÜCHER 1953, RUDORF 1958 |
| × *Ph. coccineus* | Good, reciprocally poor | MENDEL 1866, et al. |
| × *Ph. acutifolius* | Poor (embryos cultivated later in vitro) | HONMA 1956 |
| × *Ph. lunatus* | Poor (using heterozygous plants as parents) | HONMA, HEECKT 1959 |

the latest view of BURKART and BRÜCHER that *Ph. aborigineus* is a subspecies of *Ph. vulgaris* (hence, according to modern nomenclature, *Ph. vulgaris* L. ssp. *aborigineus* BURK. and *Ph. vulgaris* L. ssp. *vulgaris*) and that it is probably the long-sought ancestor of cultivated *Ph. vulgaris* L. ssp. *vulgaris*.

(2) Seeds of both subspecies of *Ph. vulgaris* are characterized by a protein character at the anode side ("*Ph. vulgaris* protein 1") which is lacking in all the other species.

(3) A pronounced protein character of the globulin fraction, phaseolin, is characteristic for both subspecies of *Ph. vulgaris*, *Ph. coccineus* and *Ph. acutifolius*, whereas it is lacking in *Ph. lunatus* and *Ph. aureus*.

(4) Thus, *Ph. coccineus* is closest to *Ph. vulgaris* sensu lato as far as protein characters are concerned. *Ph. acutifolius* is somewhat farther apart and *Ph. lunatus* and *Ph. aureus* are rather distant.

(5) The number of protein characters on immunoelectropherograms and their similarity corresponds to crossability experience between *Ph. vulgaris* and other bean species investigated here.

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Imunochemickými metodami jak kvantitativními, tak kvalitativními jsme sledovali vzájemné vztahy těchto druhů a subspecií rodu *Phaseolus: Ph. vulgaris* L. ssp. *vulgaris*, *Ph. vulgaris* L. ssp. *aborigineus* BURK., *Ph. coccineus* L., *Ph. acutifolius* A. GRAY, *Ph. lunatus* L. (američtí endemiti) a *Ph. aureus* L. (typický asijský fazol). Srovnávali jsme bílkovinné znaky děloh (tedy „zásobní“ bílkoviny) uvedených druhů pomocí antiser, připravených proti bílkovinám semen (děloh) *Ph. vulgaris* L. ssp. *vulgaris*, cv. Veltruská Saxa a sice proti: a) celému komplexu děložních bílkovin, b) albuminové frakci tohoto komplexu, c) globulinové frakci, d) krystalickému fazeolinu. Naše výsledky souhlasí s morfologickými a genetickými údaji BURKARTA a BRÜCHERA o těsném vztahu *Ph. vulgaris* L. ssp. *vulgaris* a *Ph. vulgaris* L. ssp. *aborigineus* (u obou je přítomen znak označený námi „*Ph. vulgaris* protein 1“, chybějící u ostatních, mají shodný fazeolin, kvantitativně vyjádřené rozdíly ve specifitě bílkovin jsou nepatrné). Těmto stojí nejblíže *Ph. coccineus* L. (fazeolin téměř shodný, rozdíly v albuminové a globulinové frakci, větší kvantitativně vyjádřený rozdíl ve specifitě bílkovin). *Ph. acutifolius* ve srovnání s předchozími již nemá zcela shodný fazeolin, má větší odchylky v albuminové a globulinové frakci, velký kvantitativně vyjádřený rozdíl ve specificitě bílkovin. *Ph. lunatus* a *Ph. aureus* jsou velmi odlišné ve všech těchto ukazatelích. Tyto naše údaje jsou v dobrém souladu i s fakty genetickými (křižitelností).

Й. КЛОЗ, Е. КЛОЗОВА, В. ТУРКОВА, Институт экспериментальной ботаники ЧСАН Прага: **Белковые признаки и родственные связи *Phaseolus vulgaris* L. *ssp. aborigineus* BURK. и близких ему видов рода *Phaseolus*.** — Biol. Plant. 8 : 187—196, 1966.

Мы изучали количественными и качественными иммунохимическими методами взаимоотношения следующих видов и подвидов рода *Phaseolus*: *Ph. vulgaris* L. ssp. *vulgaris*, *Ph. vulgaris* L. ssp. *aborigineus* BURK., *Ph. coccineus* L., *Ph. acutifolius* A. Gray, *Ph. lunatus* L. (американские эндемиты) и *Ph. aureus* L. (типичная азиатская фасоль). Мы сравнивали белковые признаки семядолей (следовательно „запасные“ белки) при-

веденных видов при помощи антисывороток приготовленных против белков семян (семядолей) *Ph. vulgaris* L. ssp. *vulgaris* cv. Велтруска Сакса, а именно против:

а) целого комплекса семядольных белков,
б) альбуминовой фракции этого комплекса,
в) глобулиновой фракции,
г) кристаллическому фазеолину.

Наши результаты находятся в соответствии с морфологическими и генетическими данными Буркарта и Брюхера о тесном отношении *Ph. vulgaris* L. ssp. *vulgaris* и *Ph. vulgaris* L. ssp. *aborigineus* Burk. (у обоих присутствует признак, обозначаемый «*Ph. vulgaris* протеин 1», отсутствующий у других; у них сходный фазеолин, количественно выраженные различия в специфичности белков небольшие). К ним ближе всего стоит *Ph. coccineus* (фазеолин почти сходный, различия в альбуминовой и глобулиновой фракциях, однако количественно выраженное различие в специфичности белков бóльшее). *Ph.acutofolius* по сравнению с предыдущими уже не обладает столь сходным фазеолином, имеет бóльшие уклонения в альбуминовой и глобулиновой фракциях, большое количественно выраженное различие в специфичности белков. *Ph. lunatus* и *Ph. aureus* во всех этих показателях очень отличаются. Эти наши данные находятся в хорошем соответствии и с генетическими данными (скрещиваемость).