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# Improvement of protein and amino acid contents in seeds of food legumes. A case study in *Phaseolus*

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Food legumes are considered as the major source of dietary proteins among the plant species. Protein and amino acid contents were evaluated in a wide sample of both wild and cultivated genotypes of *Phaseolus* species, with a view to investigate possibilities of genetic improvement in seed nutritional quality. Results indicate a variation in relation with taxa, biological status within species (such as in *P. lunatus*), ecological conditions, seed parts (testa, cotyledons and embryonic axis), and major protein groups. However, the sulphur containing amino acids remain a limiting factor, which could be better overcome by mixing food legumes with other plant species such as cereals.
**Keywords.** *Phaseolus*, seeds, plant protein, amino acids, genetic improvement, nutrient improvement, nutritive value.

**Amélioration des teneurs en protéines et acides aminés des graines de légumineuses vivrières. Un cas d'étude du genre *Phaseolus*.** Les légumineuses vivrières sont considérées comme la source majeure des protéines alimentaires parmi les plantes. Les teneurs en protéines et acides aminés ont été évaluées dans un large échantillon incluant des génotypes sauvages et cultivés du genre *Phaseolus*, dans le but de rechercher des possibilités d'amélioration de la qualité nutritionnelle des graines. Les résultats indiquent une variation en fonction de l'espèce étudiée, du statut biologique au sein de l'espèce (par exemple chez *P. lunatus*), des conditions écologiques, de la partie de la graine analysée (testa, cotylédons et axe embryonnaire) et des principaux types de protéines. Cependant, les acides aminés soufrés restent un facteur limitant qui peut mieux être maîtrisé par le mélange de légumineuses vivrières avec d'autres plantes, telles que les céréales.
**Mots-clés.** *Phaseolus*, graine, protéines végétales, acide aminé, amélioration génétique, amélioration de qualités nutritives, valeur nutritive.

## 1. INTRODUCTION

Plant proteins provide nearly 65% of the world supply of proteins for humans with 45% – 50% and 10 – 15% from cereals and legumes or vegetables, respectively (Mahe *et al.*, 1994). Importance of plant proteins in the average diet varies from the least developed regions (where animal proteins are scarce and poverty precludes the consumption of meat) to the highly developed regions (where animal production is particularly abundant). Nonetheless there is now an expanding consumption of protein foods of legume and vegetable origin throughout the world.

Among the plant species, grain legumes are considered as the major source of dietary proteins. Protein quality in leguminous seeds does not however reach the same level as in animal products. This is due to various factors, among them the well known are the unbalanced amino acid composition, the low true digestibility of protein and the presence of antinutritional factors in the seeds (Bressani, Elias, 1980; Norton *et al.*, 1985).

This paper deals mainly with the protein content and amino acid composition within *Phaseolus*.

## 2. MAJOR TRAITS IN PROTEIN AND AMINO ACID COMPOSITION

Characteristically, grain legume seeds have large protein contents, ranging from 20% to as much as 40% of their dry matter, according to species, genotypes within species, and environments (Otoul, 1976; Norton *et al.*, 1985; Baudoin, 1991). Storage proteins in legume seeds are mainly located in the cotyledonary tissues. Embryonic axis and testas contribute little to the total seed protein content, mainly because those components represent small proportions of the seed mass.

The major storage proteins in legume seeds are the globulins which usually account for about 70% of the total protein. Glutelins (10–20%) and albumins (10–20%) make up the remainder. The principal storage globulins in most legumes are legumin and vicilin, the latter predominating in common bean (Jansman, 1996).

Amino acid profiles of proteins in leguminous seeds are unbalanced. When compared to egg protein, the indispensable sulphur-containing amino acids are at a much lower concentration (Mahe *et al.*, 1994). Sulphur-containing amino acids (i.e. methionine and cystine) are considered as the most critical limiting components of the proteins. The various legume protein sources may differ significantly in the amino acid composition. Compared to soya bean protein, the lysine content in pulses tends to be higher and the content of sulphur-containing amino acids (methionine and cystine), and tryptophan tends to be lower (Norton *et al.*, 1985; Jansman, 1996).

Difference in amino acid composition is also observed among the protein fractions of the seeds. Globulins are relatively poor in sulphur-amino acids. Albumins are richer in sulphur-amino acids and other essential amino acids (such as lysine) than globulins. In addition, some antinutritional factors, mainly proteinase inhibitors, have shown a relatively fair content in cystine and methionine. For example, in common beans between 30 to 40% of the total seed cystine has been calculated to be present in these inhibitors (Norton *et al.*, 1985).

## 3. MEANS TO IMPROVE NUTRITIONAL QUALITY

Proper heat treatment of the seeds (to destroy heat-labile antinutritional factors) or cooking process (to remove testas containing tannins), mixture with other foods in the diet (mainly cereals), seed yield increase (with consequently a higher level of proteins) are all means to circumvent partially the low biological value of food legumes (Baudoin, 1991).

Association of the beans with other foods in the diet is surely one interesting alternative to improve the biological value of the plant proteins. A good mixture is provided by the cereal-based diets, combining adequately lysine (well represented in leguminous species) with sulphur-containing amino acids (fairly represented in cereals).

Another alternative being investigated to enhance protein quality is genetic improvement of both protein content and amino acid balance. The transfer of genes at intra- and interspecific levels may however be of limited value since nearly all grain legumes have similar biochemical and nutritional deficiencies (Norton *et al.*, 1985). Furthermore traits such as protein concentration are usually quantitatively inherited and influenced substantially by non-genetic factors, making it difficult to evaluate plant materials without extensive testing. From several studies conducted with different grain legumes and under various environments, the following trends have been pointed out (Bressani, Elias, 1980; Norton *et al.*, 1985).

– Substantial genetic variation for total seed protein concentration and amino acid composition (particularly sulphur-amino acids) exists in the gene pools of grain legumes (including primary and alien reservoirs).
– Although environment effects are large, genotype × environment interactions are often small, indicating that the relative differences between genotypes should be similar in several environments.
– There are substantial differences in amino acid composition of individual protein fractions; synthesis and accumulation of different fractions and the polypeptide composition of the fractions are under separate genetic factors.
– Heritability estimates for percentage protein range from 0.25 to 0.60, according to species, genotypes within species, and environments.
– Genetic correlation between seed yield and percent protein is small but positive while correlation between seed yield and specific amino acid contents have seldom been significant.
– Occurrence of negative correlation between total protein concentration and sulphur-amino acid content is due largely to the differential accumulation of storage proteins, with different proportion of amino acids.

## 4. RESEARCH WORKS CONDUCTED IN GEMBLOUX

In the framework of a collaborative projects with the International Institute of Tropical Agriculture (I.I.T.A., Ibadan, Nigeria), research works were developed from 1970 to 1983 in the laboratory on intertropical crop husbandry with a view to examine the following components:
– genetic diversity in protein content and amino acid composition within *Phaseolus* genus, including cultivated, weedy and wild forms;
– protein distribution among the different seed parts (testa, cotyledons and embryonic axis) and amino acid composition in different protein fractions of the seed;
– influence of environmental factors (such as climate, cultural conditions, phenology and mineral nutrition) on protein concentration and amino acid profile;
– possibilities to obtain better amino acid balance through intra- and interspecific crosses.

### 4.1. Materials and methods

Materials included a wide array of cultivars, landraces, wild and weedy forms from primary and alien gene pools of the following taxa : *P. vulgaris*, *P. lunatus*, *P. coccineus*, *P. polyanthus* and *P. acutifolius*. Among them *P. lunatus* was considered as a case study due to a collaboration between our laboratory and two international institutes: IITA (International Institute of Tropical Agriculture, Ibadan, Nigeria) and CIAT (Centro Internacional de Agricultura Tropical, Cali, Colombia). For the evaluation of protein quality, we selected among the *Phaseolus* species genotypes differing markedly from several morphological and agronomic attributes (mainly seed size, seed coat colour, plant growth habit, earliness in flowering, resistance to biotic constraints, etc.). Plants were grown either under tropical field conditions (Nigeria and Colombia, mainly) or in greenhouses within the Gembloux University campus.

Crude extracts of protein fractions were obtained by solubilisation in water and NaCl 4% or by suspension of non solubilised residue in NaOH 0,1N (Manen, Otoul, 1981). Protein content (N x 6,25) was determined by the semi micro-Kjeldahl method applied to dried flour. Amino acid spectrum was measured by means of an automatic analyser (type: Beckman Multichrom II), after an acidic hydrolysis of the seed powder (Devenyi, 1968; Otoul, 1976; Manen, Otoul, 1981). For the determination of cystine and methionine, a special hydrolysis was performed, with performic acid treatment and direct measurements of cysteic acid and methionine sulfone. Results of the amino acid analysis were expressed in percent of total amino acids determined from the sample. Electrophoresis of seed flour and lyophilisats, in order to examine protein fractions, was carried out according to the method developed by Manen and Otoul (1981).

## 4.2. Results and discussion

Results of these investigations were given in various papers, mainly Otoul (1969, 1976), Otoul and Le Marchand (1974), Otoul and Dardenne (1976), Manen and Otoul (1981), Baudoin *et al.* (1983) and Baudoin (1991). We outlined the most significant results and trends, some being drawn mainly from *P. lunatus* gene pools. **Tables 1 to 4** indicate some values of crude protein and amino acid contents in various *Phaseolus* materials.

– Chemical composition of dry seed is similar to that of many other pulses. Seeds average 20% crude protein, with variations reflecting genotypic differences and ecological conditions. Like other grain legumes, *Phaseolus* seeds are deficient in sulphur-containing amino acids and, to a lesser extent, in tryptophan, but they contain relatively large amounts of lysine.

– The amino acid profile remains fairly uniform throughout all wild and cultivated forms. This is particularly true at the intraspecific level. However, with *P. lunatus*, we noticed a higher content in protein, lysine, cystine and S-methyl-cystein (a free amino acid) for three related species belonging to the secondary gene pool: *P. polystachyus*, *P. maculatus* and *P. marechalii.*

**Table 1.** Crude protein and amino acid contents in *P. vulgaris* and *P. lunatus* seeds — *Teneurs en protéines et acides aminés dans les graines de* P. vulgaris *et* P. lunatus.

| **Parameters** | ***P. vulgaris* cv** (14 genotypes) | ***P. lunatus* cv** (10 genotypes) |
|---|---|---|
| 100 seed weight (g) | 17–62 | 30–95 |
| crude protein content (**1**) | 19.1–29.7 | 19.7–24.9 |
| Amino acids (**2**) | | |
| cystine | 0.73–1.08 | 1.03–1.27 |
| methionine | 0.94–1.25 | 1.09–1.26 |
| lysine | 7.00–7. 43 | 6.81–7.12 |
| arginine | 5.31–8.39 | 5.39–6.59 |
| glutamic acid | 16.66–18.12 | 14.71–15.37 |

(**1**) N × 6.25 in % of dry matter; (**2**) % of total amino acids.

– Ecological conditions (for example, sites of seed multiplication) markedly influence the total nitrogen content of seeds and consequently the relative proportion of essential amino acids, particularly of lysine, methionine, cystine and S-methyl-cystein. Some differences could be assumed to variation in free amino acids and peptides.

– Plant age does not influence markedly amino acid spectrum (analysing in a single plant seeds from distinct fruiting cycles). On the other side, the seed filling stage may influence the amino acid profile. No tendency could, however, be noticed among the amino acids, with the exception of proline, alanine, valine and methionine showing a decrease during seed development.

– Total nitrogen content is greater in wild and weedy forms than in cultivars, but the latter are relatively richer in sulphur-containing amino acids. No relation has been found between 100 seed weight and nitrogen content.

– A slight increase in total nitrogen content is often associated with a relative decrease in lysine and methionine values.

– Methionine concentration averages about 1% within the amino acid spectrum, a value similar throughout the whole *Phaseolus* genus.

– Differences in amino acid composition are obtained from proteins belonging to testa, cotyledons and embryonic axis. Although cotyledons represent 90% of the seed weight and 96% of the total protein, embryo remmants are richer in total protein with high percentage in arginine and methionine.

– Within the seed of both *P. vulgaris* and *P. lunatus*, globulin represents more than 50% of the flour nitrogen and constitutes the major part of storage protein; albumin and glutelin represent 30% of flour nitrogen. Amino acid composition between the three fractions is different: albumin is richer in cystine while glutelin is richer in methionine; glutamic acid is high in both glutelin and globulin. Similarities exist between the two species. High content of cystine in albumin might be related with a higher content in trypsin inhibitor.

– Progenies from intraspecific crosses do not display marked modification in amino acid profile; slight progress is observed among interspecific crosses,

**Table 2.** Crude protein and amino acid contents in *P. coccineus* and *P. polyanthus* seeds — *Teneurs en protéines et acides aminés dans les graines de* P. coccineus *et* P. polyanthus.

| Parameters | ***P. coccineus*** **cv** (13 genotypes) | ***P. coccineus*** **wild** (3 genotypes) | ***P. polyanthus*** **cv** (4 genotypes) |
|---|---|---|---|
| 100 seed weight (g) | 70–166 | 7.5–16 | 45–76 |
| crude protein content (**1**) | 20–27.4 | 22.4–26 | 21.6–25.6 |
| Amino acids (**2**) | | | |
| cystine | 0.98–1.85 | 1.17–1.56 | 1.28–1.65 |
| methionine | 0.94–1.22 | 1.03–1.06 | 0.96–1.14 |
| lysine | 7.40–7.94 | 7.31–7.59 | 7.47–7.65 |
| arginine | 5.63–6.75 | 5.46–7.28 | 5.25–5.92 |
| glutamic acid. | 16.43–18.06 | 16.90–17.47 | 15.84–17.55 |

(**1**) N x 6,25 in % of dry matter; (**2**) % of total amino acids.

**Table 3.** Crude protein and amino acid contents in *P. acutifolius*, *P. filiformis* and *P. angustissimus* seeds — *Teneurs en protéines et acides aminés dans les graines de* P. acutifolius, P. filiformis *et* P. angustissimus.

| Parameters | ***P. acutifolius*** **cv** (9 genotypes) | ***P. acutifolius*** **wild** (6 genotypes) | ***P. filiformis*** (1 genotype) | ***P. angustissimus*** (1 genotype) |
|---|---|---|---|---|
| 100 seed weight (g) | 10–22 | 1.5–5 | - | - |
| crude protein content (**1**) | 17.7–28.8 | 19.1–28.2 | 24.2 | 25.9 |
| Amino acids (**2**) | | | | |
| cystine | 0.87–1.33 | 0.76–1.13 | 0.78 | 0.98 |
| methionine | 1.10–1.28 | 0.86–1.01 | 0.81 | 0.96 |
| lysine | 6.39–6.84 | 6.62–7.06 | 7.28 | 7.39 |
| arginine | 5.27–8.10 | 5.80–8.02 | 7.31 | 7.40 |
| glutamic acid | 17.75–23.12 | 17.71–22.40 | 20.78 | 20.25 |

(**1**) N x 6.25 in % of dry matter; (**2**) % of total amino acids.

**Table 4.** Crude protein and amino acid average contents in *P. lunatus* seeds of cultivated and wild genotypes — *Teneurs moyennes en protéines et acides aminés dans les graines de* P. lunatus.

| | **Wild forms** | **Cultivated forms** | | |
|---|---|---|---|---|
| **Parameters** | (7 genotypes) | **Potato** (6 genotypes) | **Sieva** (8 genotypes) | **Big lima** (4 genotypes) |
| 100 seed weight (g) | 8.93 | 30.63 | 42.53 | 84.30 |
| crude protein content (**1**) | 25.83 | 22.09 | 21.45 | 20.42 |
| Amino acids (**2**) | | | | |
| cystine | 0.99 | 1.23 | 0.92 | 0.85 |
| methionine | 0.89 | 0.99 | 0.99 | 0.99 |
| lysine | 6.89 | 7.09 | 7.06 | 7.13 |
| arginine | 5.86 | 6.62 | 6.38 | 6.12 |
| glutamic acid | 14.88 | 15.07 | 15.19 | 15.18 |

(**1**) N x 6.25 in % of dry matter; (**2**) % of total amino acids.

mainly in the cross *P. lunatus* × *P. marechalii*. In the latter some hybrid genotypes combine good nitrogen content with increased levels of methionine and cystine.

## 5. CONCLUSIONS

Content in total nitrogen is essential and should prevail, as a criteria of selection, over amino acid spectrum. Indeed a low nitrogen content requires a higher amount of the food legume in the diet, with consequently higher antinutritional factors.

In food legumes, no significant negative relation is found between seed yield and nitrogen content. Therefore genetic improvement of protein content can be achieved in the high seed yielding varieties.

Better quality and digestibility of protein can be obtained by mixing food legumes with cereals; this association is particularly relevant to compensate deficiencies in lysine and sulphur-containing amino acids, respectively in cereals and grain legumes.

Genetic alteration of specific protein fractions provides a means for increasing total protein content, raising limiting essential amino acid concentration by differentially regulating fractions with different amino acid composition. Such alternative should however take into account the proportion of the different seed parts and their composition in protein fractions.

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