

The Protein Euphaseolin in *Phaseolinae* — a Chemotaxonomical Study

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Abstract. On the basis of immunochemical analyses of the main reserve protein of *Ph. vulgaris*, euphaseolin, in numerous cultivars of *Ph. vulgaris*, in additional 23 *Phaseolus* species, and several representatives of further genera of Viciaceae and on the basis of the comparison of these data with morphological and genetical data the authors propose to separate the section *Euphaseolus* characterized by the presence of the protein euphaseolin. The species characterised by euphaseolin are closely related and capable of being crossed. The proposal requires an additional formal completion from the point of view of the conventions of classical systematics. Further the questions of the taxonomical extent of various protein characters and the problematics of the so-called large and small protein characters are discussed.

The systematics of the *Phaseolus* genus is not elaborated in detail. In principle, the old classifications according to BENTHAM (1838), TAUBERT (ENGLER and PRANTL 1891) and PIPER (1926) are the starting points, based on single morphological characters. The systematics of this genus is rather difficult. It is a polyphyletic genus of which about 200 species have been described, mostly according to items from herbaria.

We shall endeavour to contribute to the elucidation of natural relationship of some groups of this genus by studying protein characters. Some of our earlier results (cf. KLOZ 1956, KLOZ *et al.* 1966a) and the relative independence and difference of the Asiatic group of beans, or the so-called "tropical" group were confirmed by other students of systematics; for example ÖHWI (1965) separates an independent genus *Azuki* (Asiatic species), or HUTCHINSON (1964) the genus *Macroptilium* (the "tropical" group).

In the papers mentioned below we studied the distribution of the main reserve protein of the seeds (cotyledons) isolated from *Phaseolus vulgaris* L. in the species of *Phaseolus* genus (KLOZ 1961, 1962, 1965, 1971; KLOZ *et al.* 1966a, b; KLOZOVÁ and KLOZ 1972).

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In this paper we present further results of the study of the distribution of the main reserve protein of seed cotyledons of *Ph. vulgaris* (i.e. of euphaseolin; we shall discuss the term below) in additional more than 500 cultivars of *Ph. vulgaris* and *Ph. coccineus**. This study completes those above mentioned. We shall also try to summarize the results in this paper and to formulate general rules.

Already in 1894 OSBORNE isolated a complex globuline fraction from the seeds of some species of beans, primarily from *Ph. vulgaris* (the main component of which was the protein studied by us) and he gave it the name phaseolin. In order to differentiate its main component from Osborne's complex fraction we call the main reserve protein euphaseolin.

This protein can be prepared in a practically pure state, i.e. without distinct antigenic admixtures. Euphaseolin can be isolated in various ways, for example according to JAFFÉ and HANNIG (1965), or in crystalline state according to BOURDILLON (1949), even though Bourdillon's and Jaffé's proteins are probably not structurally quite identical (Bourdillon's protein evidently does not contain a small part of the end group of the protein chain, due to the difference in the separation method), but immunochemically the two proteins cannot be differentiated. It is also possible to choose other suitable isolation methods of which there is a good selection to-day. Through some of its properties euphaseolin belongs among the so-called globulins (it is precipitated by dialysis against deionised water; it contains 50.4 % C, 15.3 % N, 0.12 % S, mol. weight about 171 000 (BOURDILLON 1949).

Using serological methods (for the preparation of the antiserum we used euphaseolin isolated from *Ph. vulgaris* L. ssp. *vulgaris* cv. Veltruská Saxa — according to BOURDILLON 1949) we found that euphaseolin is present in a number of taxons in immunochemically identical form (cf. the above references). In the preceding papers the results of the analysis of more than 650 cultivars of *Phaseolus vulgaris* L. are presented, as well as of the accessible sortiment of *Phaseolus* genus (24 species) and an additional 11 species from the *Phaseolae*, *Viciae* and *Coronillae* tribes (cf. KLOZ 1961, KLOZOVÁ and KLOZ 1972). On the basis of immunoelectrophoresis, double diffusion, and quantitative serological methods we found that euphaseolin is the typical and main reserve protein of the cotyledons of not only *Ph. vulgaris* L. ssp. *vulgaris* but also of *Ph. vulgaris* L. ssp. *aborigineus* BURK., *Ph. coccineus* L., *Ph. dumosus* MACF., *Ph. polyanthus* GREEN, and an unidentified species of which one sample comes from Columbia and the other from Venezuela⁺. In *Ph. acutifolius* A. GRAY the protein is similar to euphaseolin of the preceding species, but not identical (KLOZOVÁ and KLOZ 1972). In all other investigated species euphaseolin is missing completely, including the representatives of other studied tribes of the *Fabaceae* family; this means that convergence may almost certainly be excluded.

* The experimental work for this paper was carried out in the Botanical Institute of the Academy of Sciences of the USSR in Leningrad during our stay there in 1971.

⁺ We consider the hypothesis of Dr. Olga Brücher who supplied the second sample according to which it is a spontaneous hybrid between *Ph. vulgaris* and *Ph. coccineus* as insufficiently substantiated, even though this *Phaseolus* sp. is according to serological analyses very closely related to the species *Ph. vulgaris*, *Ph. coccineus* and *Ph. dumosus*.

In this paper we complete the preceding results by further material and we make an attempt at the generalisation of our results and conclusions.

Material and Methods

We analysed an additional 526 cultivars of *Ph. vulgaris* L. ssp. *vulgaris* kindly supplied by the Instituto de investigaciones agropecuarias, Santiago through the Czechoslovak Embassy in Chile. We should like here to express our gratitude to them. Further we analysed 10 representatives of *Ph. vulgaris* L. ssp. *aborigineus* BURK. and 30 cultivars (or wild forms) of *Ph. coccineus* L. A list of all investigated species, cultivars and forms is given in the addendum.

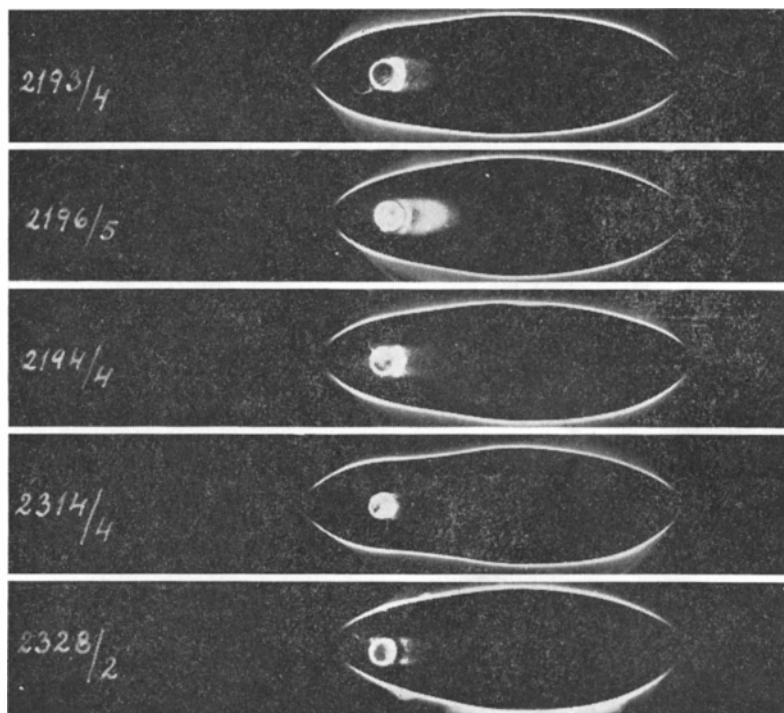


Fig. 1. Several examples of the typical euphaseolin in *Ph. vulgaris*. Total extract from seeds (cotyledons) detected with a monospecific antiserum against euphaseolin from *Ph. vulgaris* L. ssp. *vulgaris*.

The antiserum for the detection of euphaseolin was prepared by intravenous immunisation of rabbits with crystalline euphaseolin dissolved in physiological solution. Euphaseolin was prepared according to BOURDILLON (1949) from the cotyledons of the seeds of *Phaseolus vulgaris* L. ssp. *vulgaris*. The rabbits were immunised for 3—4 weeks by applying small doses, about 3—5 mg, each second or third day (cf. KLOZOVÁ and KLOZ 1972). It should be noted that pure euphaseolin is a very active antigen in comparison with seed globulins which are also good antigens, and therefore we were able to obtain sharply specific antisera with high titres even after a relatively short immunisation period.

We also used our usual method of immunoelectrophoresis in agarose-starch gel (KLOZ and KLOZOVÁ, in the press), that represents one of the modifications of gel immunoelectrophoresis.

The proteins were extracted from the flour of the ground seeds (cotyledons — always from one seed) 100 mg of the flour were extracted with 0.2 ml of buffered physiological solution for 3 hours in the cold, under occasional stirring. The extract was centrifuged at 20 000 revolutions/min ("swing out" rotor MSE Super Speed 25) and the supernatant was used for electrophoresis. After the electrophoretic separation of the mixture of extracted proteins euphaseolin was detected with the above mentioned monospecific antiserum against euphaseolin.

Results

The presence of euphaseolin was again proved unambiguously both in all cultivars of *Ph. vulgaris* L. ssp. *vulgaris* investigated (526), and in all forms of *Ph. vulgaris* L. ssp. *aborigineus* BURK. (10) and *Ph. coccineus* L. (30). Several examples are shown in Fig. 1. In all instances euphaseolin was again abundant. Polymorphism or a case of loss mutation was (in the case of euphaseolin) not observed.

Typical euphaseolins in all investigated cultivars, forms and species (with the exception of a protein similar to euphaseolin in *Ph. acutifolius*) are immunochemically identical, they evidently have identical determinant groups and the same, or slightly varying, macromolecule structure (or macromolecule populations?). Only negligible differences in the electrophoretic mobility of euphaseolins from some cultivars or forms could be observed.

Discussion

The main reserve protein of seeds (cotyledons), called euphaseolin, is typical of the group of *Phaseolus* species: *Ph. vulgaris* L. ssp. *vulgaris*, *Ph. vulgaris* L. ssp. *aborigineus* BURK., *Ph. coccineus* L., *Ph. dumosus* MACF., *Ph. polyanthus* GREEN, *Ph. sp.* (from Columbia), *Ph. acutifolius* A. GRAY. These species are endemic to America, originating mainly from Central American regions.

When comparing earlier systematical-taxonomical classification of these species according to their morphological characters by various authors it may be seen that in the majority of cases they are classified into various related groups. TAUBERT (in ENGLER-PRANTL 1891) puts *Ph. vulgaris* and *Ph. lunatus* into the *Euphaseolus* section, but *Ph. multiflorus* (= *Ph. coccineus*) into the section of *Drepanospron*. In the Cultivated Flora of USSR (IV, 1937) *Ph. lunatus* is already transferred to the section *Drepanospron*. PIPER (1926) classifies *Ph. macrolepis* (which he considers most closely related to *Ph. vulgaris*) into the section *Euphaseolus*, but *Ph. polyanthus*, *Ph. lunatus* and *Ph. acutifolius* into the group of "closely related to *Ph. coccineus*". IVANOV (1928, 1937) accepts Piper's classification but points out the possibility of common ancestors for *Ph. vulgaris* and *Ph. coccineus* (DITMER 1929—1930); BURKART (1952) includes Argentinian species from the *Drepanospron* BENTH. section into the *Euphaseolus* section. This group is close to our group of species, characterised by the presence of euphaseolin, as was mentioned above. This group is distinguished by the presence of euphaseolin. It is not necessary to stress that proteins, and hence euphaseolin as well, representing the product of primary metabolism, have a definitely higher taxonomical value than for example the products of secondary metabolism and some morphological

characters for which cases of convergence are possible. The species mentioned are evidently also in close relationship, they certainly originated from a common ancestor characterized by euphaseolin, rather recently in the history of evolution (single species are not yet much differentiated, with the slight exception of *Ph. acutifolius*). For this group of species, characterized by euphaseolin, it is also characteristic that they can be intercrossed (cf. KLOZ *et al.* 1966b). On the basis of these and some earlier data we suggest considering this distinct group of species, containing euphaseolin, as a section. We stress again that in more remote taxons euphaseolin was not found, i.e. that the convergence phenomena may be excluded unambiguously.

From our results it also follows that *Ph. lunatus* does not belong to this subsection either. As we mentioned above the views on the relationship of *Ph. lunatus* L. with *Ph. vulgaris* and also on its systematic classification were not always unanimous. In *Ph. lunatus* the main reserve protein is quite different from euphaseolin immunochemically, as was shown in our laboratory earlier (PŮLPÁNOVÁ 1967). The phenomena of ability (or inability) to cross-fertilise confirm these results (cf. KLOZ 1971).

The proposed subsection characterised by euphaseolin is thus characterised by mutual potential crossing ability. The justification of a certain observed rule can be checked by making prognoses for a new, unknown material, which would fit into this observed rule. We demonstrated this in the case of *Ph. polyanthus* in which we found euphaseolin and which would be crossed successfully with *Ph. vulgaris* (KLOZ 1961 — unpublished) and which was also crossed in Holland (1961). We also assume that exactly as all mentioned species of *Phaseolus* genus characterised by euphaseolin are potentially cross-fertilisable, those species — unknown or inaccessible so far — will also be cross-fertilisable them in which the typical euphaseolin is found in the future.

In one of the preceding papers (KLOZ and TURKOVÁ 1963a) we studied the correlation between the relationships determined by serological methods and between their mutual graft affinity. Although these correlations are evidently affected by other factors as well, and in spite of the fact that at that time we had only very few species at our disposal, the graft affinity, in that case also confirmed the relationship determined serologically. The problematics of the graft affinity in plants is still topical and it deserves further attention from this point of view as well, exactly as the problematics of the compatibility and incompatibility of tissues in medicine does.

Hence, using the protein euphaseolin we separated a relatively narrow, distinctly monophyletic group of typical American beans related to *Phaseolus vulgaris*. Certainly it would be correct to express this fact terminologically as well. It would probably be more correct to keep the older name *Drepanospron* for the broader Burkart's section *Euphaseolus*, and separate from it the mentioned group of beans, characterised by euphaseolin and the potential mutual cross-fertilising ability as a section of *Euphaseolus*. This, of course, will require additional completion of the work according to the conventions of classical systematics.

It should also be noted that in work with proteins serving as systematical-taxonomical characters two types of proteins may be met with in principle: the first (where euphaseolin belongs) comprises proteins typical without ex-

ception of the population of related organisms, or at least their possible variability — for example within the species, probably negligible, is not serologically demonstrable. Such are proteins that prevail to a certain extent in seeds or generally in storage organs. They may be called "large" proteinic characters. As the second type we may consider those seed proteins that vary in population and display polymorphism not only within the species but even within the variety (cultivar). Such an example is *Ph. vulgaris* protein 1 and 2 for which we demonstrated some time ago the occurrence of polymorphism and genotype variability (but not phenotypical, for example under the effect of the environment which is not common in seed proteins) in some *Phaseolus* species — both in self-pollinating and cross-pollinating species (KLOZ and KLOZOVÁ 1968). These proteins may be called "small" protein characters. The relation and correlations of the last type of proteins with other characters, including habitual ones, are not yet clear.

Let us notice another phenomenon; Euphaseolin occurs in relatively small number of species within the widespread *Phaseolus* genus. We are convinced that this situation cannot be changed even by the fact that we had the opportunity of analysing a mere 24 species of this genus, which is relatively little in view of the fact that the *Phaseolus* genus — possibly even after revision — will have many times more than this number of species. Hence, euphaseolin occurs in a relatively small group of beans when compared with other "large" protein characters, as for example reserve proteins of pea (*Pisum sativum* L.), legumin and vicilin, which are present in all other genera of the *Viciae* tribe (to which the *Pisum* genus belongs) and even partly exceed the limits of this tribe (KLOZ *et al.* 1961, KLOZ and TURKOVÁ 1963b). This probably also proves the relatively great differentiation of the present *Phaseolus* genus. This again is a case of the uneven "taxonomical width" of various proteins. Whether it is caused more by the fact that the phylogenetic differentiation of various proteins took place at an unequal rate or by the lack of uniformity of the systematical-taxonomic categories (units) of the more or less artificial system must be judged separately in single cases on the basis of further material and additional experimental results.

Conclusions

1) One of the main reserve proteins of seeds (cotyledons) of *Ph. vulgaris* — euphaseolin — is present without exception and in a distinct form in all additional 526 analysed cultivars of this species. Including the earlier investigated selection of *Ph. vulgaris* we proved the presence of euphaseolin in 1170 cultivars of *Ph. vulgaris*, without a single exception.

2) Euphaseolin is also typical of other species close to *Ph. vulgaris*: *Ph. vulgaris* L. ssp. *aborigineus* BURK., *Ph. dumosus* MACF., *Ph. polyanthus* GREEN, *Ph. sp.* — COLUMBIA, *Ph. coccineus* L.; euphaseolin from *Ph. acutifolius* A. GRAY is slightly different; in *Ph. lunatus* L. and other investigated species, as well as in the representatives of other genera, euphaseolin was never found.

3) The group of species characterised by the characteristic euphaseolin is cross-fertilisable.

4) All other species in which the typical euphaseolin will be found must be closely related to the former species and it may be assumed that they will be also cross-fertilisable with them; we disregard here the cases of incompatibility which are not based on relationship, *i.e.* on systematics.

5) On the basis of our results we propose that an independent section should be separated, called for example *Euphaseolus*, from the section *Drepanospron*, where the above mentioned American species may be classified which are characterised by the presence of euphaseolin.

6) On the basis of the comparison of the taxon-specificity of proteins with which we have worked so far the conclusion may be made that the proteins may have different values as systematic characters, and we divide them into "large" and "small" protein characters.

References

- Annu. Rep. Agr. Exp. Stat. Holland, 1961.
- BENTHAM, G.: *Euphaseoleae*. — Ann. Wien. Mus. **11** : 113, 1838.
- BOURDILLON, J.: Crystalline protein from commercial beans (*Phaseolus vulgaris*. — J. biol. Chem. **180** : 553—556, 1949.
- BURKART, A.: Las leguminosas Argentinas. — ACME Agency, Soc. de Resp. Ltda., Buenos Aires, 1952.
- DITMER, E. E.: [On the question of the origin of cultivated beans]. In Russ. — Tr. prikl. Bot. Genet. Selekt. **3**(5) 1929—30. Cited after: IVANOV, N. R.: Tr. prikl. Bot. Genet. Selekt. **1**(2) : 41 to 106, 1937.
- HUTCHISON, J.: The Genera of Flowering Plants. — Vol. I. Oxford Univ. Press, Oxford, 1964.
- IVANOV, N. R.: [Peculiarities in the originating of forms of *Phaseolus* L. in the old and the new world]. In Russ. Tr. Prikl. Bot. Genet. Selekt. **19**(2) : 185—212, 1928.
- IVANOV, N. R.: [Geographical regularities in the distribution of cultivated *Phaseolinae*]. In Russ. Tr. Prikl. Bot. Genet. Selekt. **1**(2) : 41—106, 1937.
- JAFFÉ, W. G., HANNIG, K.: Fractionation of proteins from kidney beans *Phaseolus vulgaris*. — Arch. Biochem. Biophys. **109** : 80—90, 1965.
- KLOZ, J.: The use of quantitative ring precipitation reaction for determining the intensity of cross reaction with special reference to comparative serology. — Biol. Plant. **3** : 127—227, 1961.
- KLOZ, J.: An investigation of the protein characters of four *Phaseolus* species with special reference to the question of their phylogenesis. — Biol. Plant. **4** : 85—90, 1962.
- KLOZ, J.: Protein characters and their genesis in lower taxons. — In: LANDA, Z. (ed.): Mechanism of Mutation and Inducing Factors. Pp. 477—483. Academia Praha, 1966.
- KLOZ, J.: Serology of the Leguminosae. — In: HARBORNE, J. G., BOULTER, D., TURNER, B. L. (ed.): Chemotaxonomy of the Leguminosae. Pp. 309—365. Acad. Press, London—New York, 1971.
- KLOZ, J., KLOZOVÁ, E.: Variability of proteins I. and II. in the seeds of species of the genus *Phaseolus*. — In: HAWKES, J. G. (ed.): Chemotaxonomy and Serotaxonomy. Pp. 93—102, Acad. Press, London—New York, 1968.
- KLOZ, J., TURKOVÁ, V.: Affinity and serodiagnostical relationships. — Serol. Mus. Bull. **29** : 8, 1963a.
- KLOZ, J., TURKOVÁ, V.: Legumin, vicilin and proteins similar to them in the seeds of some species of the *Viciaceae* family (a comparative serological study). — Biol. Plant. **5** : 29—40, 1963b.
- KLOZ, J., KLOZOVÁ, E., TURKOVÁ, V.: Chemotaxonomy and genesis of protein characters with special reference to the genus *Phaseolus*. — Preslia **38** : 229—236, 1966a.
- KLOZ, J., KLOZOVÁ, E., TURKOVÁ, V.: Protein characters and relationships between *Phaseolus vulgaris* ssp. *aborigineus* BURK. and related taxons of the genus *Phaseolus*. — Biol. Plant. **8** : 187—196, 1966b.
- KLOZOVÁ, E., KLOZ, J.: Distribution of the protein "phaseolin" in some representatives of *Viciaceae*. — Biol. Plant. **14** : 264—272, 1972.
- OSBORNE, T. B.: The proteids of kidney beans. — J. Amer. chem. Soc. **16** : 633—643, 1894.
- OHWI, J.: Flora of Japan. — Smiths. Inst., Wash. USA, Washington D.C., 1965.
- PIPER, C. V.: Studies in American *Phaseolineae*. — Contr. U.S. Herbar. **22** : 663—701, 1926.
- PULFÁNOVÁ, J.: Proteins characters and family relationships of *Phaseolus lunatus* and some others species of the genus *Phaseolus*. — Thesis. Charles University, Praha, 1967.
- TAUBERT, P.: *Phaseolineae*. — In: ENGLER, A., PRANTL, K. (ed.): Natürliche Pflanzen-Familien. Vol. III-2. Pp. 379—380, 1894.
- VULF, E. V. (ed.): Kulturnaya Flora SSSR. [Cultivated flora of the USSR]. — Vol. 4. Pp. 457 to 603. Leningrad, 1937.

The list of used plant material

1. Cultivars of *Phaseolus vulgaris* ssp. *vulgaris* (received from: Instituto de investigaciones agropecuarias, Santa Rose Paradero 33, Santiago, Chile).

2. Astro (74/1); 3. Algarrobo (74/2); 4. Alabama 1 (74/3); 5. Amarillo (74/4); 6. Amarillo chico (76/6); 7. Amarillo chaucha (75/1); 8. Ameliore (75/2); 9. Aparecido (75/3); 10. Alemanas (75/4); 11. Almidón (75/5); 12. Araucanos (78/1); 13. Amancy (78/2); 14. Argentina Alubia (78/3); 15. Arvejilla (78/4); 16. Algarrobes (78/5); 17. Avalitos (79/1); 18. Aviles (79/2); 19. Aragon (79/3); 20. Asgrow Valentine (79/4); 21. Asgrow Stringless Green Pod. (79/5); 22. Azulillos (82/1); 23. Azufrados (82/2);

24. Bayos 1941 (82/3); 25. Bayos Rancaguinos (82/4); 26. Bayos Cobquecura (82/5); 27. Bayos Alegres (83/1); 28. Bayos El Llano (83/2); 29. Bayos "B" (83/3); 30. Bayos Achatados (83/4); 31. Bayos Chatos (83/5); 32. Bayos Redondo (86/1); 33. Bayos 5032/34 (82/2); 34. Bayos Palos (86/3); 35. Bayos Coihuecos (86/4); 36. Belga (86/5); 37. Belga Nueva Imperial (87/1); 38. Beurre Blans Nain Ameliore (87/2); 39. Beurre Blue Lake (87/3); 40. Beurre Doré Nain (87/4); 41. Beurre Aiguillette (87/5); 42. Beurre Rapide (90/1); 43. Beurre Sure Crop (90/2); 44. Beurre Gloire Ollainville (90/3); 45. Beurre Nain Merville Marché (90/4); 46. Beurre Sans Rival (90/5); 47. Beurre Du Roi (91/1); 48. Beurre De Paulinat (91/2); 49. Beurre De Montfavéd (91/3); 50. Beurre Noir a Longue Cossé (94/1); 51. Beurre Du Digoín (91/4); 52. Beurre Nain Du Mont D'Or (91/5); 53. Barbuchos (94/2); 54. Bañeza Oro (94/3); 55. Baja California 1.A (94/4); 56. Balin D'Albenga (94/5); 57. Bueyes Rojos (95/1); 58. Burritos (95/2); 59. Bio-Bio (95/3); 60. Borlotta Vigevano (95/4); 61. Brittle Wax (95/5); 62. Brown Beauty (98/1); 63. Boyacá 5 (98/2); 64. Bueyes (98/3); 65. Borlotta della Regina (98/4); 66. Boris (98/5); 67. Bondadoso (99/1); 68. Bunford Blue D. 32 (99/2); 69. Blanco Redondo (99/3); 70. Blanco Grande (99/4); 71. Blue Lake (99/5); 72. Blanco Jaspeado (102/1); 73. Black Valentine (102/2); 74. Black Marvel (102/3); 75. Bueyes Temuco (102/4); 76. Bountiful (102/5); 77. Bayos (103/1); 78. Burros Colchagua (103/2); 79. Burros Argentinos (103/3); 80. Burros 806 (103/4); 81. Burros 802/32 (103/5); 82. Burros Españoles (106/1); 83. Burros 1502 (106/2); 84. Burros Común (106/3); 85. Burritos Buchupuerto (106/4); 86. Bushbohne (106/5); 87. Bayos Agronomía (107/1); 88. Bayos Camaná (107/2); 89. Blanco de Chinchá (107/3); 90. Bachicha 10405 (107/4); 91. Bataaf (107/5); 92. Black Mexican (110/1); 93. Bush Bean Tenderpod (110/2); 94. Bush Bean Burpees Britte Wax (110/3); 95. Bush Bean Rush Proof Black Wax (110/4); 96. Bush Bean Burpees Stringless Wax (110/5); 97. Bush Bean Stringless Green Pod (111/1); 98. Bush Bean Supergreen (111/2); 99. Bush Bean Tendergreen (111/3);

100. Canario Perú (111/4); 101. Cabritos (111/5); 102. Canarios (114/1); 103. California Pink Var Sutter (114/2); 104. Canadian Wonder (114/3); 105. California White (114/4); 106. Caballeros (114/5); 107. Cauquenes (115/1); 108. Cabros (115/2); 110. Canario Divew 8120 (115/4); 111. Cauca 34. (115/5); 112. Canela (118/1); 113. Capuchinos (118/2); 114. Carnaval de Venecia (118/3); 115. Cesante (118/4); 116. Chapingo 1. 86 (118/3); 117. Chapingo 1. 94 (119/1); 118. Chapingo 1. 95 (119/2); 119. Chapingo 1. 96 (119/3); 120. Cochacho (119/4); 121. Coyunda Cauquenes (119/5); 122. Coscorron (122/1); 123. Coscorron Aleman (122/2); 124. Coscorron Blanco El Llano (122/3); 125. Coscorron Alto Jahuel (122/4); 126. Coscorron Blanco (122/5); 127. Coscorron Mendez (123/1); 128. Crema C. 84 (123/2); 129. C. 89 (123/3); 230. C.C.G.B. 44 (123/4); 131. Cocona (123/5); 132. Coco Nain Rose D'Eryrague (126/1); 133. Coco Bicolore du Papa (126/2); 134. Coco Blans Precoc (126/3); 135. Coco Rose (126/4); 136. Complet (126/5); 137. Corden (127/1); 138. Cornell 51/686/1 (127/2); 139. Coyunda (127/3); 140. Coyunda Nueva Imperial (127/4); 141. Cubano (127/5); 142. Cundinamarca 116.A (130/1); 143. Cristal Bayo (130/2); 144. Cristal Blanco (130/3); 145. Chiapa 96.3. (130/4); 146. Chiquito Colorado (130/5); 147. Cherokee (131/1); 148. Cheviert Vert (131/2); 149. Chiapa 4.A (131/3); 150. Cordillera (131/4,5); 151. Columbia Pinto (134/1); 152. Contesse de Chambord (134/2); 153. Contender (134/3);

154. Dark Red-Kidney mahogany (134/4); 155. Dark Red Kidney (134/5); 156. Danisse Nain Blanc Hatif (135/1); 157. D'Argel (135/2); 158. Dobbies Select Canadian Wonder (135/3); 159. Domina French Germany 68.A (135/4); 160. Dauphin (135/5); 161. De Soisson (138/1); 162. Delice Vert (138/2); 163. De Liguereux (138/3); 164. De Liguereux Improved (138/4); 165. De Liguereux Improved Stringless (138/5); 166. Drumming Small (139/1); 167. Dia blito de Mata (139/2); 168. Dwarf French Bean Bountess (139/3); 169. Dwarf Horticultural (139/4); 170. Du Perreux (139/5);

171. Enreradera (142/1); 172. Enrame (142/2); 173. Enchilar (142/3); 174. Essax (142/4); 175. Excelsior (142/5; 146/1,2); 176. Everbeanning Stringless (146/3); 177. Español de Leugo-

toma (146/4); 178. Extender (146/5); 179. Extra Hatif (147/1); 180. Emerson 51 (147/2); 181. Essos (147/3); 182. Español (147/4); 183. Earliest of All (147/5) Enano Grumpy (150/1); 185. Emamé (150/2);

186. Furtilla (150/3); 187. Full Measure (150/4); 188. Florida Belle (150/5); 189. Francés "A" (151/1) 190. Francés "B" (151/2); 191. Flight (151/3); 192. Flageolet Rouge (151/4); 193. Flageolet 1 (151/5); Flageolet 2. (154/1); 195. Flageolet 2.A. (154/2); 196. Flageolet 3. (154/3); 197. Flageolet 5. (154/4); 198. Flageolet 12. (154/5); 199. Flageolet 25. (158/1); 200. Flageolet Blanc a Longue Cossé (158/2); 201. Flageolet Blanco (158/3); 202. Flageolet X (158/4); 203. Flageolet Amarillo (158/5); 204. Fiskeby Haricot Nain Sans Fils (159/1); 205. Florigreen (159/2); 206. Fin de Bagnol (159/3); 207. Fin de Linas (159/4); 208. Fideos (159/5);

209. Green Snap (162/1); 210. Guarzo Amarillo (162/2); 211. Guernica (162/3); 212. Great Northern (162/4); 213. Great Northern 15 (162/5); 214. Great Northern 16. (163/1); 215. Great Northern 31. (163/2) 216. Great Northern (163/3); 217. Great Northern 59. (163/4); 218. Great Northern 1140. (166/1); 220. Great Northern 1143.2.B. (168/2); 221. Great Northern U.S. 164557. (166/3); 222. Gorrión (166/4); 223. Gratiot (166/5); 224. Golden Green (167/1) 225. Golden Gate Wax (167/2); 226. Golden Snap (167/3); 227. Giant Stringless Green Pod (167/4) 228. Giant Stringless Green Pod x 22659 (167/5); 229. Gloire Devil (170/1); 230. Garza (170/2); 231. Gato (170/3, 174/1); 232. Gelandrone (170/4); 233. Ganadato (170/5); 234. Ganso (171/1);

235. Hallados Chicos (171/2); 236. Hallados Cauquens (171/3); 237. Hallados Los Andes (171/4); 238. Hallados Talca (171/5); 239. Hallados Grandes (174/2); 240. Hallados Nueva Imperial (174/3); 241. Habilla Pinto Crema (174/4); 242. Harvester (174/5); 243. Hawksbury Wonder (175/1); 244. Hilacha (175/2); 245. Hidalgo 32.A. (175/3); 246. Huerto (175/4);

247. Isla (175/5); 248. Iran Flatbean (178/1) 249. Italianos (178/2); Infante (178/3); 251. Improved Kidney Wax (178/4); 252. Imuna (178/5, 182/4); 253. Inglés (179/1); 254. Incomparable (179/2); 255. Indiano (179/3); 256. Idaho Refugee (178/4, 182/2); 257. Idaho Wade (179/5); 258. Idaho 1. (182/3); 259. Idaho 2.A. (182/4); Idaho (182/5); 261. Idaho Bountiful 183/1; 262. Indigenas Regionales Petruhue (183/2);

263. Japonés Bayo (183/3); 264. Japonés Blanco (183/4); 265. Jaune Cent Pour Un (183/5); 266. Jalisco 2.A. (186/1); 267. Jalisco 11.A. 1. (186/2); 268. Jamaica Pea R. 32. (186/3);

269. Kentucky Wonder Wax (186/4); 270. Kentucky Wonder Asgrow 17. (186/5); 271. Kentucky Wonder Asgrow 21. (187/1); 272. Kentucky Wonder (187/2); 273. Kinghorns Special (187/3);

274. Lot-One (187/4); 275. Louise Nain Blanc Hatif (187/5); 276. Loreto 17. (190/1); 277. L'Inépuisable (190/2); 278. London Horticultural (190/3); 279. Logan (190/4); 280. Lighting (190/5); 281. Llico (191/1); 282. Llena Canasta (191/2); 283. Landreht Improved (191/3);

284. Mangetout Saint Fiacre (191/4); 285. Mangetout Saint Fiacre Blanc. (191/5); 286. Mangetout De La Vallé (194/1); 287. Mangetout Du Maine (194/2); 288. Mangetout Enfant Du Mont Calmé (194/3); 289. Mangetout Phoenix (194/4); 290. Mangetout Plein Le Pamier (194/5); 291. Mangetout Fin des Fin (195/1); 292. Mangetout Nain Extra Hatif (195/2); 293. Mangetout Saxa (195/3); 294. Mangetout Roi D'Été (195/4); 295. Mangetout Du Bua (195/5); 296. Milanés (198/1); 297. Michoacán 9.A. (198/2); 298. Mil x Uno (198/3); 299. Milarro (198/4); 300. Morados (198/5); 301. Mc. Costa (199/1); 302. Mantequillero (199/2); 303. Metis (199/3); 304. Mc. Caslan (199/4); 305. Mercado Ideal (199/5); 306. Marfil 202/1; 307. Marrow (202/2); 308. My Hatif de Belgique (202/3); 309. Michoacán S.A. (202/4); 310. Magdalena 3. (202/5); 311. Mutatos (203/1); 312. Muselina (203/2); 313. Mutilla (203/3); 314. Monroe Selection (204/4); 315. Monroe 1960 (203/5); 316. Morados Linares (206/1); 317. Michelite a Longue Cossé (206/2); 318. Michelite (206/3); 319. Moisson (206/4); 320. Mexico 11.B. (206/5); 321. Mexico 13.B. (207/1); 322. Mexico 13.A. 2 (207/2); 323. Mexico 16.J. (207/3); 324. Mexiko 16 K. (207/4); 325. Mexico 53. A.2. (207/5); 326. Mantecoso de Aragón (210/1); 327. Mantequilla (210/2); 328. Maravilla de Venecia (210/3); 329. Masterpiece 210/4; 330. Metis Café (210/5); 331. Mexicanos (211/1); 332. Mariné (211/2); 333. Mantecoso (211/3);

334. No. 1. (211/4); 335. No. 1. (China) (211/4); 336. No. 2 (214/1); 337. No. 2.A. (214/2); 338. No. 4. (214/3); 339. No. 20. (214.4); 340. No. 47. (214/5); 341. No. 55. (215/1); 342. No. 68.

(215/2); 343. No. 71.B. (215/3); 344. No. 74. (215/4); 345. No. 93. 346. No. 630. (218/1); 347. No. 643. (218/2); 348. No. 650. (218/3); 349. No. 765. (218/4); 350. No. 780. (Russia) (218/5); 351. No. 814. (Russia) (219/1); 352. No. 1482 (Russia) (219/2); 353. No. 8438. (Russia) (219/3); 354. No. 8440 (Russia) (219/4); 355. No. 8442 (Russia) (219/5); 356. No. 851 (Russia) (222/1); No. 10262 (Russia) (222/2); 358. No. 10425 U.S.A. (222/3); 359. No. 10506 (Russia) (222/4); 360. No. 10507 (Russia) (222/5); 361. No. 97717 (Russia) (223/1); 362. Noir Hatif de Belgique (223/2); 363. Nouvel Ermitage (223/3); 364. Negro Veracruz (223/4); 365. Negro Nuevo Imperial (223/5); 366. Negro de Cuba (226/1); 367. Negro San Miguel (226/2); 368. Negro Mexicano (226/3); 369. Negro de Guatemala (226/4); 370. Negritos (226/5); 371. Negro Argel (227/1); 372. Nagafsa (227/2);

373. Olé (227/3); 374. Ojo de Cabra (227/4); 375. Ocañero (227/5); 376. Oro (230/1); 377. Unknown origin (230/2);

378. Perdigones (230/3); 379. Panguipulli No. 3. (230/4); 380. Panguipulli No. 1. (230/5); 381. Panameño (231/1); 382. Pajarito (231/2); 383. Paledrum (231/3); 384. Presto (231/4); 385. Parrón (231/5); 386. Pepa de Zapallo (234/1); 387. Pencil Pod Black Wax (234/2); 388. Peerry Marrow (234/3); 389. Perdices (234/4); 390. Perelle de Marcoichez (234/5); 391. Peumo (235/1); 392. P. I. 203958 (235/2); 393. Phenomenon a Ramés (235/3); 394. Phenomenon (235/4); 395. Pico de Oro (235/5); 396. Pinto 5 (238/1); 397. Pink (238/2); 398. Pinto Bean (238/3); 399. Pinto 78 (238/1); 397. Pink (238/2); 398. Pinto Bean (238/3); 399. Pinto 78 (238/4); 400. Pinto 14 (238/5); 401. Pinto Rosado (239/1); 402. Pinto U.I. 111. (239/2); 403. Piririte (239/3); 404. Ponderoso (239/4); 405. Pole London (239/5); 406. Plentiful (242/1); 407. Prince R. French Type (242/2); 408. Potomac (242/3); 409. Portugues (242/4);

410. Quinchamali (242/5);

411. Rocha 437/36 (243/1); 412. Roi de Belges (243/2); 413. Rosas (243/3); 414. Ricos 2015 (243/4); 415. Rocha (243/5); 416. Ricos (246/1); 417. Red Mexican U.I. 35. (246/2); 418. Red Mexican U.I. 3. (246/3); 419. Red Mexican U.I. 34. (246/4); 420. Red-Kidney Maine (246/5); 421. Red-Kidney Miami (247/1); 422. Red Valentine Stringless (247/2); 423. Rangers (247/3); 424. Ramillete (247/4); 425. Red Kidney California (247/5); Robust (250/1); 427. Kentucky Wonder R.R. (250/2); 428. Red-Mexican (250/3); 429. Red Kidney (250/4);

430. Supermetis (250/5); 431. Superviolet (251/1); 422. Sure Crop Wax (251/2); 433. Suisse Nain Black Hatif (251/3); 434. Suprême (251/4); 435. Stringless Burpees (251/5); 436. Stella de Weibull (510/1); 437. Streamliner (510/2); 438. Stringless Green Refugee (510/3); 439. Sietesemanas (510/4); 440. Soisson Blanc a Ramés (510/5); 441. Señorita (511/1); 442. Seaway (511/2); 443. Seaway Type (511/3); 444. Seminole 511/4); 445. Saxea Zonder Prout (511/5); 446. Shell Red-Kidney (514/1); 447. Saxea Zonder Draap (514/2); 448. Sanchez (514/3); 449. Sapito (514/4); 450. Sangretoporo (514/5); 451. San Rafael "A" (515/1); 452. Sanilac (515/2); Saint Fiacre (515/3); 454. Saint Fiacre Beurré (515/4); 455. Saint Marcellini (515/5); 456. Salve Decroit (518/1); 457. Saint Sprit a Oeil Noire (518/2); 458. Saint Andrés Nain Sans Fils (518/3);

459. Triumph (518/4); 460. Tropero (518/5); 461. Twed Wonder (519/1); 462. Trés Hatif de Estampe (519/2); 463. Trés Hatif de Massy (519/3); 464. Triumphe de Farcy (519/4); 465. Tous les Jours (519/5); 466. Traros (522/1); 467. Triguito (522/2); 468. Top-Crop (522/3); 469. Topmost (522/4); 470. Top-Notch Golden Wax (522/5); 471. Tennessee Green Pod (523/1); 472. Te Temuco (523/2); 473. The Prince Dwarf French Type (523/3); 474. Tenepantla 64. (523/4); 475. Tenepantla 68. (523/5); 476. Tenderlong (526/1); 477. Tendergreen (526/2); 478. Tejuelas Café (526/3); 479. Tejuelas Blancas (526/4); 480. The Prince (526/5); 481. Tendercrop (527/1); 482. Tender and True (527/2); 483. Talca 5 (527/3); 484. Tallarines (527/4); 485. Teófiles (527/5); 486. Tacho Cauquenes (530/1); 487. Tacho Paine (530/2); 488. Talca 3. (530/3); 489. Tórtola (530/4); 490. Temucano (530/5);

491. U.S. 3. (531/1); 492. U.S. 4. (531/2); 493. U.S. 5. Refugee (531/3); 494. Uno (531/4); 495. Uribe Redondo (531/5);

496. Veracruz I.A. (534/1); 497. Venadito (534/2); 498. Vara Argentina (534/3); 499. Villa rica (534/4); 500. Verde Temuco; 501. Vert Aiguillete (535/1); 502. Vroege Wagennar (535/2); 503. Voorluck (535/3); 504. Valcesia (535/4);

505. Wells Red-Kidney (535/5); 506. Wax (538/1); 507. Wade (538/2); 508. Wonderful (538/3); 509. Windsor Long Pod (538/4); 510. Wisconsin Refugee (538/5); 511. White Navy (539/1); 512. White Marrow (539/2); 513. Westralia Pole Bean (539/3); 514. White Kidney (539/4); 515. Widusa (539/5);

516. Yellow Eye (554/1); 517. Yellow Eye Bean (554/2); 518. Zacatecas Bayos (544/3); 519. Zepelin (544/4); 520. Zeus (544/5);

521. Chaucho Eleodoro (543/1); 522. Furore (543/2); 523. Fedette ed Maraychez (543/3); 524. Huila 15. (543/4); 525. Pinto 72. (543/5); 526. Sable Verde (549/1); 527. Semienanos (549/2); 528. Zacatecas 1.A. (549/3);

2. Forms of *Phaseolus vulgaris* ssp. *aborigineus*

10-3 (School of Agriculture Cambridge) (621/1); 10-4 (Institute f. Hort. Plant Breeding Wageningen Nr. 61006) (621/2); 10-5 (VIR Leningrad Nr. 10659) (621/3); 10-8 (Instituto de Botanica San Isidro) (621/4); 10-10 (Univer. Centr. de Venezuela Caracas "Aglutininfrei Linie") (621/5); 10-11 (Univer. Centr. de Venezuela Caracas "Linie mit Agglutinin") (622/1); 10-12 (University of Southampton AB 946 PI 266910) (622/2); 10-14 (VIR Leningrad Nr. K-10947) (622/3); 10-15 (VIR Leningrad Nr. K-10656) (622/4); 10-16 (VIR Leningrad Nr. 10656 with black seeds) (622/5);

3. Cultivars of *Phaseolus coccineus*

100-1 (Botan. Garden Montreal) (623/1); 100-2 (black seeded and with red flowers, Bot. Garden Prague) (623/2); 100-3 (Weisse Riesen (Inst. f. Kulturpflanzenforschung Gatersleben Nr. 757/61) (623/3); 100-4 var. *albonanus* Niedrige weissblühende Feuerbohne (Inst. f. Kulturpflanzenforschung Gatersleben Nr. OHA 101/60) (623/4); 100-9 Hammonds Dwarf Scarlet (Inst. f. Kulturpflanzenforschung Gatersleben PHA 8003/63) (623/5); 100-11 Zweifärbigblühende Prunk (Inst. f. Kulturpflanzenforsch. Gat. PHA) (260/62) (624/1); 100-12 (Bulgarische Handelsaat Gatersleben PHA 798/63) (624/2); 100-16 f. *formosus* (Inst. for Plantbreeding Wageningen Nr. (64002) (624/3); 100-18 Prof. Lamprecht's L 25/62 (Inst. f. Plantbreeding Weibullsholm Landskrona) (624/4); 100-19 Prof. Lamprecht's L 27/58 (Landskrona) (624/5); 100-20 Ph. *multigaris* Prof. Lamprecht's L 43/60 (Landskrona) (625/1); 100-21 Ph. *multigaris* Prof. Lamprecht's L 148/61 Typ B (Landskrona) (625/2); 100-24 L 73/63 (Landskrona) (625/3); 100-25 Prunkbohnen Rotblühende (Quedlinburg Tgl 14197) (625/4); 100-26 Prunkbohnen Erfo (Quedlinburg T 1 14197) (625/5); 100-29 ssp. *formosus* HBK (School of Agriculture, Cambridge) (626/1); 100-32 Désirée (Inst. f. Kulturpflanzenforschung Gatersleben L 647/66) (626/2); 100-39 Arabische Schneekönigin (Gatersleben PHA 816/65) (626/3); 100-41 Erfo (Gatersleben L 641/65) (626/4); 100-42 Grünhülsige Dwarf French (Gatersleben PHA 8002/65) (626/5); 100-45 Rotblühende Prunk (Gatersleben PHA 799/65) (627/1); 100-47 (Bulgarische Handelsaat Gatersleben PHA 8006/66) (627/2); 100-50 (Gatersleben 8013/66) (627/3); 100-57 Lopata (VIR Leningrad K 9262) (627/4); 100-68 Prince Runner Bean (Inst. de Invest. Agropec. Santiago) (627/5); 100-71 Morado Jaspe (Inst. Nac. Invest. Agr. Mexico) (628/1); 100-72 Café rosado Jaspe (Mexico) (628/2); 100-73 Gois (Mexico) (628/3); 100-74 Morado oscuro jaspado (Mexico) (628/4); 100-82 Jas (Poland) (628/5).

J. KLOZ, EVA KLOZOVÁ, Ústav experimentální botaniky ČSAV, Praha: Bílkovina euphaseolinu a tribu *Phaseolinae* — chemotaxonomická studie. — Biol. Plant. 16 : 290—300, 1974.

Na základě imunochemických analys hlavní zásobní bílkoviny *Ph. vulgaris* euphaseolinu a velkého počtu kultivarů *Phaseolus vulgaris*, dalších 23 druhů *Phaseolus* a několika zástupců dalších rodů *Viciaceae* a srovnáváním těchto údajů s údaji morfologickými a genetickými autofi navrhuji vydělení sekce *Euphaseolus*, charakterisované přítomností bílkoviny euphaseolinu. Druhy charakterisované euphaseolinem jsou si blíže příbuzné, jsou křížitelné. Návrh vyžaduje ještě formální doplnění z hlediska zvyklostí klasické systematiky.

V článku jsou dále diskutovány otázky taxonomické šíře různých bílkovinných znaků.