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Variability of Essential Amino Acid Content in Seeds of 22 *Phaseolus* Species

G. **BALDI** and F. **SALAMINI**

Istituto sperimentale per l'Orticoltura, Sezione di Montanaso Lombardo (Italy)

Summary. The reserve protein composition of 22 *Phaseolus* species has been studied. The non-sulphur-containing amino acids were present at values higher than those suggested for animal (and human) nutrition, but the sulphur-containing amino acids, with some exceptions, were under the minimum accepted requirement. However, taking into account the variability in the percentages of methionine and cystine, as well as the cystine/methionine ratio, it is concluded that the genus *Phaseolus* has a theoretical possibility for synthesizing a reserve protein with a balanced sulphur-containing amino acid content. An accession from Mexico of the species *Ph. phyllanthus* possesses a protein characterized by a high sulphur-containing amino acid content (3,84%). The possible utilization of this species in breeding for legume protein quality is suggested. Some observations about the percentage of arginine in wild and cultivated forms of *Ph. vulgaris* are also presented.

Introduction

The seed proteins of the *Leguminosae* family are globulins (Pant and Tulsiani, 1969). Their amino acid composition is different from that of cereal proteins (for a recent review, see Baldi *et al.*, 1972). The biological value of the legume proteins is negatively influenced by their low content of sulphur-containing amino acids (Pant and Tulsiani, 1969; Zimmermann and Levy, 1962; Bandemer and Evans, 1963; Evans and Bandemer, 1967; Abu-Shakra *et al.* 1970).

In the cultivated species of *Phaseolus* the search for seed proteins with a high content of sulphur-containing amino acids may be carried out by making use of intraspecific variation or by studying wild species, with the aim of improving, by interspecific hybridization, the amino acid pattern of the cultivated species.

This may open the possibility of basic improvement in the legume protein quality, considering the present promises of *in vitro* interspecific hybridization.

Materials and Methods

The species analysed were the following:

- *Ph. aconitifolius*, 2 accessions from India;
- *Ph. acutifolius*, 3 accessions from Mexico, Nicaragua, El Salvador;
- *Ph. adenanthus*, 1 accession from Argentina;
- *Ph. angularis*, 3 accessions from Korea, Oklahoma;
- *Ph. angustifolius*, 2 accessions from El Salvador, Mexico
- *Ph. atropurpureus*, 2 accessions from Australia;
- *Ph. bracteatus*, 3 accessions from Brazil, Paraguay;
- *Ph. calcaratus*, 3 accessions from Belgian Congo, India;
- *Ph. caracalla*, 1 accession from Brazil;
- *Ph. coccineus*, 2 accessions from Italy;
- *Ph. dumosus*, 1 accession from Mexico;
- *Ph. lathyroides*, 1 accession from Australia;
- *Ph. lunatus*, 1 accession from Italy;
- *Ph. mungo*, 2 accessions from India;
- *Ph. panduratus*, 1 accession from Brazil;
- *Ph. phyllanthus*, 1 accession from Mexico;
- *Ph. radiatus*, 2 accessions from India, China;
- *Ph. sp. 239355*, 1 accession from Belgian Congo;
- *Ph. sp. 286307*, 1 accession from Ivory Coast;
- *Ph. sp. 312195*, 1 accession from Mexico;
- *Ph. trilobatus*, 1 accession from Ivory Coast;
- *Ph. vulgaris*, 3 accessions from Italy;
- *Ph. vulgaris* (wild) 2 accessions from Mexico.

All the accessions studied were obtained from the Plant Introduction Station of Pullman, Wash., except for the 3 *Phaseolus vulgaris*, the 2 *Ph. coccineus* and the *Ph. lunatus* varieties collected in Italy, and one of the 2 *Ph. vulgaris* wild accessions obtained from O. W. Norvell, Palo Alto, Calif. All the species were grown in the greenhouse during the 1970/71 autumn-winter period since some of them were short-day requiring species. For each accession, 10 plants were grown in 5 large pots and the seeds obtained at different times were grouped. After grinding, samples were submitted to two different types of hydrolysis: for the analysis of all the amino acids, except cystine and methionine, 6% HCl hydrolysis was used; a second hydrolysis was carried out with ground samples previously treated with performic acid and, in this case, the hydrolisates contained cysteic acid and methionine sulphoxide instead of cystine and methionine (Moore, 1963). Therefore, these two sulphur-containing amino acids were evaluated on the basis of their products of oxidation. The amino acid analyses were carried out with an automatic Bekman Unichrom amino acid analyzer. The internal standards for the *long* and *short* columns were norleucine and α-amino β-guanidin propionic acid, respectively. Sometimes the chromatogram showed a peak located at the probable elution point of 5-methyl-cysteine. The chromatography of an authentic sample of 5-methyl-cysteine and co-chromatography of this amino acid with one of our samples confirmed the presence of this compound in some of the species analysed.

Results

Table 1 reports, for the 22 species, the percentage of proteins, the cystine, methionine, and S-methyl-cysteine contents, the cystine/methionine ratio and the cystine plus methionine content. The percentage of protein varies between 22.57% and 36.66%, with minimum and maximum values for the species *Ph. phyllanthus* and *Ph. panduratus*, respectively. Among the cultivated species, *Ph. lunatus* has a low protein content of 23.81%. There seems to be a difference in the protein content between the cultivated and wild forms of *Ph. vulgaris* (29.55 *versus* 35.21%). The percentage of cystine is highly variable, with the species *Ph. aconitifolius* showing a low content of 0.55%, while the species *Ph. phyllanthus* shows a percentage of 2.28. It is interesting to note that this species has the maximum cystine content, but the minimum percentage of proteins. The cultivated species have values of 1.08, 1.02 and 1.19% for *Ph. vulgaris*, *lunatus* and *coccineus*, respectively.

The percentage of methionine does not show such wide variability as cystine. *Ph. adenanthus* has a protein with the lowest methionine content (0.70%), while *Ph. mungo* has the highest content (1.59%). None of the species analyzed has a methionine content equal to or higher than the minimum content (2.2%) suggested by the FAO/WHO Expert-group (1965) for a diet adequate for animal or human nutrition.

S-methyl-cysteine is almost absent in the species *Ph. adenanthus, angularis, calcaratus, caracalla, mungo, sp. 239355, sp. 286307, trilobatus*, while the species *Ph. acutifolius* and *Ph. panduratus* show very high values.

The cystine/methionine ratio is highly variable, with values from 0.47 to 2.45. This ratio is around 1 for the three cultivated species.

Table 1. *The crude protein and sulphur-containing amino acid contents in the seeds of 22 Phaseolus species*[1]

Species	Number of samples	Percent of proteins[2]	Cystine[3]	Methionine[3]	S-methylcysteine[3]	Cystine/methionine ratio	Cystine plus methionine[3]
Ph. aconitifolius	2	28.87	0.55	0.96	0.15	0.57	1.51
Ph. acutifolius	3	33.95	0.96	0.98	2.91	0.97	1.94
Ph. adenanthus	1	29.77	1.72	0.70	T[6]	2.45	2.42
Ph. angularis	3	29.66	1.18	1.53	T	0.77	2.71
Ph. angustifolius	2	27.17	1.40	1.16	1.58	1.21	2.56
Ph. atropurpureus	2	32.18	1.51	1.14	0.54	1.32	2.65
Ph. bracteatus	3	31.44	1.35	1.23	0.73	1.09	2.58
Ph. calcaratus	3	27.52	0.99	1.42	T	0.70	2.41
Ph. caracalla	1	25.94	1.24	0.92	T	1.34	2.16
Ph. coccineus	2	25.32	1.19	1.08	1.01	1.10	2.27
Ph. dumosus	1	32.03	1.34	1.25	0.37	1.07	2.59
Ph. lathyroides	1	29.87	1.02	1.11	0.81	0.91	2.13
Ph. lunatus	1	23.81	1.02	1.40	0.54	0.72	2.42
Ph. mungo	2	32.39	0.76	1.59	T	0.47	2.58
Ph. panduratus	1	36.66	1.08	1.21	2.21	0.89	2.28
Ph. phyllanthus	1[4]	22.57	2.28	1.56	0.32	1.46	3.84
Ph. radiatus	2	29.88	0.92	1.39	0.14	0.66	2.21
Ph. sp. 239355	1	30.26	0.63	1.28	0.40	0.49	1.89
Ph. sp. 286307	1	29.32	0.94	1.46	T	0.64	2.40
Ph. sp. 312195	1	27.55	1.80	1.36	T	1.32	3.16
Ph. trilobatus	1	25.98	0.90	1.55	T	0.58	2.45
Ph. vulgaris	3	29.55	1.08	1.25	1.60	0.86	2.33
Ph. vulgaris (wild)	2	35.21	1.24	1.07	0.79	1.15	2.31
FAO pattern[5]				2.2			4.20

[1] The mean values have been reported for the species with more than one accession.
[2] Kjeldahl nitrogen per 6.25.
[3] Grams of amino acid per 16 g of nitrogen.
[4] For this accession the values reported are the mean of 4 analyses.
[5] FAO pattern of amino acid requirements (FAO, 1957; FAO/WHO Expert Group, 1965).
[6] Trace amount = *T*.

The last column of table 1 presents the cumulative values for cystine and methionine. It can be seen that *Ph. phyllanthus* is the species with the highest sulphur-containing amino acid content (3.84%). This value is still low but near to the 4.2% suggested by the FAO/WHO Expert-group (1965) for the sum of the two sulphur-containing amino acids.

Table 2 shows the essential amino acid (other than sulphur-containing) contents of the 22 species of *Phaseolus* studied. The eight amino acids considered (arginine, histidine, isoleucine, leucine, lysine, phenylalanine, threonine, valine) are always present in large quantities. In fact, comparing the minimum values suggested by the FAO/WHO Expert-group, the species analyzed possess, with rare exceptions, proteins with a favourable non-sulphur-containing amino acid balance. The values reported for the amino acids, histidine, isoleucine, lysine, phenylalanine, threonine, and valine, are only slightly variable for the different species but some variations are detectable when we consider the arginine content. The three cultivated species have the lowest amounts of this amino acid (5.71, 5.63 and 5.91%, respectively, for *Ph. coccineus, lunatus* and *vulgaris*), while the wild species show higher levels. It is interesting that the wild accession of *Ph. vulgaris* also has a high arginine content.

Table 3 reports the mean weight of the seeds of the 22 species. It may be noted that the cultivated species show higher weight per seed than the wild species. The difference between the wild and cultivated *Ph. vulgaris* accessions is remarkable (28.7 *versus* 535 mg respectively). It is interesting to note that *Ph. phyllanthus*, the species showing the higher sul-

Table 2. *The essential amino acid (other than sulphur-containing) contents in seeds of 22 Phaseolus species*[1]

Species	Arginine	Histidine	Iso-leucine	Leucine	Lysine	Phenyl-alanine	Threo-nine	Valine
Ph. aconitifolius	7.11	2.74	4.24	8.27	7.03	6.14	3.50	5.05
Ph. acutifolius	7.37	2.64	3.73	7.02	6.47	5.34	4.03	4.52
Ph. adenanthus	6.10	2.40	2.96	6.00	5.58	4.18	2.99	3.59
Ph. angularis	7.72	3.31	4.19	8.54	7.86	6.18	3.76	4.95
Ph. angustifolius	5.68	2.52	3.99	7.41	6.61	5.86	4.56	4.89
Ph. atropurpureus	8.23	3.19	3.89	7.61	6.74	5.37	4.05	4.46
Ph. bracteatus	7.23	2.99	4.12	8.26	7.11	5.54	4.05	4.76
Ph. calcaratus	6.30	2.93	4.16	8.28	7.31	6.02	3.55	4.63
Ph. caracalla	8.62	3.85	3.79	7.69	6.85	5.44	3.68	4.28
Ph. coccineus	5.71	2.81	4.25	7.89	7.13	5.42	4.81	5.07
Ph. dumosus	6.18	3.18	4.27	8.27	7.10	5.92	4.66	4.82
Ph. lathyroides	8.22	3.00	4.25	8.60	7.22	5.67	3.59	4.77
Ph. lunatus	5.63	2.67	4.89	8.56	6.00	6.07	4.91	5.40
Ph. mungo	6.43	2.84	4.30	8.52	6.76	6.14	3.63	5.19
Ph. panduratus	7.06	2.94	3.95	8.22	6.89	6.30	3.81	4.63
Ph. phyllanthus	5.66	3.27	4.13	7.66	7.46	5.62	4.23	4.71
Ph. radiatus	6.92	2.96	4.01	7.96	7.41	6.14	3.63	4.80
Ph. sp. 239355	6.82	2.75	3.92	8.19	7.41	6.19	3.47	4.93
Ph. sp. 286307	6.79	3.13	3.87	7.95	8.23	5.79	3.56	4.47
Ph. sp. 312195	5.26	3.21	3.38	7.37	6.79	9.22	3.87	4.03
Ph. trilobatus	7.69	2.99	3.90	7.91	8.08	5.84	3.85	4.56
Ph. vulgaris	5.91	2.91	4.42	8.07	6.83	5.78	4.36	4.96
Ph. vulgaris (wild)	8.05	2.70	3.97	7.83	6.54	5.91	4.53	4.57
FAO pattern[2]	2.0[3]	2.4[4]	4.2	4.8	4.2	2.8	2.8	4.2

[1] The mean value has been reported for the species with more than one accession. Grams of amino acid per 16 grams of nitrogen.
[2] FAO pattern of amino acid requirement (FAO, 1957).
[3] Amino acid requirement by Rose (1937), cited by Evans and Bandemer (1967).
[4] Requirement of infant (from Food and Nutrition Board, 1963, cited by Evans and Bandemer, 1967).

phur-containing amino acid content, has a mean seed weight (21.6 mg) comparable with that of the wild *Ph. vulgaris*. As reported elsewhere, the *Ph. vulgaris* wild form has a higher arginine content than the cultivated one; the obvious difference in seed weight of the two forms, and therefore the possible negative correlation between seed weight and arginine content, suggested the control of this correlation over all the species considered. In fact the calculated value of the correlation coefficient is −0.473, significant at the 5% level.

Discussion

The globulins are characterized by a low content of sulphur-containing amino acids. They are not homogeneous, as demonstrated for glycinin, the soybean globulin. Naismith (1955), in ultracentrifuge studies, separated the glycinin in 4 components (2, 7, 11 and 15 S). The 7 S component has a low methionine content (Roberts and Briggs, 1965), thus the improvement of the nutritive value of legume proteins might be obtained by genetically suppressing the synthesis of the 7 S component (Nelson, 1969). However, no data substantiating this approach have been reported to date.

We found some variability in the methionine and cystine contents in the analyzed *Phaseolus* species. Taking into account the variable cystine/methionine ratios (from 0.47 to 2.45), it can be concluded that the genus *Phaseolus* possesses a theoretical potential for the synthesis of a reserve protein with a balanced sulphur-containing amino acid content. Obviously, the practical realisation is conditioned by the existence of incompatibility barriers which restrict crosses among the *Phaseolus* species.

Viable interspecific hybrids have been obtained from crosses between the cultivated species *Ph. vul-*

Table 3. *Mean weight of the seeds of the 22 Phaseolus species*[1] *and correlation coefficient (r) between mean weight and the percentage of arginine*

Species	weight (mg)	Species	weight (mg)
Ph. aconitifolius	23.0	*Ph. lunatus*	1126
Ph. acutifolius	106	*Ph. mungo*	44
Ph. adenanthus	31	*Ph. panduratus*	71
Ph. angularis	140	*Ph. phyllanthus*	21.6
Ph. angustifolius	156	*Ph. radiatus*	61
Ph. atropurpureus	15.5	*Ph. sp. 239355*	43
Ph. bracteatus	9.4	*Ph. sp. 286307*	81
Ph. calcaratus	113	*Ph. sp. 312195*	119
Ph. caracalla	51	*Ph. trilobatus*	8.7
Ph. coccineus	1362	*Ph. vulgaris*	535
Ph. dumosus	399	*Ph. vulgaris* (wild)	28.7
Ph. lathyroides	8.7		

correlation coefficient between seed weight and % of arginine (r) = −0.473*

[1] means obtained from 3 samples of 50 seeds.
* $P < 0{,}05$

garis, *coccineus* and *lunatus*. Some other interspecific hybrids came out from cultivated and wild species, the compatibility relationships among wild species not having been established at present (for a review see Yarnell, 1965, and Al-Yasiri and Coyne, 1966).

The percentages of the non-sulphur-containing essential amino acids are higher than or equal to the minimum contents suggested for human or animal diet in all the cultivated species considered. On the other hand, cystine and methionine are present in the proteins of the 3 cultivated species only at low values, slightly variable around 1%. *Ph. acutifolius*, a species now disappearing from cultivation (Kaplan, 1965), is also low in sulphur-containing amino acids.

Among the wild species, the *Ph. phyllanthus* derived from Mexico has a protein with a high sulphur-containing amino acid content (3.84%); this species also has the minimum protein content (22.57%). Its use in interspecific hybridization to improve the protein quality of the cultivated species may be proposed. Crossing compatibility with the cultivated species may be low, but this has not been proved yet. A second way of utilizing this species could be direct breeding towards an economically cultivable form. This seems unlikely, considering the mean seed weight of the plant (21.6 mg). Clearly, the present yield of this species is too low, and may not justify a breeding effort.

Nevertheless, it must be taken into account that the wild *Ph. vulgaris* accessions considered were characterized by a mean seed weight of 28.7 mg, a value only slightly higher than that found for the *Ph. phyllanthus*.

The non protein amino acid S-methyl-cysteine has been suspected of being an antimetabolite for methionine and cystine, because of its relation to these 2 amino acids. S-methyl-cysteine content, however, is high only for *Ph. acutifolius* and *Ph. panduratus*, the cultivated species showing values around 1%. This amino acid, should not present a problem in breeding for protein quality, since it has not shown any harmful effect in feeding experiments with rats (Evans and Bandemer, 1967).

Some interesting observations can be made on the observed differences in arginine content between wild (8.05%) and cultivated (5.91%) accessions of *Ph. vulgaris*. It is evident that the other two cultivated species, *Ph. coccineus* and *Ph. lunatus*, characterized by a high mean seed weight, possess a relatively low arginine (5.71 and 5.63%, respectively) content. In fact, the correlation coefficient between mean seed weight and percentage of arginine, calculated from all the accessions, is significantly negative. This finding may simply mean that the lowering of the arginine content in the reserve protein has been common in the genus *Phaseolus* for the species that, during their evolution, have increased their seed weight.

It can not be excluded, however, that the negative correlation may be only a casual finding. Consequently, the observed difference in arginine content between the cultivated and wild forms of *Ph. vulgaris* may be interpreted in a different way. For instance, it is possible to think of a *Ph. coccineus* introgression into the *Ph. vulgaris* species. Some observations may be in favour of this hypothesis: *Ph. coccineus* has a lower arginine content and a heavier seed weight than *Ph. vulgaris*; it can hybridize naturally with *Ph. vulgaris* (Miranda Colin, 1967) and it might have been domesticated before *Ph. vulgaris*, although this last assumption is not completely acceptable (Kaplan, 1965).

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Dr. G. Baldi
Centro di Ricerche sul Riso
Ente nazionale Risi
Mortara (Italy)

Professor Francesco Salamini
Istituto sperimentale per l'Orticoltura,
Sezione di Montanaso Lombardo
Milano (Italy)