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Enhanced available methionine concentration associated with higher phaseolin levels in common bean seeds

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Summary. The relationship between available methionine concentration and the levels of phaseolin – the major seed storage proteins of the common bean – was studied using three groups of genetic materials: First, the F_2 progenies of interspecific crosses between *P. vulgaris* cultivars and a *P. coccineus* subsp. *coccineus* line (cv. 'Mexican Red Runner') having no detectable phaseolin; second, the F_2 progenies and segregating F_3 families of crosses between cultivated *P. vulgaris* lines and a Mexican wild bean accession (PI 325690-3) carrying a gene producing a reduction in phaseolin content; third, two inbred backcross populations: 'Sanilac' × 'Bush Blue Lake 240' (population 2) and 'Sanilac' × '15R 148' (population 6). Total seed N levels were determined by micro-Kjeldahl, phaseolin levels by rocket immunoelectrophoresis and available methionine levels by the *Streptococcus zymogenes* bioassay. Our results indicate that in all the genetic materials studied, with the exception of population 6, higher phaseolin levels lead to increased available methionine concentration. Although phaseolin has a low methionine concentration, it is actually a major source of available methionine in common bean seeds, because it represents a large part of total seed nitrogen and because limited differences exist between the methionine concentrations of the different protein fractions. This contrasts with the situation in cereals such as maize, barley and sorghum, where increased levels of the major limiting amino acid (lysine) can be achieved through a decrease in the amounts of the main seed storage protein fraction (prolamines). In population 6, no relationship was observed between available methionine and phaseolin content. Other factors, such as additional methionine-rich polypeptides or the presence of tannins, might obscure the positive relationship between phaseolin and available methionine content in population 6.

Key words: *Phaseolus vulgaris* L. – *Streptococcus zymogenes* bioassay – Rocket immunoelectrophoresis – Micro-Kjeldahl – Limiting essential amino acid

Introduction

The common bean, *Phaseolus vulgaris* L., is a major protein source in most countries of Latin America and Eastern Africa. Seed protein of common bean accounts for up to 32% and 20% of total protein intake in Rwanda and Brazil, respectively (FAO 1980). However, the biological value of bean seed proteins is limited by their inadequate methionine and cystine contents. The concentration of these two sulfur amino acids is only one third of that in hen's egg protein which is considered to be the nutritionally ideal protein (Kelly 1973). Supplementation of maize-bean or cassava-bean diets with methionine leads to higher average weight gains and protein efficiency ratios in rat trials (Bressani 1973).

One of the strategies which has been suggested to improve the biological value of seed proteins is a modification of the ratios among the different seed protein fractions (Bright and Shewry 1983; Bliss and Brown 1983). Seed protein fractions which are poor in the major limiting amino acids can be decreased in favor of other protein fractions with a higher concentration of these amino acids. In cereals, the major seed storage protein is generally the alcohol-soluble prolamine fraction, characterized by a low lysine concentration compared to the glutelin, globulin, and albumin fractions. Mutants such as opaque-2 and floury-2 (maize; Mertz et al. 1964; Misra et al. 1975), Risø 1508 and Hiproly (barley; Shewry et al. 1980; Miflin and Shewry 1979), IS-11167 and P-721-N (sorghum; Singh and Axtell 1973; Guiragossian et al. 1978) show a reduced prolamine content and a simultaneous increase in other protein fractions. The result of this modification of the ratios among seed protein fractions in favor of lysine-rich fractions is a higher lysine concentration.

In the common bean, the major seed storage protein fraction is the phaseolin fraction (globulin-1 or G1) which accounts for 35 to 50% of total seed nitrogen. Other seed protein fractions include – in decreasing order of importance – the alkali-soluble fraction (20–30% of total seed N), albumins (11–20%), the free amino acid pool (5–9%), the globulin-2 (G2) fraction (4–7%) and the prolamine fraction (2–4%) (Ma and Bliss 1978). Differences in methionine concentration of these seed protein fractions have been identified. Whereas the phaseolin fraction contained 8.8 mg methionine/g protein, the alkali-soluble and the prolamine fractions contained 19.9 and 15.5 mg methionine/g protein, respectively (Ma and Bliss 1978). Differences in methionine content between the seed protein fractions are a prerequisite to insure a modification in the overall amino acid balance of the seeds by altering the ratios between the contents of the different seed storage protein fractions.

In the work reported here, we have used several genetic stocks to produce a modification in the ratios between the phaseolin protein fraction on one hand and the other seed protein fractions. Our results indicate that phaseolin is a major source of available methionine in common bean seeds.

Materials and methods

Plant materials

Three different groups of plant materials were investigated in this study. The first type of material consisted of the F_2 progenies of crosses between cultivated *Phaseolus vulgaris* lines as female parents and a *P. coccineus* subsp. *coccineus* line (cv. 'Mexican Red Runner') as the male parent. 'Mexican Red Runner' (MRR) contains no detectable phaseolin as assessed by rocket immunoelectrophoresis. The absence of phaseolin in this line is under the control of a single recessive gene (our unpublished data). Initially crosses with 'Mexican Red Runner' were attempted using 12 *P. vulgaris* cultivars representing different genetic backgrounds: 'Porrillo 70', 'State Half Runner', 'Redkote', '15R-148', 'Protop-P_1', 'Cornell 49-242', 'Pinto U.I. 111', 'PI 368737', 'ICA-Bunsi', 'Black Turtle Soup', 'Sanilac', and a lectin-free derivative of 'Sanilac'. Only crosses with 'Redkote', 'Protop-P_1', and 'ICA-Bunsi' produced F_1 hybrids with reasonably good viability and fertility and were therefore retained for further study. In addition, 'Mexican Red Runner' was also crossed to another *P. coccineus* subsp. *coccineus* line ('PI 255573'), which contains phaseolin. The F_1 hybrids of this cross showed normal viability and fertility.

The second group of materials included the F_2 progenies of crosses between cultivated *P. vulgaris* lines used as the female parent and a Mexican accession of wild *P. vulgaris*, 'PI 325690-3', as the male parent. This line carries a dominant gene causing a reduction in phaseolin concentration and concomitantly inducing the synthesis of a novel protein (Romero-Andreas and Bliss 1982; Blake and Bliss 1983). Five cultivated *P. vulgaris* lines were used in the crosses with 'PI 325690-3': 'Sanilac', 'Bush Blue Lake 240' (BBL 240), 'Pinto U.I. 111', '15R-148', and 'Porrillo 70'. All F_1 hybrids showed normal viability and fertility. For one of these crosses – 'Sanilac' × 'PI 325690-3' – segregating F_3 progenies were also studied.

The third group of materials investigated contained 2 inbred backcross populations resulting from crosses between 'Sanilac' as the female parent and either 'BBL 240' (a high methionine line) or '15R-148' (a high non-phaseolin protein line). The inbred backcross procedure as well as the characteristics of these two populations in terms of seed protein traits have been described by Sullivan and Bliss (1983).

Growth conditions of plant materials

The F_1 hybrids of the crosses with 'Mexican Red Runner' and 'PI 325690-3', as well as the F_2 generation of the 'Sanilac' × 'PI 325690-3' cross were grown in the greenhouse. F_1 seeds were germinated on moist filter paper in Petri dishes. After appearance of the radicle, seeds were transplanted into a soil-sand-peat mixture. At the primary leaf stage (V2 stage; Fernandez et al. 1983), the seedlings were transplanted into a soil-sand-peat moss-perlite mixture in 18 cm diameter pots. After transplantation, plants were fertilized with Osmocote (14N: 14P: 14K) and treated with Temik (10% aldicarb). Throughout their vegetative cycle plants were fertilized at regular intervals. Flowers of the F_1 hybrids of the crosses with 'Mexican Red Runner' were tripped to improve seed set. Inbred backcross populations 2 and 6 were grown in the field at the Hancock Experiment Station in 1980 and 1979, respectively. Growing conditions and cultural practices were reported previously (Sullivan and Bliss 1983).

Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed on protein extracted from individual seeds as described by Romero et al. (1975) and Ma and Bliss (1978). Flour from the raphe end of individual seeds was soaked in 0.5 M NaCl for at least 30 min. After centrifugation equal volumes of supernatant and cracking buffer (0.625 M Tris-HCl, pH 6.8, 2 mM EDTA, 2% (w/v) SDS, 40% (w/v) sucrose, 1% (v/v) 2-mercaptoethanol, and 0.01% (w/v) bromophenol) were mixed and boiled for 5 min. This mixture was then subjected to electrophoresis in 0.75 mm thick slab gels with a 15% acrylamide running gel and a 3% acrylamide stacking gel.

Protein determination

Percentage protein, calculated as percentage N × 6.25, was determined by the micro-Kjeldahl procedure (Association of Official Agricultural Chemists 1960) for the progenies of the crosses with 'Mexican Red Runner' and 'PI 325690-3'.

For populations 2 and 6, protein concentration had been measured previously using a GQA infra-red protein analyzer. The analyses of the protein data for the two populations were published earlier (Sullivan and Bliss 1983).

Phaseolin determination

Phaseolin concentrations in the progenies of the crosses with 'Mexican Red Runner' and 'PI 325690-3' were determined using rocket immunoelectrophoresis (Mutschler and Bliss 1981). Each entry was determined in duplicate.

Phaseolin concentrations in populations 2 and 6 had been determined previously using rocket immunoelectrophoresis and the results of the analysis reported by Sullivan and Bliss (1983).

Available methionine determination

Available methionine content was measured by the *Streptococcus zymogenes* bioassay as described by Kelly and Bliss (1975) with the following modifications. In the progenies of crosses with 'Mexican Red Runner' and 'PI 325690-3', seedcoats were removed manually before grinding to eliminate any confounding effect die to tannins in the seedcoats. In popula-

tion 2, seedcoats were not removed as both parents ('Sanilac' and 'BBL 240') and the progenies are white-seeded. In population 6 resulting from a cross between white-seeded 'Sanilac' and red-seeded '15R-148', only white-seeded progeny lines were analyzed. Each entry was represented by four replicate tubes, two of which were inoculated with the *S. zymogenes* culture. After an incubation period of 24 h the optical densities (OD) at 580 nm of the four tubes were measured. The OD's of the uninoculated tubes were then subtracted from the OD's of the inoculated tubes. The resulting values were then compared to the reference curve to obtain the amount of available methionine of each entry.

Results

Crosses with P. coccineus subsp. coccineus cv. 'Mexican Red Runner' (MRR)

The protein extracted from individual seeds harvested from a single F_1 plants of one intraspecific *P. coccineus* cross ('PI 255573' × 'MRR') and three interspecific *P. vulgaris* × *P. coccineus* subsp. *coccineus* crosses ('Redkote' × 'MRR', 'Protop P-1' × 'MRR', and 'ICA-Bunsi' × 'MRR') were analyzed by SDS-PAGE to determine the presence or absence of phaseolin. Seeds with or without phaseolin were then bulked separately and analyzed for protein, phaseolin and available methionine content.

With the exception of the 'Protop P-1' × 'MRR' cross, seeds without phaseolin showed a small but significant reduction in protein concentration (Table 1). Phaseolin concentration measurements by rocket immunoelectrophoresis merely confirmed the presence or absence of phaseolin as determined earlier by SDS-PAGE. In each of the crosses studied, seeds containing phaseolin had significantly higher available methionine levels compared to seeds without phaseolin.

Crosses with wild P. vulgaris, 'PI 325690-3'

'PI 325690-3' carries a dominant gene which simultaneously produces a reduction in phaseolin concentration and the appearance of a novel protein (Romero-Andreas and Bliss 1982; Blake and Bliss 1983). By means of SDS-PAGE, appearance of the novel protein was used as a marker to separate the F_2 seeds, produced from single F_1 plants of five crosses between cultivated *P. vulgaris* and 'PI 325690-3' into a high phaseolin (absence of the novel protein) and a low phaseolin (presence of the novel protein) group.

For each cross, these two groups were then analyzed for protein, phaseolin, and available methionine concentration (Table 2). No significant differences in protein concentration were observed between the seeds with and without the novel protein. As predicted, seeds with the novel protein had markedly lower levels of phaseolin compared to seeds without. The latter had significantly higher available methionine levels. For the 'Sanilac' × 'PI 325690-3' cross, segregating F_3 families were also analyzed following the same methods used for the F_2 generation (Table 3). As for the F_2 generation, seeds with higher phaseolin levels had higher available methionine levels compared to seeds with lower phaseolin levels.

Table 1. Protein, phaseolin and available methionine concentrations in F_2 seeds of crosses with *P. coccineus* subsp. *coccineus* cv. 'Mexican Red Runner' (MRR)

Cross	Phaseolin phenotype	Protein	Phaseolin	Available methionine
		mg/g flour		
'PI 255573[a]' × 'MRR[a]'	+[c]	235	83	1.7
	−	221	n.d.[d]	1.5
'Redkote[b]' × 'MRR'	+	295	135	2.3
	−	272	n.d.	1.6
'Protop-P1[b]' × 'MRR'	+	316	135	2.6
	−	317	n.d.	2.2
'ICA-Bunsi[b]' × 'MRR'	+	268	106	1.9
	−	261	n.d.	1.8
Paired *t*-test: t		2.69*	6.53***	3.85**
df		11	7	7

[a] *P. coccineus* subsp. *coccineus*
[b] *P. vulgaris*
[c] +: presence of phaseolin; −: absence of phaseolin
[d] n.d.: not detectable by rocket immunoelectrophoresis

Table 2. Protein, phaseolin and available methionine concentrations in F_2 seeds of crosses with 'PI 325690-3' (PI)

Cross	G3[a] phenotype	Protein	Phaseolin	Available methionine
		mg/g flour		
'Sanilac' × 'PI'	–	237	139	2.2
	+	262	75	1.6
'Pinto U.I. 111' × 'PI'	–	267	145	2.0
	+	263	80	1.5
'BBL 240' × 'PI'	–	277	160	2.4
	+	268	77	1.8
'15R148' × 'PI'	–	307	156	2.4
	+	297	75	1.8
'Porrillo 70' × 'PI'	–	292	180	2.2
	+	297	100	2.0
Paired *t*-test	t	0.35 n.s.	25.36***	4.41**
	df	9	9	9

[a] +: presence of G3; –: absence of G3

Table 3. Protein, phaseolin and available methionine concentrations in F_3 seeds of the cross between 'Sanilac' and 'PI 325690-3'

Family no.	G3[a] phenotype	Protein	Phaseolin	Available methionine
		mg/g flour		
3	–	259	142	1.8
	+	245	86	1.6
4	–	266	131	2.0
	+	253	81	1.8
14	–	244	134	1.9
	+	290	111	1.7
16	–	275	155	2.0
	+	262	89	1.5
21	–	288	152	2.5
	+	291	65	1.9
33	–	264	136	2.1
	+	270	75	1.7
35	–	274	136	1.9
	+	283	78	1.6
37	–	265	148	1.9
	+	271	95	1.6
40	–	282	134	2.1
	+	260	72	1.5
Paired *t*-test	t	0.18 n.s.	9.85***	5.89***
	df	17	17	17

[a] +: presence of G3; –: absence of G3

Inbred-backcross populations

Two inbred backcross populations developed by Sullivan and Bliss (1983) were analyzed for available methionine concentration. Analysis of variance was then performed on the available methionine data and on other seed protein data taken from Sullivan and Bliss (1983). In population 2, derived from the cross between 'Sanilac' and 'BBL 240' – a high methionine line – significant differences were observed among the different lines for available methionine concentration per unit of flour, protein concentration per unit of flour and phaseolin concentration per unit of flour or per unit of protein. No significant differences among lines were observed however for available methionine concentration per unit of protein (Table 4). Although some of the progeny lines had relatively high available methionine levels (around 2.3 to 2.4 mg available methionine/g flour), none of these lines reached the available methionine levels achieved by 'BBL 240' (around 2.6 mg available methionine/g flour). In population 6, resulting from a cross between 'Sanilac' and '15R-148' – a high non-phaseolin protein line – significant differences among lines were observed for available methionine concentration per unit of four, protein content per unit of flour and phaseolin concentration per unit of flour or pr unit of protein. In contrast to population 2 however, significant differences were observed among lines in terms of available methionine concentration per unit of protein (Table 4).

Correlation coefficients between various seed protein traits were also calculated. In population 2, a high correlation was observed between available methionine concentration and both protein and phaseolin concentration per unit of flour. A moderately high correlation was observed between available methionine concentration per unit of flour and phaseolin concentration per unit of protein. Only a low

Table 4. F values from analyses of variance of seed protein traits inbred-backcross populations 2 and 6

Trait	Population 2[a] ('Sanilac' × 'BBL 240')	Population 6[b] ('Sanilac' × '15R148')
Available methionine/flour	2.32***	5.07***
Available methionine/protein	0.73 n.s.	3.10***
Protein/flour	7.48***	3.65***
Phaseolin/flour	13.35***	4.16***
Phaseolin/protein	8.23***	3.80***
Non-phaseolin protein/flour	3.23***	3.47***

[a] Degrees of freedom: 45
[b] Degrees of freedom: 38

correlation was observed between available methionine concentration per unit of flour and non-phaseolin protein concentration per unit of flour. No significant correlations were observed between available methionine concentration per unit of protein, and protein and phaseolin concentration per unit of flour or phaseolin concentration per unit of protein (Table 5). In population 6, a rather low correlation was observed between available methionine concentration per unit of flour and protein concentration per unit of flour. No correlations were observed between available methionine concentration per unit of flour and phaseolin concentration per unit of flour or protein, and non-phaseolin protein concentration per unit of flour. Likewise no correlations were observed between available methionine concentration per unit of protein and protein concentration per unit of flour, phaseolin concentration per unit of flour or protein, and non-phaseolin protein concentration per unit of flour (Table 5).

Discussion

In the crosses involving 'Mexican Red Runner' and 'PI 325690-3' seeds from individual F_1 or F_2 plants were analyzed for protein, phaseolin and available methionine concentration. Because of the strong maternal control on protein levels in the seeds, it was expected that seeds would contain similar total protein levels in spite of widely different phaseolin contents. This would allow total protein levels to be eliminated as a factor in available methionine concentration variation and to focus the interpretation of our results on the relationship between phaseolin and available methionine concentrations. As expected, in the crosses with 'PI 325690-3', no significant differences in total protein levels were observed between seeds with high or low levels of phaseolin. However, in three out of four crosses with 'Mexican Red Runner', a small but significant reduction in total protein was observed in seeds without phaseolin compared to seeds with phaseolin. This slight reduction in total protein levels might be due to a failure of the other seed protein fractions to compensate for the lack of phaseolin.

In all the populations studied, with the exception of inbred backcross population 6, increased levels of phaseolin led to higher levels of available methionine. In the F_2's of each of the four crosses with 'Mexican Red Runner', seeds with phaseolin had higher available methionine levels than seeds without phaseolin. Although the latter also showed slightly reduced protein levels, the magnitude of this reduction was smaller than the magnitude of the reduction in available methionine concentration between seeds with and without phaseolin. This suggests that the reduction in available methionine in the non-phaseolin seeds, compared to the

Table 5. Correlation coefficients between seed protein traits in inbred-backcross populations 2 and 6

	Protein/flour	Phaseolin/flour	Non-phaseolin protein/flour	Phaseolin/protein
Population 2 ('Sanilac' × 'BBL 240')				
Available methionine/flour	0.78***	0.74***	0.37**	0.52***
Available methionine/protein	0.06 n.s.	0.14 n.s.	−0.11 n.s.	0.16 n.s.
Population 6 ('Sanilac' × '15R148')				
Available methionine/flour	0.45**	0.26 n.s.	0.30 n.s.	0.05 n.s.
Available methionine/protein	−0.04 n.s.	−0.06 n.s.	0.02 n.s.	−0.05 n.s.

seeds with phaseolin, was due primarily to the absence of phaseolin, rather than to a reduction in total protein content. The F_2 generations of each of the crosses with 'PI 325690-3', as well as the segregating F_3 families of 'Sanilac' × 'PI 325690-3', showed increased levels of available methionine associated with higher phaseolin levels. The segregates with lower phaseolin also contained a novel protein, in contrast to the high phaseolin segregates. Because the low phaseolin segregates also have reduced available methionine, the novel protein is probably methionine poor.

Populations 2 and 6 showed distinctly different relationships between available methionine content and other seed protein traits. In population 2, a high correlation was observed between seed protein or phaseolin concentrations and available methionine levels. No significant differences among inbred backcross lines were observed for available methionine concentration per unit of protein indicating that the variation in available methionine resulted from variation in the concentrations of the same proteins. Phaseolin is a major determinant of total protein levels (Ma and Bliss 1978). A higher phaseolin content in common bean seeds will lead to higher phaseolin concentrations per unit of flour or per unit of protein and therefore under our hypothesis, to higher available methionine concentrations per unit of flour. No correlation was observed, however, between available methionine concentrations per unit of protein and phaseolin concentrations per unit of protein. We attribute this lack of correlation to the limited differences in available methionine concentration between phaseolin on one hand and the other protein fractions on the other hand. In population 6, on the other hand, small or no correlations were observed between seed protein or phaseolin concentrations and available methionine levels. Significant differences in available methionine concentration per unit of protein were observed among inbred backcross lines. This might indicate that fractions other than phaseolin, such as the alkali-soluble fraction – might also be contributing significantly to the available methionine concentration in the seeds. '15R-148', one of the donor parents of population 6, was selected as a high non-phaseolin protein line. Some proteins or polypeptides of the non-phaseolin protein fractions might have an available methionine concentration that is sufficiently high to influence the overall available methionine concentration in the seeds. Alternatively, some non-protein factors such as polyphenolic pigment in the seedcoats, may obscure the positive relationship between phaseolin and available methionine contents. '15R-148' is a red-seeded line. Although only white-seeded progeny lines of population 6 were analyzed, some of these lines might carry anthocyanin precursors which do not pigment the seedcoat but nevertheless could bind either to the seed proteins or to the proteases involved in the *Streptococcus zymogenes* bioassay. It has been suggested by Bressani and Elias (1980) that polyphenolic compounds in the common bean decrease protein digestibility either by inhibiting digestive enzymes or by reacting with proteins, reducing the availability of amino acids.

Our finding that higher levels of phaseolin, the major storage protein of the common bean, are associated with increased levels of methionine the major limiting essential amino acid, are in contrast with results from analogous work in cereals (Nelson 1980; Bright and Shewry 1983). The opaque-2 and floury-2 (maize), Risø 1508 and Hiproly (barley), P721 opaque and IS 11167 (sorghum) mutants produce a reduction in the prolamine fraction (the most abundant seed protein fraction in these cereals) and concomitantly increase other nitrogen fractions such as glutelins, globulins, albumins or the free amino acid pool. The latter fractions have lysine concentrations which are up to 50 times higher than the lysine content of the prolamine fraction (maize: Sodek and Wilson 1971; barley: Shewry et al. 1979). Consequently, a reduction in the major storage protein fraction, prolamine, leads to substantially higher levels of lysine, the major limiting essential amino acid of these cereals. In the common bean, differences in methionine concentration among protein fractions are much smaller (Ma and Bliss 1978). While the phaseolin fraction contains 8 to 9 mg methionine/g protein, the alkali-soluble fraction, the second most important seed protein fraction, contains about 20 mg methionine/g protein. Although phaseolin has a low methionine concentration, it is a major source of available methionine in common bean seeds, because it represents an important part of total seed nitrogen (36% to 50%) and because only small differences exist between the methionine concentrations of the different protein fractions.

It must be determined whether higher phaseolin levels and the resulting higher available methionine levels, will improve the nutritional value of common bean seeds. It may be necessary to improve phaseolin not only quantitatively but also qualitatively by searching for specific phaseolin polypeptides with a higher methionine content.

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