GRAIN QUALITY AND BIOCHEMISTRY

Chickpea and Pigeonpea

Amino Acid Composition

Prepared by:

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FOREWORD

This is the second progress report of ICRISAT Grain Quality and Biochemistry Support Program. In this report the work on the amino acid composition of chickpea and pigeonpea has been described. The work has been carried out during 1977-82. In addition to this report, results on this aspect have appeared in ICRISAT ANNUAL REPORTS. Our program has closely collaborated with Genetics Resources Unit, Pigeonpea Breeding, Chickpea Breeding and Pulse Physiology programs at ICRISAT and their contribution and assistance are gratefully acknowledged. Also, I thank Dr. R. Jambunathan for his comments on the earlier draft of this report.

This is not a formal publication of the institute and should not be cited.

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SUMMARY

1. Methods of estimation of methionine, cystine and tryptophan:

The procedure of ion exchange column chromatography using the amino acid analyser was first examined for the determination of these amino acids. Two methods of hydrolysis which are commonly used were compared in order to know the extent of losses of methionine and cystine. Results indicated that refluxing was better than sealed-tube hydrolysis particularly from the viewpoint of recovery of sulphur containing amino acids. Performic acid oxidation (PAO) procedure resulted in higher methionine and cystine recovery values.

When compared statistically with the amino acid analyser results, microbiological method was found satisfactory for the estimation of methionine in case of both chickpea and pigeonpea but not for cystine. Although the microbiological method produced reliable methionine results for both the crops, it was not found suitable for screening large numbers of samples because it was observed to be tedious and slow and also required careful handling by an experienced person.

For methionine, rapid colorimetric method involving nitroprusside reaction produced results which did not differ significantly from those obtained by amino acid analyser. Cystine values estimated by the Goa method were highly correlated with the results of amino acid analyser. The hydrozinolysis of the sample in screw cap tubes instead of using small ampules and buffer system with reduced pH were the important modifications introduced in the Goa method.

Two rapid colorimetric procedures of tryptophan estimation were compared with the amino acid analyser. In case of both chickpea and pigeonpea, the results of these two rapid procedures were significantly correlated with the results of amino acid analyser. Although it appeared that both the rapid colorimetric procedures can be used for accuracy of analysis for screening purpose, in view of the simplicity and rapidity of the analysis, the Spies and Chamber procedure was sugested for screening large numbers of samples.

2. Total sulphur and sulphur amino acids:

The procedures of wet-digestion and Leco-sulphur analyser were compared for the estimation of total sulphur in chickpea and pigeonpea. These procedures were highly and significantly correlated with each other. Total sulphur was positively and significantly correlated with the sulphur

amino acids in pigeonpea but not in chickpea. This indicated the possibility of using total sulphur content as an index of the levels of sulphur amino acids in pigeonpea.

When expressed as percent of protein methionine was significantly correlated with cystine in both chickpea and pigeonpea. Moreover, cystine and methionine were correlated with each other when expressed either as percent of protein or as percent of sample suggesting that screening for one of these two sulphur amino acids would be sufficient.

3. Variabilty for amino acid composition:

Sulphur amino acids, methionine and cystine when considered together were the first limiting amino acids of chickpea and pigeonpea. Based on the analysis of several genotypes, tryptophan was not found to be the limiting amino acid of chickpea whereas pigeonpea cultivars were invariably deficient in this amino acid. Besides these amino acids, threonine was observed to be the second limiting amino acid of chickpea.

Some wild speices of genus Cicer were also studied for their amino acid composition. Sulphur amino acids, methionine and cystine, when considered together varied from

2.12 to 3.42 g/16g N for wild species whereas the cultivated species had 2.38 g/16g N.

Together, methionine and cystine values were highest ranging from 2.55 to 3.43 with mean being 2.93 g/16g N for 28 accessions of A. scarabaeoides and lowest ranging from 2.34 to 2.79 with mean being 2.55 g/16g N for 10 accessions of A. albicans when the results of many accessions representing 19 wild species of pigeonpea were compared.

4. Seed protein fractions and amino acid composition:

Studies on protein fractionation in seed coat, embryo, cotyledons and whole seed of chickpea and pigeonpea were carried out. Results indicated that globulin was the major fraction of embryo and cotyledons of chickpea and pigeonpea. Seed coat nitrogen was observed to be mostly comprised of nonprotein nitrogen and glutelin fractions.

While no noticeable differences between chickpea and pigeonpea were apparent with respect to the levels of various protein fractions, the higher levels of sulphur containing amino acids in glutelin than in globulins of these pulse crops suggest that cultivars with a higher ratio of glutelin to globulin should be identified to improve their seed protein quality.

1. INTRODUCTION

Amino acid composition is the first approximation by which one measures the protein quality of a given crop. In general, the sulphur containing amino acids, methionine and cystine, and tryptophan limit the nutritive value of pulse crops. Improvement of nutritional quality of pigeonpea and chickpea through effective breeding program is one of the objectives of ICRISAT. The progress of such breeding programmes would in part depend on the availability of rapid and accurate analytical procedures for the estimation of these amino acids. Therefore, we have initially concentrated on the identification and standardization of rapid and reliable screening methods for the estimation of these amino acids in chickpea and pigeonpea. In order to know the genetic variability for these amino acids, several cultivars of chickpea and pigeonpea were analysed. In addition, several other related aspects that affect the protein quality of these crops were also examined. In this report, the following aspects concerning the amino acids of pigeonpea and chickpea are described.

I. Methods of estimation of methionine and cystine
II. Rapid colorimetric procedures for the estimation of tryptophan
III. Genetic variability for methionine, cystine and tryptophan
IV. Seed protein fractions and amino acid composition

2. Methods of estimation of methionine and cystine:

2.1. Determination of methionine and cystine by using the amino acid analyser:

Even though the procedure of ion exchange column chromatography using the amino acid analyser is rather slow and cumbersome for the determination of amino acids, it is still regarded as a standard procedure. Before attempting to evaluate the rapid methods for the estimation of sulphur containing amino acids, methionine and cystine, it is necessary to ensure that these amino acids are determined accurately using the amino acid analyser. So this procedure was first examined.

The hydrolysis of protein into a mixture of amino acids by splitting the peptide linkages is an essential first step which influences the results in this procedure. Therefore, two methods of hydrolysis which are commonly used were compared in order to know the extent of losses that occur, particularly with respect to the levels of methionine and cystine. The procedures of refluxing and sealed-tube hydrolysis were compared. For sealed-tube hydrolysis, specially made tubes having constricted necks were used. About fifty mg of defatted material were accurately weighed into the tube and 10 ml 6 N HCl were added and the tube was partially evacuated and sealed. The hydrolysis was carried out for 24 hr at 110°C in an oven which had a built in fan

to keep the temperature uniform. In the case of refluxing, about 50 mg defatted sample were accurately weighed into a 250 ml round bottom flask and the sample was refluxed with 50 ml 6 N HCl for 24 hr. After the hydrolysis, the excess acid was removed in a rotary flash evaporator. The residue was dissolved in a suitable amount of citrate buffer (pH 2.2). Protein hydrolysate thus obtained was analysed in a Beckman model 120C amino acid analyser.

Table 1. Results of essential amino acid composition of six pigeonpea cultivars obtained by two different methods of hydrolysis.

Essential amino acid (g/16g N)	Methods of Hydrolysis			
	Refluxing		Sealed Tube	
	Range	Mean	Range	Mean
Lysine	7.26-8.17	7.80	7.04-7.84	7.58
Threonine	3.86-4.28	4.04	3.79-4.29	4.02
Methionine	0.93-1.07	1.01	0.88-1.06	0.96
Cystine[a]	Trace-1.09	0.93	0.50[b]	0.50[b]
Phenylalanine	9.78-12.31	11.25	9.95-10.76	10.86
Valine	4.58-5.19	4.89	4.51-5.35	4.66
Leucine	8.27-8.76	8.60	9.95-10.76	8.89
Isoleucine	3.91-4.27	4.18	2.93-3.88	3.19

a, Five cultivars; b, Only one sample.

Six cultivars of pigeonpea were analysed for this study and the results are given in Table 1. Except for leucine which showed a lower value, all other values were higher in the results obtained by the refluxing than sealed-tube technique. Our observations indicated that refluxing is better than sealed-tube hydrolysis particularly from the view-point of recovery of sulphur containing amino acids. This was found to be the case for chickpea as well.

2.1.1. Performic acid oxidation of methionine and cystine:

Unfortunately cystine and methionine are destroyed to a certain extent during the process of acid hydrolysis. Therefore, the actual values of these amino acids cannot be determined by the normal acid hydrolysis procedure. Methionine and cystine are therefore determined after oxidation into their acid stable compounds, methionine sulfone and cysteic acid, respectively using the performic acid oxidation procedure (PAO) as described below.

The performic acid reagent was prepared by the addition of 1 ml of 30% (w/w) hydrogen peroxide to 9 ml of 88% formic acid. The solution was allowed to stand for 1 hr at room temperature to permit the performic acid concentration to reach the maximal value. To 20 to 25 mg of defatted sample weighed into a round bottom conical flask, 2 ml of the

performic acid solution was added. After standing for 4 hr at 0°C, 0.3 ml of 38% HBr was added to stop the oxidation. The excess bromine was removed by placing the flask in a dessicator containing pellets of NaOH under vacuum. Then the flask was attached to a rotary flash evaporator and the contents of the flask were dried completely. Then, hydrolysis was conducted by adding 50 ml of 6 N HCl to the residue and refluxing the sample for 24 hr. After the hydrolysis, HCl was removed by using a rotary flash evaporator. The residue was dissolved in 5 ml of citrate buffer (pH 2.2). Methionine sulphone and cysteic acid thus formed were analysed in the amino acid analyser.

The results obtained by the PAO procedure were compared with those obtained by normal (unoxidized) procedure. Recovery values in oxidized samples were higher than the unoxidized sample for both methionine (Table 2) and cystine (Table 3). It is to be noted here that in case of unoxidized samples very small peaks for methionine sulphoxide, and cysteic acid were also recorded and these were calculated and added to methionine and cystine content respectively. This could lead to lower recovery values for these amino acids in the case of unoxidized samples. Dimethyl sulphoxide (DMSO) is also used as an oxidizing agent in place of performic acid. The DMSO method although

Table 2. Recovery of added methionine by amino acid analyser and microbiological method[a]

Crop	Amino acid analyser		Microbiological assay
	Control	Performic acid oxidation	
	..... Recovery (%)		
Chickpea	76.5 - 85.2	90.8 - 94.2	92.8 - 98.6
(G-130)	80.5 ± 4.0	92.6 ± 1.8	96.8 ± 2.7
Pigeonpea	74.6 - 82.7	86.5 - 94.6	102.2 - 110.1
(ICP-1)	78.0 ± 3.8	89.7 ± 2.0	104.4 ± 2.6

a, Range and mean based on five determinations on defatted dhal.

simpler, faster and more convenient for cystine determination, cannot be used for the simultaneous determinatin of methionine. It has also been reported that the presence of trace amounts of dimethyl sulphoxide in samples loaded on to the amino acid analyser can interfere with subsequent amino acid analyses due to the production of ninhydrin positive degradation products. Therefore PAO method was followed in our laboratory for the determination of methionine and cystine.

Table 3. Recovery of added cystine by amino acid analyser and microbiological method[a]

Crop	Amino acid analyser		Microbiological assay
	Control	Performic acid oxidation	
	..... Recovery (%)		
Chickpea	64.5 - 71.8	92.5 - 97.0	83.7 - 110.6
(G-130)	68.3 ± 3.4	94.2 ± 2.0	97.8 ± 7.4
Pigeonpea	67.5 - 73.4	91.6 - 95.8	84.5 - 105.8
(ICP-1)	70.5 ± 3.1	93.6 ± 2.4	91.6 ± 6.9

a, Range and mean based on five determinations on defatted dhal.

2.2. Rapid procedures for the estimation of methionine and cystine:

As mentioned earlier, the slow and cumbersome procedure of amino acid analyser is unsuitable for screening methionine and cystine in a crop improvement programme where the analyses of large numbers of samples are required. Therefore, there was a need to identify and standardize suitable rapid procedures for the estimation of these amino acids in chickpea and pigeonpea. Keeping this in mind, the following rapid procedures were studied:

(I) Microbiological method for the estimation of methionine and cystine

(II) Colorimetric procedure involving nitroprusside reaction for methionine estimation

(III) Colorimetric estimation of cystine using the Goa procedure

2.2.1. Microbiological method for the estimation of methionine and cystine:

Microbiological method is based on the principle that certain microorganisms require specific nutrients for growth. Using a basal medium complete in all respects except for the nutrient (amino acid) under study, growth responses of organisms are compared quantitatively with a standard and unknown solutions. Either the acid or the turbidity produced by the microorganisms is measured to determine the extent of growth and thereby indirectly the amount of nutrient present in the test solution.

Total methionine content was determined by the microbiological assay using the organism Leuconostac mesenteroides P-60 ATC 8042. The defatted sample (0.25 g) was weighed into a conical flask and 2.5 ml of 2.5 N HCl was added and the mixture was autoclaved for 6 hr at 15 lbs pressure and cooled. The pH was then adjusted to 4.5 with 10 N NaOH and the solution was made up to 25 ml, filtered

and stored in cold and toluene was added as preservative. An aliquot of 2 ml was taken, and the pH was adjusted to 6.8 with dilute NaOH (0.1 N) and made up to 10 ml. Then suitable aliquots (0.2, 0.4, 0.6, 0.8 and 1.0 ml) of the solution were pipetted out and made up to 25 ml with distilled water and this was followed by the addition of 2.5 ml of methionine assay medium (Difco-B-423). This medium contains all other factors and amino acids necessary except methionine for the normal growth of L. mesenteroides. Suitable aliquots of standard solutions of methionine (0-15 ugs) were pipetted out and diluted to 2.5 ml. Then 2.5 ml of basal medium was added. After preparing the standards and the samples, the assay tubes were covered with muslin cloth, then with absorbent cotton, packed well and sterilized in an autoclave for 10 minutes at 15 lbs pressure and cooled.

One day prior to the planned assay, a subculture was prepared from the stock culture of the organism by inoculating the organism into 10 ml of Bacto-micro broth. After incubation at 35-37°C for 16-24 hr, the cells were centrifuged under asceptic conditions and the supernatant was discarded. The cells were washed repeatedly with 0.9% sodium chloride solution and finally the cell suspension was diluted 5-100 times with sterile solution of isotonic sodium chloride. One drop of this suspension was added to each of the tubes with a sterile syringe and needle, under sterile condition in a inoculation chamber. The inoculated tubes

were then incubated at 35-37°C for 16-20 hr and the turbidity was measured in a nephelometer (Evans Electro Selenium Ltd., Halstead, Essex, England). The standard readings were then plotted on a graph and calculations were carried out by using this graph. This procedure was studied for its accuracy and reproducibility. The standard error and coefficient of variation of this procedure for chickpea and pigeonpea are given in Table 4. Methionine recovery values ranged between 92.8 and 98.6% for chickpea and between 102.2 and 110.1% for pigeonpea (Table 2).

Using the microbiological method, 30 cultivars of chickpea and 24 cultivars of pigeonpea were analysed for their methionine content and the results were compared with those of amino acid analyser (Appendix 1). A comparison of results obtained by these two methods for methionine estimation is shown in Appendix 1. A highly significant correlation was observed between the values obtained by the microbiological and amino acid analyser procedures for both chickpea ($r = 0.82^{**}$) and pigeonpea ($r = 0.89^{**}$).

Cystine was estimated microbiologically using Leconostac mesenteroides as test organism using the procedure similar to that of methionine assay except that protein hydrolysis was carried out in 2.5 N HCl for 1 hr. Standard error of the method was worked out and recovery assays were conducted before analysing the cultivars of chickpea and pigeonpea. A large variation in the recovery

values for both chickpea and pigeonpea was noticed (Table 3). Coefficient of variation of estimation by this procedure was high, being 7.4% for chickpea and 6.9% for pigeonpea. Considerable differences were also observed when equal amounts of cysteine and cystine were assayed by this procedure. Earlier workers have also examined this procedure and have reported it to be unsuitable for cystine estimation (Hannah et al. 1977). It has been reported that since cystine supports more growth than cysteine, the relative amounts of both forms of the amino acid must be known before this microbiological assay can be used to quantify the levels of these amino acids. So in view of this report it was not considered worthwhile to continue this procedure for cystine determination. One draw back of microbiological procedure is that the procedure is slightly tedious and slow and needs careful handling by an experienced person for analysing about 10 samples per day.

2.2.2. Rapid colorimetric procedure for methionine:

The rapid method of MacCarthy and Sullivan (1941) involving nitroprusside reaction, was evaluated for methionine estimation in chickpea and pigeonpea. The procedure outlined below was investigated and modified suitably for these crops. As the -S-CH_3 group of methionine takes part in the reaction, the amount of this amino acid

Table 4. Standard error and coefficient of variation of amino acid analyser and microbiological assay[a]

Crop		Methionine (g/16g N)	
		Amino acid analyser	Microbiological assay
Chickpea	Range	1.29 - 1.40	1.00 - 1.07
(G-130)	Mean	1.37	1.06
	SE	0.05	0.03
	CV	3.59	2.96
Pigeonpea	Range	1.23 - 1.34	0.91 - 0.98
(HY-3C)	Mean	1.27	0.94
	SE	0.05	0.03
	CV	3.78	3.23

a, Based on five determinations on defatted dhal.

can be determined in peptides as well as this group is free to react with sodium nitroprusside. So the hydrolysis of protein is an important step and efforts were first made to find out the suitable methods of protein hydrolysis for this procedure. Two commonly used procedures for protein hydrolysis, as described below, were studied to find out their suitability for methionine determination.

I Acid hydrolysis of protein:

Defatted sample (1.0 g) was taken in a hydrolysis tube and 10 ml of 40% HCl was added according to the procedure

described by Lunder (1973). After partial evacuation, the tube was sealed and hydrolysed at 120°C for 12 hr. The sample was cooled and treated with about 5 g of charcoal to decolourize the hydrolysate which would otherwise interfere in the colorimetric procedure. The content was filtered and the residue was washed thoroughly with 25% ethyl alcohol to liberate the absorbed methionine on charcoal. Final volume was made to 25 ml.

II. Enzymatic hydrolysis of protein:

Partial protein hydrolysis using the enzyme papain (Sigma Chemical Co., USA) was carried out according to Gehrke and Neuner (1974). These workers have shown that partially hydrolysed protein material was enough to allow quantitative reaction of sodium nitroprusside with the exposed sulphur. To 0.75 g of defatted sample taken in a conical flask, 10 ml of 0.1 M tris buffer pH 7.2 containing 0.5% mercaptoethanol, 0.002 M ethylene diaminotetra acetic acid (EDTA) were added. Hydrolysis was carried out at 50°C for 4 hr. The reaction was stopped by adding a few drops of phosphoric acid. Then the contents were transferred and the final volume was made to 25 ml with distilled water and filtered. Aliquots of this solution was taken for analysis. The influence of different buffer systems, viz. phosphate

buffer (0.1 M) sodium acetate buffer (0.1 M) and borate buffer (0.1 M) and different durations of hydrolysis were studied.

Methionine assay procedure:

To suitable aliquots (1.0 ml) of acid and enzyme hydrolysates, 1 ml of 13 N NaOH and 1 ml of 5 N NaOH were added respectively followed by 1 ml of 1% glycine. After mixing, 1 ml of 1% aqueous solution of sodium nitroprusside was added and the contents were mixed well and incubated in a water bath at 40°C for 10 min. Then the tubes were cooled, 5 ml of phosphoric acid (85%) was added to develop the colour. The contents of the tubes were mixed well and read at 520 nm. Standard methionine solutions were treated similarly for the preparation of a standard graph.

2.2.3. Comparison of enzyme and acid hydrolysis for the estimation of methionine by nitroprusside reaction:

Methionine by nitroprusside reaction could be carried out on acid hydrolysate provided the hydrolysates are discolorized by charcoal treatment (Lunder, 1973). In order to find out the usefulness of acid hydrolysate, the samples were hydrolysed and after partial discolorization of the hydrolysate by charcoal treatment as described earlier, aliquots were analysed for methionine content. Chickpea and pigeonpea samples were analysed for both enzyme and acid

hydrolysis procedures and the results are shown in Table 5. For both chickpea and pigeonpea, much lower methionine values were obtained by the acid hydrolysis procedure. When the methionine recovery assays were carried out, only about 70 percent of the added methionine to acid hydrolysate was recovered. It was considered that charcoal treatment could result in lower values because of the absorption of methionine on charcoal. Recovery values did not improve even when excessive washings with hot water and ethyl alcohol were given to charcoal to liberate absorbed methionine. Moreover, charcoal treatment of acid hydrolysate did not remove the extraneous color completely. The remaining colour was pale yellow and this might have interfered in the colorimetric estimation.

It is also quite possible that during the acid hydrolysis, part of methionine could be oxidised to methionine sulphone or sulfoxide if the hydrolysis was not carried out under an atmosphere of nitrogen. As methionine sulfone and sulfoxide do not develop colour with nitroprusside reaction, this could also result in the under-estimation of methionine. An indication to this effect was observed when lower methionine values of the sample and lower recovery percent were recorded with increasing duration of hydrolysis (Table 5). It was quite obvious from these experiments that the acid hydrolysis procedure was unsuitable for the estimation of methionine.

Table 5. Comparison of procedures of acid and enzyme hydrolysis for methionine estimation by the nitroprusside method[a]

Crop	Acid hydrolysis time (hr)				Enzyme hydrolysis time (hr)			
	4	8	16	24	2	4	8	16
Chickpea (cv G-130)								
Methionine (g/16g N)	0.67	0.84	0.70	0.71	0.92	1.26	1.28	1.20
Recovery (%)	50.52	70.54	68.30	59.51	90.78	96.15	94.74	93.60
Pigeonpea (cv ICP-1)								
Methionine (g/16g N)	0.50	0.74	0.52	0.54	0.86	1.16	1.09	1.14
Recovery (%)	58.40	74.03	66.56	60.38	94.46	95.00	95.10	92.45

a, Defatted dhal samples were used and results are averages of two determinations.

The enzyme papain was found to be quite suitable for the hydrolysis purpose. This enzyme is easily available, not expensive and does not contain methionine. The different buffer systems consisting of borate, phosphate and acetate solutions at pH 7.2 were studied and the results were compared with that of tris-buffer, pH 7.2. Results indicated that there was no interaction due to different buffer systems employed for enzyme hydrolysis (Table 6). In the absence of any significant advantages of other buffers, 0.1 M tris-buffer (pH 7.2) was followed for further analysis.

Table 6. Effect of different buffer systems on methionine estimation[a]

	0.1 M buffer (pH 7.2)			
Crop	Phosphate	Borate	Acetate	Tris
	..... Methionine (g/16g N)			
Chickpea (G-130)	1.28 ± 0.03	1.27 ± 0.04	1.30 ± 0.03	1.29 ± 0.02
Pigeonpea (ICP-1)	1.09 ± 0.02	1.04 ± 0.03	1.10 ± 0.03	1.12 ± 0.02

a, Analysis of dhal, Results are averages of five determinations.

Further attempts were made to study other favourable conditions for enzyme hydrolysis. Sodium cyanide is generally used as an enzyme activator. In our study, the use of this compound did not reveal any improvement in methionine values so the use of this compound was not considered. EDTA is used to bind heavy metals which would otherwise inactivate the enzyme by binding to the active sites. The use of mercaptoethanol has been recommended to improve the recovery of methionine by preventing the oxidation or loss of the -S- CH_3 group. Experiments were conducted to study the usefulness of these compounds in our assay procedure (Table 7). Even though there were no large differences in the methionine values when EDTA and mercaptoethanol were used, both these compounds were used as a precautionary measure.

Table 7. Effect of mercaptoethanol and ethylenediaminotetra-acetic acid (EDTA) on methionine estimation[a]

Crop	Control	Mercaptoethanol	EDTA
	.....	Methionine (g/16g N)	.....
Chickpea	1.28	1.29	1.29
(G-130)	± 0.03	± 0.03	± 0.02
Pigeonpea	1.09	1.10	1.09
(ICP-1)	± 0.02	± 0.03	± 0.03

a, Analysis of dhal, Results are averages of five determinations.

Different durations of enzyme hydrolysis were studied in order to find out their effects on methionine estimation. Results of such an experiment are presented in Table 5. No large differences in methionine values were observed except that the samples hydrolysed for 2 hr produced slightly lower values. Lower values for recovery assays were obtained when the hydrolysis was continued beyond 8 hr. This study suggested that prolonged period of hydrolysis was not necessary and enzyme hydrolysis for 4 hr was used for quantitative methionine estimation by nitroprusside reaction.

The nitroprusside reaction for methionine has a high degree of specificity. We have investigated the possible interference due to other sulphur containing amino acids such as cysteine, cysteic acid, methionine, and methionine sulfoxide. Only negligible amount of interference was noticed in case of methionine sulfone, methionine sulfoxide, cysteine, and cysteic acid. It has been reported that cystine and cysteine may complex with nitroprusside but the products are highly unstable and break down during a short period.

Using the standard assay conditions, the precision of this rapid procedure was determined and the results are shown in Table 8. The procedural error was more when the

determinations were carried out on different days as compared to those conducted on the same day. The coefficient of variation for the estimations that were carried out on different days was 6.48 and 4.80 for chickpea and pigeonpea respectively. However, the procedure appears to be more accurate for pigeonpea as compared to chickpea. One reason for this could be that in the case of pigeonpea, the filtrate was more clear than chickpea.

Finally this assay procedure was tested to know if it was suitable for the analysis of dhal and whole seed samples. Therefore, whole seed samples of cultivars having different testa colour were analysed and the values were compared with their respective dhal samples. Considerably higher values were obtained for whole seed samples which have darker seed colour in pigeonpea as shown in Table 9. This was also found to be true for chickpea. This shows some interference due to seed coat pigments suggesting that this procedure would be suitable for dhal samples only. Further, it was also observed that this procedure would be suitable for the analysis of defatted samples as undefatted samples did not yield a clear filtrate and this interfered in the colorimetric estimation.

Table 8. Standard errors and coefficients of variation of estimation of methionine (nitroprusside reaction) in chickpea and pigeonpea

Methionine	Chickpea (G-130)		Pigeonpea (ICP-1)	
	a	b	a	b
	..... Methionine (g/16g N)			
Min	1.04	0.91	0.98	0.99
Max	1.15	1.17	1.12	1.13
Mean	1.09	1.08	1.03	1.04
SE	0.06	0.07	0.04	0.05
CV %	5.50	6.48	3.88	4.80

a, Ten determinations on the same day; b, Ten determinations on different days during two weeks period.

Table 9. Effect of seed coat colour of pigeonpea on methionine estimation by the nitroprusside method

Cultivar	Seed colour	Protein (%)[a]		Methionine (g/16g N)	
		Dhal	Whole-seed	Dhal	Whole-seed
MY-3C	White	23.2	20.6	1.00	0.96
MP(WR)-15	White	26.1	23.0	1.07	0.79
DN-1	Light brown	26.0	21.9	0.86	1.20
-11	Brown	23.2	20.9	1.06	1.34

, Defatted sample; N x 6.25.

2.3 Estimation of cystine by Goa method:

Of the various analytical procedures available in the literature, Goa method for cystine estimation has been reported to work satisfactorily for several grain legumes (Goa, 1961). The procedure is based on the reduction of cystine or cysteine sulphur by hydrazine hydrate to hydrogen sulphide which is determined by the colorimetric method.

Cystine + Hydrazine --- $2H_2S$ + NH_3 + Unknown substances

This is obtained by leading the hydrogen sulphide formed by hydrozinolysis into a solution of bismuth nitrate whereby bismuth sulphide is formed and this is measured colorimetrically. This method was examined and modified suitably.

Reagents:

a. Hydrazine hydrate 99 - 100%

b. Bismuth nitrate solution: Dissolve 2.2 g bismuth nitrate pentahydrate in 250 ml of a 3.2 percent solution of mannitol in water. To this solution, add 80 ml glycerol and 360 ml of 2.5 percent solution of gum arabic in water. Dilute to 1 litre with 0.2 N acetate buffer (pH 4.4) and filter. The reagent at this pH was found to be stable for at least three weeks whereas the previously reported reagent of pH 5.2 was found stable only for 2-3 days.

c. Sulphuric acid 6 N.

Assay Procedure:

Defatted sample (40-50 mg) was weighed and transferred into a screw cap tube (15 x 100 mm). The sample was dispersed by adding 1.0 ml of distilled water and was followed by an addition of 2 ml of hydrazine hydrate (99-100%). Hydrazine hydrate should be added in such amounts so that the final concentration is 50 to 100%. Standard cystine solutions (0 to 500 ug) were treated similarly. The tube was stoppered tightly with a screw cap and heated at 120°C for 18 hr. After cooling, 1 ml water was added and the tube was connected to a glass manifold. The system was so designed that with the help of a continuous flow of nitrogen gas, hydrogen sulphide evolved on addition of sulphuric acid could be trapped into bismuth nitrate solution. The manifold had 8 outlets for the analysis of 8 samples at a time. Ten ml of bismuth nitrate reagent was placed and nitrogen gas was immediately passed through the system after adding 5 ml of 6 N sulphuric acid to the tubes containing the sample. Under these conditions, the liberated hydrogen sulphide gas was quantitatively taken into a bismuth nitrate reagent within 15 min. The yellow colour of the bismuth sulphide thus formed was read in a spectrophotometer at 400 nm while using bismuth reagent as a blank.

The important modification of this procedure was the hydrozinolysis of samples in screw cap tubes instead of using small ampules. After hydrozinolysis, the screw cap tube itself was connected to the aeration manifold. This modification had two important advantages: Firstly, a considerable amount of time was saved which would otherwise have been spent in transferring the material from the ampule into some other tube. Secondly, there is no loss of the hydrozinolysed material which otherwise could have occurred as a result of transfer of the material from an ampule to an aeration tube.

Another modification that was introduced in this procedure is the reduction of pH of bismuth nitrate solution from 5.2 to 4.4. The bismuth nitrate solution prepared in acetate buffer pH 4.4 was found to be quite stable for about three weeks whereas the same solution at pH 5.2 developed turbidity within 2-3 days. Cystine results were compared by using bismuth nitrate solutions of different pH buffers as shown in Table 10. No noticeable differences were observed as a result of use of different pH buffers. Keeping in mind the stability of bismuth nitrate solution, the acetate buffer of pH 4.4 was used.

Using this modified procedure, the recovery assays were conducted and the results were compared with the old procedure. It was noted that the recovery values for cystine were considerably lower in case of old procedure

Table 10. Effect of pH of bismuth nitrate solution on cystine estimation by the Goa procedure[a]

Cultivar	pH of acetate buffer		
	4.4	4.8	5.2
	... Cystine (g/16g N) ...		
L-550	1.20	1.18	1.21
Rabat	1.26	1.27	1.26
850-3/27	0.98	1.05	1.05
Annigeri	1.17	1.04	1.04
G-130	1.23	1.19	1.22

a, Results are averages of two determinations on defatted dhal.

than the modified procedure (Table 11). The higher recovery values could be attributed to the fact that no material was lost in case of modified procedure. The modified procedure was further examined and different durations of hydrozinolysis were studied. Increasing the time of hydrozinolysis up to 18 hr, resulted in higher cystine values (Table 12). Cystine values decreased considerably when the hydrozinolysis was continued beyond 24 hr.

Table 11. Comparison of procedures of hydrozinolysis for cystine estimation[a]

Crop	Goa Procedure	Modified Goa Procedure
	..... Cystine Recovery (%)	
Chickpea (G-130)	88.90	96.63
Pigeonpea (ICP-1)	90.53	98.75

a, Results are averages of four determinations on defatted dhal.

Although, a very slight reduction in cystine values was noted when samples were hydrozinolysed for 24 hr as compared to 18 hr, the hydrozinolysis period of 18 to 24 hr can be used for screening purpose. The results of our studies are reported based on 18 hr hydrozinolysis (Table 12).

The interference of other sulphur containing amino acids in the colorimetric estimation of cystine was examined, by adding these amino acids to the samples. The results showed that minimal interference was obtained when methionine sulfone, methionine sulfoxide and cysteic acid were present in the solution.

Table 12. Effect of duration of hydrozinolysis on the estimation of cystine by the modified Goa procedure[a]

Hydrozinolysis Time (hr)	Cystine (g/16g N)		Recovery (%)	
	Chickpea (G-130)	Pigeonpea (ICP-1)	Chickpea (G-130)	Pigeonpea (ICP-1)
6	0.84	0.98	60.35	54.53
12	0.96	1.03	69.76	80.40
18	1.32	1.12	93.84	97.56
24	1.32	1.13	92.00	96.18
30	1.30	1.10	86.57	91.27

a, Hydrozinolysis was carried out at 120°C using defatted dhal.

Pigment interference in colorimetric assay procedures is a well known fact. Since there was no direct involvement of seed coat pigment in the assay procedure described here, effect of seed coat content on the estimation of cystine was studied (Table 13). The amount of seed coat influenced the protein content of the seed. However, no large differences in the cystine content of dhal and whole seed were noticed when the results were expressed as g/16g N. This suggests that cystine analysis using this procedure could also be satisfactorily conducted for whole seed samples.

Precision of the method was worked out by calculating the standard error and coefficent of variation of cystine

estimation. Independent determinations were carried out on the same day and on different days. Coefficient of variation was higher when the samples were analysed on different days (Table 14). For determining accuracy of the method, 65 cultivars of pigeonpea and 57 cultivars of

Table 13. Effect of seed coat content on the estimation of cystine by the Goa method in chickpea[a]

Cultivar	Seed color	Seed coat (%)	Protein (%)		Cystine (g/16g N)	
			Dhal	Whole-seed	Dhal	Whole-seed
L-550	Salmon white	4.8	24.32	22.80	1.23	1.13
NP-34	Salmon white	14.6	25.80	23.76	1.18	1.05
JG-62	Light brown	15.1	22.45	19.58	1.30	1.16
P-6292	Brown	16.4	21.60	18.07	1.29	1.18
Kaka	Black	16.0	20.57	16.75	1.42	1.26
Hema	Green	15.8	25.67	22.54	1.10	0.97

a, Results are averages of two estimations.

chickpea were analysed by this method and results were compared with amino acid analyser (Table 15). The correlation coefficients between this rapid method and amino acid analyser method were 0.87** and 0.85** for chickpea and pigeonpea, respectively (Table 16). This suggests that this rapid method may be used for initial screening of the material depending on the objectives of the programme.

Table 14. Standard error and coefficient of variation of estimation of cystine (modified Goa procedure) in chickpea and pigeonpea

	Chickpea (G-130)		Pigeonpea (ICP-1)	
	a	b	a	b
	Cystine (g/16g N)			
Min	1.08	1.16	1.10	1.04
Max	1.30	1.40	1.35	1.26
Mean	1.22	1.29	1.20	1.16
SE	0.05	0.09	0.07	0.08
CV	4.46	6.89	5.67	7.10

a, Ten estimations were carried out on the same day; b, Ten estimations were carried out on different days during a two week period.

Table 15. Methionine and cystine values of chickpea and pigeonpea cultivars obtained by the amino acid analyser and rapid colorimetric procedure.

	Chickpea (n=57)				Pigeonpea (n=65)			
	Methionine		Cystine		Methionine		Cystine	
	a	b	a	b	a	b	a	b
	..... (g/16g N)							
Min	0.85	0.91	0.85	0.96	0.83	0.89	0.70	0.85
Max	1.40	1.43	1.47	1.48	1.30	1.28	1.34	1.31
Mean	1.17	1.20	1.16	1.19	1.05	1.08	0.99	1.05

a, Analysis of defatted dhal samples; n = number of cultivars analysed; a - Ion exchange chromatography (amino acid analyser); b - Colorimetric procedures for methionine (Nitroprusside reaction) and cystine (Goa procedure).

In order to know the accuracy of this procedure for chickpea and pigeonpea, 65 cultivars of pigeonpea (Appendix-3) dhal samples representing different maturity groups and 57 cultivárs of chickpea dhal (Appendix-2) samples were analysed for methionine content by the colorimetric procedure and the results were compared statistically with those obtained by an amino acid analyser. This study showed a considerable variation for methionine among these cultivars. In the case of chickpea, methionine (g/16g N) ranged between 0.91 and 1.43 with a mean value of 1.20 (Table 15) when samples were analysed by the nitroprusside procedure. A similar variation was observed when the samples were analysed using the amino acid analyser. However, mean values were slightly lower in the case of amino acid analyser. Similar results were obtained when pigeonpea samples were analysed using the amino acid analyser and nitroprusside procedures. Statistical comparison of these methods indicated significant correlations for chickpea (0.92**) and pigeonpea (0.93**) as given in Table 16. Standard errors of estimate were 0.54 and 0.57 for chickpea and pigeonpea respectively. This study indicated that rapid colorimetric procedure for methionine estimation can be used satisfactorily for screening purpose.

Table 16. Correlation coefficients between rapid colorimetric and amino acid analyser procedures for methionine and cystine in chickpea and pigeonpea[a]

Crop	Correlation coefficient	Syx	Regression Equation
Chickpea (n=57)			
Methionine	0.92**	0.54	Y = 0.09 + 0.86 x
Cystine	0.87**	0.62	Y = 0.74 + 0.38 x
Pigeonpea (n=65)			
Methionine	0.93**	0.57	Y = -1.13 + 1.97 x
Cystine	0.85**	0.63	Y = -0.85 + 1.65 x

a, Analysis of defatted dhal samples; n = number of cultivars analysed; ** Significant at 1% level.

3. Relationship between total sulphur and sulphur amino acids:

Total sulphur content has been suggested as an indicator of sulphur amino acids in some grain legumes, whereas for others a poor correlation has been reported. In order to ascertain if total sulphur content could be used as a screening technique for sulphur amino acids in chickpea and pigeonpea, the relationship between these variables was studied using defatted dhal samples of 30 cultivars of chickpea and 24 cultivars of pigeonpea. Methionine and cystine were estimated according to the method of performic

acid oxidation as described earlier. Total sulphur was determined by the procedures as described below. We have also published data concerning this aspect (Jambunathan and Singh, 1981).

3.1 Methods of total sulphur estimation:

In order to ensure that total sulphur was determined accurately two methods were compared. Total sulphur was determined by the wet digestion method as follows: A suitable amount of sample (1 g) was digested with 10 ml of nitric acid in a Tecator digestion tube (250 ml) for 30 min at 100 °C in a block digestor. After cooling, 6 ml of 70 percent perchloric acid was added and digestion continued for 60 min at 235 °C. The contents were allowed to cool and 10 ml of 6 N HCl was added before making volume to 250 ml. To 15 ml of this aliquot, 250 mg of finely ground barium chloride was added and after mild shaking for 10 min, the percent transmittance (T) of the turbid suspension was measured at 420 nm in a spectronic-20 spectrophotometer. The quantity of sulphate in the aliquot was read from a standard graph prepared by using potassium sulphate.

Total sulphur content of the dhal samples was also determined by the Leco Sulphur Analyser (Leco Corporation, St. Joseph, Michigan, USA). Samples were subjected to combustion in a stream of oxygen and the released sulphur dioxide was measured colorimetrically according to the procedure described in the manual. Recovery experiments using methionine and cystine were also carried out by both the methods.

The standard errors and coefficients of variation of the wet digestion and the Leco analyser procedures are shown in Table 17. The means, standard errors and coefficients of variation were higher for the Leco sulphur analyser than

Table 17. Sulphur estimation by the wet digestion method and the Leco sulphur analyser (g/100g meal): standard errors and coefficients of variation[a]

	Wet digestion		Leco analyser	
	Chickpea	Pigoenpea	Chickpea	Pigeonpea
Minimum	0.205	0.125	0.217	0.133
Maximum	0.225	0.150	0.251	0.162
Mean	0.215	0.136	0.238	0.146
SE ±	0.007	0.006	0.014	0.013
CV (%)	3.16	4.48	5.60	8.92

a, Mean values of 10 determinations.

for the wet digestion method but the results obtained by both the methods were highly correlated (r = 0.94**) with each other. The results of recovery experiments are shown in Table 18. Methionine gave a slightly lower recovery by the wet digestion procedure. Cystine, and methionine together with cystine gave excellent recoveries by both the methods. Based on these findings, the wet digestion method results were used for correlation studies between total sulphur and sulphur amino acids.

Table 18. Recovery of sulphur from methionine and cystine[a]

	Wet digestion		Leco analyser	
Compound	Chickpea	Pigeonpea	Chickpea	Pigeonpea
	..... Recovery (%)			
Methionine	91.3 ± 5.7	91.6 ± 3.5	95.4 ± 3.2	97.3 ± 4.7
Cystine	99.9 ± 3.6	96.5 ± 4.0	97.1 ± 4.6	97.2 ± 5.3
Methionine and Cystine	94.3 ± 4.4	101.0 ± 6.2	98.8 ± 6.6	97.8 ± 4.0

a, Mean values of 8 determinations.

3.2. Variation in total sulphur and sulphur amino acids:

The individual values of total sulphur, methionine, cystine and the sulphur content of sulphur amino acids in relation to the total sulphur content in 30 chickpea cultivars are shown in Appendix 4. Total sulphur as percent

of samples varied between 0.17 and 0.27 with a mean value of 0.22, showing a difference of about 57% between the lowest and highest values. Total sulphur as percent of protein varied between 0.82 and 1.41 with a mean value of 1.13 and the relative difference between the lowest and highest value was about 72%. Total sulphur amino acids as percent of protein varied between 2.02 and 2.63 with a mean of 2.31 while the values expressed as percent of sample were found to vary between 0.36 and 0.57, the variation being about 57%. The sulphur content of methionine and cystine acounted for 54.8% of total sulphur while the individual values ranged between 41.0 and 67.6%. This indicated that a considerable amount of the total sulphur was present in forms other than sulphur amino acids.

A comparison of total sulphur, methionine, cystine and the sulphur content of sulphur amino acids, in relation to the total sulphur content in 24 pigeonpea cultivars are shown in Appendix 5. Total sulphur as percent of pigeonpea sample varied between 0.14 and 0.19, the variation being about 30 percent between the lowest and highest values. Total sulphur amino acids as percent of protein varied between 1.76 and 2.55 with a mean of 2.11. When expressed as percent of sample they varied between 0.38 and 0.57 with a mean of 0.47, the variation being about 50%. The amount of sulphur in methionine and cystine together accounted for 75.5% of total sulphur, ranging from 59.2 and 84.6%.

Chickpea showed a larger variation in protein content than pigeonpea, though the mean protein content of chickpea was lower. The mean values for total sulphur expressed as percent of sample (0.22) or as percent of protein (1.13) (indicated above) were higher in chickpea than in pigeonpea which had the values 0.17 and 0.74 respectively. However, the two species did not differ much in the mean values for sulphur amino acids expressed either as percent of sample or as percent of protein. Also, the sulphur content of methionine and cystine accounted for a higher proportion of the total sulphur in pigeonpea (75.5%) than in chickpea (54.8%). It was obvious that both crops had considerable amounts of other sulphur compounds in addition to methionine and cystine and apparently chickpea had higher extraneous sulphur compounds.

3.3. Correlation between total sulphur and sulphur amino acids:

The correlation coefficients between total sulphur and sulphur amino acids of chickpea and pigeonpea are shown in Table 19. In chickpea, on a whole sample basis, the percentage protein and total sulphur were significantly and positively correlated with percentage cystine, methionine and cystine plus methionine and with each other. The correlation between percentage protein and cystine plus methionine was 0.809**, indicating that about 65% variation in these amino acids can be attributed to the levels of protein in the sample. When expressed as percentage

Table 19. Correlation coefficients among protein, total sulphur and sulphur amino acids in 30 chickpea and 24 pigeonpea cultivars.

	Protein (%)	Cystine (g/100 g sample)	Methionine (g/100 g sample)	Cystine+ Methionine (g/100 g sample)	Cystine (g/16g N)	Methionine (g/16g N)	Cystine+ Methionine (g/16g N)
Chickpea							
Protein (%)	--	0.719**	0.845**	0.809**	-0.309	-0.645**	-0.487
Total sulphur (g/100g sample)	0.476**	0.402*	0.578**	0.494**	0.043	0.094	0.016
Total sulphur (g/100g protein)	-0.611**	-0.460**	-0.361	0.466**	0.390**	0.612**	0.522**
Pigeonpea							
Protein (%)	--	0.269	0.489*	0.392*	-0.308	-0.262	-0.214
Total sulphur (g/100g sample)	-0.150	0.554**	0.453*	0.534**	0.616**	0.612**	0.651**
Total sulphur (g/100g protein)	-0.745**	0.175	-0.064	0.096	0.593**	0.458**	0.566**

* Significant at 5% level; ** Significant at 1% level.

of protein in sample, the correlation of protein with methionine was significant and negative while with cystine and cystine plus methionine, it was negative but insignificant. The correlation of total sulphur as percent of sample with cystine, methionine, cystine plus methionine as percent of protein was insignificant indicating that any rapid method of estimating total sulphur may not yield reliable information on the sulphur amino acid contents of the sample. However, as the number of cultivars tested in this study was small, further evaluation with more number of samples is necessary to ascertain these observations.

In pigeonpea, total sulphur was correlated with cystine, methionine and cystine plus methionine on whole sample basis while protein was correlated with methionine and to a lesser magnitude ($r = 0.392^{*}$) with cystine plus methionine.

When expressed as a percentage of protein in the sample, the correlation of protein with sulphur amino acids was insignificant. Total sulphur as percent of sample showed a significant positive correlation with cystine, methionine and cystine plus methionine when expressed as percent of protein. This differs from the results obtained with chickpea and indicates the possibility of using total

sulphur content as an index of sulphur amino acids in pigeonpea. This needs to be further verified with large numbers of samples.

4. Evaluation of rapid methods of tryptophan estimation:

Tryptophan has been reported to be the second limiting essential amino acid in chickpea and pigeonpea. This amino acid is, therefore, of considerable importance in breeding programmes that are aimed at improving the protein quality of these food legumes.

Tryptophan, however, is destroyed by acid hydrolysis even under conditions that are best suited for other amino acids. Therefore, several methods have been developed for its determination and among the various procedures, alkaline hydrolysis, biological and enzymatic assays, various colorimetric methods have been employed for different crop material and have been published (Hugli and Moore, 1972., Rama Rao et al., 1974., Lewis et al., 1976., and Concon, 1975).

4.1. Methods of tryptophan estimation:

The following two rapid colorimetric methods of tryptophan estimation were examined and compared with the

standard method to find out their suitability in terms of accuracy and rapidity of the procedures for screening purpose in chickpea and pigeonpea.

(a) Standard conventional method (Method 1):

The method involving the principle of ion exchange chromatography after alkaline hydrolysis was followed (Hugli and Moore, 1972) and designated as Method I.

Procedure:

Finely ground defatted sample (100 to 150 mg) was taken in a polypropylene tube and 3 ml of 5 N NaOH was added. The polypropylene tube was kept inside another glass tube which was sealed after evacuation. Then the tube was kept in an oven for hydroysis for 24 hr at 110°C. The hydrolysate was adjusted to pH 4.25 and tryptophan was determined by the ion exchange chromatography procedure using the amino acid analyser.

(b) Estimation of tryptophan by Concon procedure (Method 2):

This method is based on the principle of Hopkins Cole-reaction wherein iron in the presence of sulphuric acid converts acetic acid to glyoxylic acid that in turn reacts with biological material (Friedman and Finely, 1971).

Reagents:

Acetic acid - ferric chloride solution: Dissolve 0.54 g of ferric chloride in 1.0 ml of water containing a few drops of acetic acid to prevent the formation of insoluble ferrous hydroxide. To 0.5 ml of this solution, glacial acetic acid containing 2% acetic anhydride was added, to a final volume of one litre. This reagent is stable indefinitely. Sulphuric acid (25.8 N) and sodium hydroxide (0.075 N) were also prepared.

Procedure:

For extraction of protein, defatted sample (100 to 150 mg) was weighed in a test tube. Ten ml of 0.075 N sodium hydroxide was added and the sample was mixed well on a vortex mixer. The tube was then shaken on a mechanical shaker for 1 hr. Then the content was centrifuged (12,000 x g for 15 min) and the supernatant was saved. Protein in the supernatant was determined by the microKjeldahl method. To one ml protein extract in a test tube, 3 ml of glacial acetic acid - ferric chloride solution was added. To this 2.0 ml of 25.8 N sulphuric acid was added rapidly and mixed well to a homogenous solution. The colour developed was stabilized by incubating the sample at 60 °C for 45 min. Then the sample was cooled to room temperature in an ice-water bath and the absorbance was recorded at 545 nm against the reagent blank. For preparing

a standard curve, different concentrations of standard tryptophan solutions (0-40.0 ug/ml) were also treated in a similar way.

(c) Determination of tryptophan by the Spies and Chamber Procedure (Method 3):

This method is based on the estimation of tryptophan by its reaction with p-dimethylaminobenzaldehyde (DAB) and subsequent development of a blue colour by oxidation with sodium nitrite.

Reagents: P-dimethylaminobenzaldehyde (DAB),
Sulphuric acid, 19 N
Sodium nitrite, 0.045%

Procedure:

Suitable amount of sample (20-25 mg) was taken in a test tube. Then 10 ml of solution of dimethyl aminobenzaldehyde (3 mg/ml in 19 N sulphuric acid) was added and the sample was incubated in dark at room temperature (25 $\pm$ 2°C) for 18 hr. After incubation, 0.1 ml of 0.045 percent solution of sodium nitrite was added and content was shaken to a homogenous mixture. After keeping the solutions at room temperature for 30 min, readings were taken at 590 nm in spectronic-21 spectrophotometer. Standard tryptophan solutions (0 to 120 ug/ml) were analysed in a similar way and a standard curve was prepared.

In order to test if Methods 2 and 3 described above can be used for the estimation of both protein bound and free tryptophan, enzymatic hydrolysis of pigeonpea and chickpea samples was carried out as mentioned below. To 200 mg defatted sample 15 ml of pronase (Sigma Chem. Co., USA) solution (0.4 mg/ml) in 0.1 M phosphate buffer (pH 7.5) was added. A few drops of toluene were added to prevent the microbial growth. Enzymatic hydrolysis was carried out for 24, 48 and 72 hr to know the optimum time of hydrolysis. The sample was then centrifuged at 12,000 x g for 15 min and the protein content in the supernatant was determined by the microKjeldahl method. Recovery of added tryptophan was also calculated.

The usefulness of any method for screening purpose can be evaluated in terms of its rapidity and simplicity within the acceptable limits of accuracy and precision. Also, the cost of analysis per sample should be taken into consideration while working out the suitability of a procedure for screening large numbers of samples. It should be mentioned here that analysis of samples by Method I is very costly, slow and cumbersome, whereas Method 2 and Method 3 are simple and rapid and also the cost of analysis per sample is substantially lower than the Method I. More number of analyses per person per day are possible by using the Method 3 as compared to Method 2.

4.2. Comparison of different methods of tryptophan determination:

Keeping in view, the simplicity and rapidity of Methods 2 and 3 some assay conditions for these procedures were examined. Values obtained on protein bound and free tryptophan by both these methods were estimated after enzymatic hydrolysis as described above. Different durations of enzymatic hydrolysis were also studied. No large differences were observed when the tryptophan estimation was carried out on enzyme hydrolysates and both the methods gave similar results. In the case of pigeonpea and chickpea enzyme hydrolysis produced slightly higher results when assayed by Method 2 (Table 20). It is interesting to note that the tryptophan values obtained on unhydrolysed and hydrolysed samples of chickpea and pigeonpea were similar. This suggests that enzyme hydrolysis was not a necessary prerequisite for these two methods.

In the case of Method 2, the use of acetic anhydride in the reaction mixture is important as traces of water in the acetic acid will affect the tryptophan values. Higher values for tryptophan were obtained when acetic anhydride was not used in the reagent (Table 21). Tryptophan values

Table 20. Effect of hydrolysis on tryptophan values (g/16g N) obtained by Methods 2 and 3[a]

Method	Protein bound tryptophan (unhydrolysed)	Enzyme hydrolysis for different durations (hr)		
		24	48	72
Method 2:				
Chickpea (G-130)	1.16 (98.7)	1.28 (104.4)	1.26 (97.5)	1.30 (100.8)
Pigeonpea (ICP-1)	0.98 (100.6)	1.17 (99.2)	1.18 (102.5)	1.17 (96.7)
Method 3:				
Chickpea (G-130)	1.09 (104.5)	1.12 (116.3)	1.05 (110.8)	0.94 (108.9)
Pigeonpea (ICP-1)	0.96 (97.8)	0.89 (102.2)	0.75 (98.3)	0.90 (100.5)

a, Analysis of defatted dhal sample, tryptophan values (g/16g N) are averages of three determinations; Values within parenthesis show the recovery (%).

Table 21. Effect of acetic anhydride on the estimation of tryptophan by Method 2.

	Acetic anhydride conc. (%)	Tryptophan (g/16g N)		Recovery (%)	
		Range	Mean	Range	Mean
Pigeonpea[a]	0	1.09-1.11	1.10	118.4-122.1	120.3
(ICP-1)	1	0.79-0.82	0.81	107.4-108.9	108.0
	2	0.74-0.77	0.76	99.9-103.6	101.5
	3	0.68-0.69	0.69	95.0-98.3	96.6
Chickpea[a]	0	1.54-1.58	1.56	116.3-118.7	117.2
(L-550)	1	1.30-1.32	1.31	106.8-108.3	107.6
	2	1.24-1.27	1.26	99.5-100.8	110.4
	3	1.23-1.24	1.24	95.3-97.6	96.1

a, Defatted dhal samples.

decreased when the concentration of acetic anhydride was increased and this was found to be the case for both chickpea and pigeonpea. Minimal concentration of acetic anhydride which resulted in almost complete recovery of added tryptophan was observed to be 2% and this concentration was used in all our procedures.

The concentration of P-dimethylaminobenzaldehyde (DAB) plays an important role in the estimation of tryptophan by Method 3. Different concentrations of DAB were examined. Tryptophan values increased up to a concentration of 2 mg/ml

Table 22. Effect of concentration of p-dimethylaminobenzaldehyde on the estimation of tryptophan by Method 3[a]

DAB conc. (mg/ml)	Tryptophan (g/16g N)	
	Chickpea (G-130)	Pigeonpea (ICP-1)
1	0.86	0.60
2	1.12	0.75
3	1.13	0.74
4	1.08	0.73
5	1.10	0.75

a, Results are averages of two estimations.

reaction mixture. Higher concentration of DAB did not result in any measurable increase in tryptophan value (Table 22). Based on these results, DAB concentration of 3 mg/ml reaction mixture is suggested for tryptophan estimation in chickpea and pigeonpea. In the procedure described, it is required that meal samples should be kept overnight in DAB solution to solubilize proteins to form a stable complex with tryptophan. Results obtained with different incubation periods showed that tryptophan values increased up to an incubation period of 20 hr and afterwards there was no change (Table 23). The concentration of sodium nitrite which is used as an oxidizing agent in this procedure also plays an important role. Different concentrations of sodium nitrite were studied and a concentration of 0.045% was found to be satisfactory for

Table 23. Effect of incubation periods on the estimation of tryptophan by Method 3[a]

Incubation period (hr)	Tryptophan (g/16g N)	
	Chickpea (G-130)	Pigeonpea (ICP-1)
8	0.61	0.43
16	1.03	0.74
20	1.10	0.75
24	1.08	0.75

a, Results are averages of two estimations on defatted dhal

both chickpea and pigeonpea. Studies were also conducted to find out the error of estimation of tryptophan by Methods 2 and 3 in comparison with the standard Method I (Table 24). The coefficients of variation ranged from 1.69 for Method 2 to 5.11 for Method I. The data further show that reproducibility of the Method 3 was slightly lower than Method 2. Tryptophan recovery assays were conducted using these three methods. A considerable variation in percent recovery values was observed between Method 2 and Method 3. While recovery results obtained by Method I and Method 3 were comparable, lower recovery values was obtained in case of chickpea for Method 2 (Table 25).

Table 24. Precision of different methods used for the estimation of tryptophan in chickpea and pigeonpea[a]

Method	n	Range	Mean	S.E.	C.V. (%)
		...(g/16g N) ...			
Chickpea (G-130):					
Method I	9	0.94 - 1.03	0.98	0.033	3.35
Method 2	16	0.92 - 0.98	0.95	0.016	1.70
Method 3	12	0.85 - 0.96	0.90	0.032	3.56
Pigeonpea (ICP-1):					
Method I	17	0.70 - 0.81	0.75	0.038	5.11
Method 2	14	0.73 - 0.76	0.75	0.013	1.69
Method 3	20	0.63 - 0.71	0.67	0.023	3.42

a, Analysis of defatted dhal sample.

Forty cultivars of chickpea (Appendix-6) and 5[illegible] cultivars of pigeonpea (Appendix-7) were analysed for tryptophan using these methods. A large variation in tryptophan content appeared to exist among the cultivars of pigeonpea and chickpea (Table 26). The result of correlation coefficients standard errors of estimation and regression equation obtained between the Method I and the other two methods are shown in Table 27. In the case of chickpea, Method I was significantly correlated with Method 2 (r= 0.87**) and Method 3 (r= 0.79**). However, it

Table 25. Recovery of added tryptophan obtained by different methods in chickpea and pigeonpea

Method	No of estimations	Recovery (%) Range	Mean
Chickpea:			
Method I	5	95.8 - 102.5	100.2
Method 2	4	91.8 - 93.5	93.0
Method 3	5	98.8 - 100.7	99.9
Pigeonpea:			
Method I	5	103.8 - 106.1	105.0
Method 2	4	93.8 - 105.5	99.4
Method 3	4	99.9 - 103.6	101.5

was observed that Method 2 produced results with higher standard errors of estimation in comparison with Method 3. Correlation coefficient between Method 2 and Method 3 were higher than those between Method 1, and rapid methods (Method 2 and Method 3). Similar observation was obtained for pigeonpea that indicate a close agreement between the standard method and these rapids tested. This might have happened due to slightly higher error of determination of the standard method itself as shown in Table 24. These

Table 26. Tryptophan content of chickpea and pigeonpea analysed by three different methods

Crop	Protein (%)		Tryptophan (g/16g N)	
	Range	Mean	Range	Mean
Chickpea (n=40)				
Method I	19.8 - 29.9	24.0	0.85 - 1.64	1.15
Method 2	"	"	0.80 - 1.67	1.16
Method 3	"	"	0.78 - 1.53	1.14
Pigeonpea (n=54)				
Method I	18.8 - 26.9	23.5	0.64 - 0.93	0.74
Method 2	"	"	0.65 - 0.89	0.75
Method 3	"	"	0.64 - 0.95	0.74

n = number of cultivars analysed using defatted dhal sample.

studies clearly show that Method 2 and Method 3 are comparable with each other and the tryptophan value obtained by either of these two methods do not differ significantly from those of the standard method. Therefore, it seems that both Method 2 and Method 3 can be used for screening purpose. Considering the simplicity and rapidity of the procedure, Method 3 is suggested for screening large numbers of samples.

Table 27. Statistics for comparing the degree of correlation between rapid methods and standard method for the estimation of tryptophan content

Crop	Method	Correlation coefficient	Syx	Regression equation
Chickpea	1 vs 2	0.87**	0.67	y = 0.23 + 0.71x
	1 vs 3	0.79**	0.59	y = 0.35 + 0.68x
	2 vs 3	0.92**	0.47	y = 0.52 + 0.42x
Pigeonpea	1 vs 2	0.85**	0.55	y = 0.41 + 0.54x
	1 vs 3	0.81**	0.56	y = 0.18 + 0.79x
	2 vs 3	0.90**	0.53	y = 0.48 + 0.50x

** Significant at 1% level.

5. Relationship between protein content and amino acids in chickpea and pigeonpea:

In cereal grains, an inverse relationship between protein content and its quality, particularly in terms of lysine levels, has received much attention. This relationship is due to the observation that during

maturation of cereal seeds, proteins which are deficient in limiting essential amino acids (eg. lysine) are deposited in the grains. So far as grain legumes are concerned, this aspect has not been thoroughly investigated. The relationship between protein content and essential limiting amino acids of chickpea and pigeonpea was studied and several cultivars of chickpea and pigeonpea were analysed for these constituents.

A lot of 80 chickpea germplasm accessions grown during 1977-78 were analysed for methionine content by the microbiological method (Appendix-8). Protein content was determined by the TAA procedure which ranged from 15.2 to 29.6 percent as shown in Table 28. Methionine (g/100g meal) ranged between 0.21 and 0.41 with a mean value of 0.29 and methionine as g/16g N varied from 1.10 to 1.63 with mean being 1.29 (Table 28).

Protein content was positively and significantly correlated (r= 0.861**) with methionine content as percent of meal (g/100g meal), but a significant negative correlation (r= -0.451**) was obtained between protein content and methionine as percent of protein (g/16g N).

The significant positive relationship between methionine (g/100g meal) and protein content indicates that about 74 percent of the variation in methionine content may be due to the variation in protein. The implication of this

Table 28. Methionine and protein content of 80 chickpea germplasm lines grown during 1977-78

	Minimum	Maximum	Mean	Correlation coefficient between protein and methionine
Protein (%)	15.2	29.6	22.5	-
Methionine (g/100g sample)	0.21	0.41	0.29	+ 0.861**
Methionine (g/16g N)	1.12	1.51	1.29	- 0.451**

** Significant at 1% level.

observation is that the variation in methionine content may appear larger if the results are expressed as methionine content g/100g meal. In order to investigate the genetic variability for this trait in the germplasm collection, it is suggested that methionine be expressed as g/16g N. A similar relationship was found to exist in the case of pigeonpea as well.

5.1 Relationship between methionine and cystine contents:

Thirty cultivars of chickpea (Appendix-3) and 24 cultivars of pigeonpea (Appendix-4) grown during 1978-79 were analysed for protein content and sulphur amino acids. Methionine and cystine were determined by PAO method using the amino acid analyser and protein was determined by TAA procedure. As shown in Table 29, while nonsignificant negative correlation between cystine (g/16g N) and protein was observed, methionine, and cystine plus methionine when expressed as g/16g N showed significant and negative correlations with protein content in case of chickpea. Surprisingly, no significant correlation was obtained between protein content and these amino acids in case of pigeonpea even though there was a large variation in the protein content (19.4-28.0%) among the cultivars. This observation indicated that chickpea and pigeonpea differ from each other as far as the relationship between protein and sulphur containing amino acids are concerned.

Using the same data, information was obtained on the relationship between cystine and methionine. Methionine, cystine, and methionine plus cystine together when expressed

Table 29. Correlation coefficients between cystine and methionine in 30 chickpea and 24 pigeonpea cultivars grown during 1978-79.

	Correlation coefficients				
	Methionine	Cystine+ Methionine	Cystine	Methionine	Cystine+ Methionine
	(g/100g sample)			(g/16g N)	
Chickpea					
Cystine[a]	0.756**	0.969**	0.201	-0.277	0.009
Methionine[a]	--	0.893**	0.079	-0.148	-0.130
Cystine[b]	--	--	--	0.686**	0.941**
Methionine[b]	--	--	--	--	0.890**
Pigeonpea					
Cystine[a]	0.801**	0.956**	0.829**	0.743**	0.956**
Methionine[a]	--	0.940**	0.516**	0.838**	0.693**
Cystine[b]	--	--	--	0.780**	0.958**
Methionine[b]	--	--	--	--	0.926**

a, g/100g sample; b, g/16g N; ** Significant at 1% level.

as percent of protein or as percent of sample showed highly significant correlations with and among each other (Table 29). Methionine and cystine when expressed as percent of sample were significantly correlated with each other for both chickpea ($r= 0.756^{**}$) and pigeonpea ($r= 0.801^{**}$). When expressed as percent of protein, methionine was significantly correlated with cystine in chickpea ($r= 0.686$) as well as in pigeonpea ($r= 0.780$). Moreover, cystine and methionine were correlated with each other when expressed either as percent of protein or percent of sample.

In order to confirm these results, cultivars grown during 1979-80 were analysed for these amino acids and the results were examined for the relationship between protein and amino acids as summarised in Table 30. The analysis of these cultivars has already been discussed in this report. These cultivars showed a large variation in their protein contents in case of both chickpea and pigeonpea (Appendix-9). In the case of chickpea, the protein content was highly and significantly correlated with cystine ($r= -0.854^{**}$), methionine ($r= -0.700^{**}$) and tryptophan ($r= -0.671^{**}$) (Table 30). The correlation coefficients between these amino acids and protein were significant though lower in magnitude in the case of pigeonpea. Again, methionine and cystine were found to be highly and

Table 30. Relationships between cystine, methionine, tryptophan, and protein in chickpea and pigeonpea cultivars grown during 1979-80.

	Correlation coefficients[a]			
	Methionine	Cys+Met	Tryptophan	Protein (%)
Chickpea (n=57)				
Cystine	0.796**	0.941**	-0.186	-0.854**
Methionine	-	0.943**	0.201	-0.700**
Cys + Met	-	-	0.105	-0.813**
Tryptophan[b]	-	-	-	-0.671**
Pigeonpea (n=65)				
Cystine	0.535**	0.895**	0.201	-0.438**
Methionine	-	0,849**	0.162	-0.294**
Cys + Met	-	-	0.085	-0.423**
Tryptophan[b]	-	-	-	-0.564**

** Significant at 1% level; a, Results are based on g/16g N of amino acids; b, Chickpea (n=40), Pigeonpea (n=54).

significantly correlated with each other for both chickpea and pigeonpea cultivars. The tryptophan results showed no significant correlations with cystine or methionine content in case of both chickpea and pigeonpea.

Based on these observations following conclusions can be drawn. Increase in protein content will be accompanied by a decrease in the levels of sulphur amino acids and tryptophan in the case of chickpea, but this may not be the case with pigeonpea. Estimation of either of the two sulphur amino acids would be sufficient for screening large numbers of samples depending on the facility available for this purpose. No relationship exists between tryptophan and sulphur amino acids for chickpea and pigeonpea.

6. Variability for amino acids in chickpea and pigeonpea:

6.1. Varietal differences in the amino acid composition of chickpea seed proteins:

Considerable variation appears to exist in the amino acid composition of chickpea cultivars. The cultivars studied differed with respect to the levels of essential and nonessential amino acids (Table 31). Sulphur amino acids, methionine and cystine when considered together were the first limiting amino acids of chickpea and pigeonpea and ranged between 1.90 and 2.53 g/16g N among these cultivars. Tryptophan values varied from 0.99 to 1.22 g/16g N and this showed that some of these cultivars were not deficient in this amino acid. Although the legumes are a rich source

Table 31. Amino acid composition (g/16g N) of ten cultivars of chickpea

Amino acid	JG-62	Pyroz	NEC-240	NEC-1639	USA 613	L-550	NEC-34	Laba-nese	NEG-10	NEC-143
Lysine	6.70	6.82	6.89	5.64	6.55	7.54	6.96	7.22	6.39	5.84
Histidine	2.37	2.23	2.28	2.29	2.43	2.40	2.57	2.52	2.09	1.97
Arginine	9.14	7.86	8.34	8.97	8.56	7.73	9.08	9.43	7.70	7.49
Aspartic acid	9.80	10.28	10.52	9.72	10.95	11.00	11.69	11.59	12.47	10.83
Threonine	3.21	3.64	3.61	3.24	3.63	3.79	3.85	3.96	3.77	3.31
Serine	4.57	4.78	4.85	4.53	4.88	4.96	5.17	5.30	5.02	4.50
Glutamic acid	15.75	16.11	15.94	15.25	16.28	16.79	17.61	17.52	16.97	16.63
Proline	3.55	3.91	4.17	3.64	3.94	4.02	4.16	4.19	3.98	3.67
Glycine	3.72	4.00	4.13	3.67	4.14	4.21	4.35	4.93	4.27	3.88
Alanine	3.77	4.07	4.20	3.76	4.22	4.31	4.4	4.57	4.36	3.92
Cystine	0.98	1.32	1.27	0.92	1.17	1.19	1.12	1.23	1.07	1.05
Valine	3.94	4.32	4.32	3.70	4.27	4.36	4.46	4.68	4.49	4.03
Methionine	0.92	1.21	1.21	1.15	1.30	1.29	1.30	1.30	1.10	0.90
Isoleucine	3.80	4.01	4.14	3.71	4.17	4.21	4.34	4.38	4.31	3.99
Leucine	7.37	7.57	7.70	6.99	7.87	8.06	8.13	8.36	8.10	7.34
Tyrosine	2.63	3.02	3.01	2.53	2.86	3.00	3.02	3.12	3.01	2.68
Phenylalanine	4.97	5.31	5.45	4.75	5.26	5.34	5.73	5.87	5.61	5.24
Tryptophan	1.06	1.10	1.05	0.99	1.06	1.22	1.21	1.09	1.13	1.04
Protein (%)[a]	22.28	19.39	22.02	23.94	20.84	19.09	21.30	21.40	21.38	24.12

a, Defatted and moisture free, N x 6.25.

of lysine, it remains an important amino acid in cereal/legume diets. Lysine content (g/16g N) of these cultivars ranged from 5.64 to 7.54 (Table 31) and this indicates that there are cultivars with lower levels of this amino acid. Besides these amino acids, thereonine is another amino acid of considerable interest as far as chickpea is concerned. Some workers have also reported this amino acid as the second limiting amino acid of some cultivars of chickpea. From this study, it is also noted that the seed proteins of all the cultivars are deficient in threonine, the value for which ranged between 3.21 and 3.96 g/16g N. Some differences in the levels of other amino acids were also observed (Table 31).

Some wild species of genus Cicer were also studied for their amino acid composition (Table 32). This attempt was made to know whether the amino acid profiles of different wild species differ from that of the cultivated species. Lysine values (g/16g N) ranged between 6.22 and 7.37 among the wild species whereas the cultivated one had 6.95 when compared under similar conditions. Sulphur amino acids, methionine and cystine, when considered together varied from 2.12 to 3.42 g/16g N for wild species whereas the cultivated species had 2.38 g/16g N.

Table 32. Amino acid composition (g/16g N) of wild species of chickpea

Amino acid	Cicer arietinum	C.biju-gum	C.choras-sanicum	C.cune-atum	C.juda-icum	C.mont-bretti	C.pun-gens	C.reticu-latum	C.yama-shitae	Cicer arieti num (cv. G-130)
Lysine	6.98	7.37	6.90	7.02	6.22	7.16	6.78	6.38	6.81	6.95
Histidine	2.35	2.42	2.39	2.34	2.35	2.16	2.40	2.50	2.56	2.25
Arginine	8.71	11.05	9.57	7.29	7.85	8.36	8.98	8.49	9.78	8.78
Aspartic acid	12.08	11.06	9.75	10.76	11.42	10.69	11.52	11.06	11.55	11.34
Threonine	3.50	3.06	2.62	3.64	3.44	3.12	3.45	3.31	3.43	3.55
Serine	5.11	4.57	3.24	4.69	4.59	4.50	4.97	4.92	5.08	4.96
Glutamic acid	22.80	19.18	21.65	20.24	20.30	19.40	21.09	19.33	22.38	21.80
Proline	4.14	3.96	4.01	3.61	3.83	3.25	4.06	3.55	3.98	4.30
Glycine	4.14	4.28	4.22	4.21	4.31	4.20	5.09	3.95	4.21	4.84
Alanine	4.85	4.31	5.09	4.43	4.41	4.86	4.47	4.05	4.39	4.67
Cystine	1.04	1.09	0.93	1.18	1.12	0.96	1.08	0.94	0.85	1.10
Valine	5.10	4.49	4.88	4.49	4.79	4.35	4.55	4.03	4.64	4.50
Methionine	1.21	1.74	2.08	2.00	2.30	1.16	1.33	1.19	1.29	1.28
Isoleucine	4.28	3.47	4.09	4.16	3.80	4.03	4.31	3.84	4.01	3.86
Leucine	8.60	7.50	7.88	8.23	7.58	7.55	7.80	7.32	8.06	8.15
Tyrosine	3.06	3.08	2.35	3.36	2.52	2.84	2.71	2.52	2.75	2.78
Phenylalanine	5.91	5.09	4.66	5.81	5.09	5.60	5.34	5.03	5.35	5.40
Total	104.36	97.74	96.31	97.50	96.03	94.19	99.94	92.52	101.34	100.51
N.Recovery (%)	91.54	86.50	87.80	84.50	84.81	83.90	88.75	81.48	90.18	88.64
Protein (%)[a]	31.70	25.60	29.60	27.15	22.82	29.83	27.76	29.34	30.07	22.85
Chemical score										
Met + Cys (%)[b]	64	81	86	91	98	61	69	61	61	68

a, Moisture free (N x 6.25); b, FAO/WHO, 1973

C. judaicum had the highest value for sulphur amino acids followed by C. cuneatum and C. chorassanicum. The chemical scores of 98, 91 and 86 were obtained for C. judaicum, C. cuneatum and C. chrossanicum, respectively. These chemical score values were remarkably higher than that of the cultivated species, indicating that wild species may be good sources of high sulphur amino acids. However, these results have to be confirmed by growing these wild species in one or two more seasons. If they are sources of higher sulphur amino acids, attempts could be made to use such species in a breeding programme for improving protein quality. No large variation with respect to the levels of other amino acids was observed in the wild and cultivated species.

6.2. Varietal differences in amino acid composition of pigeonpea seed proteins:

By following the refluxing procedure of hydrolysis, 10 cultivars of pigeonpea grown during kharif 1975-76 were analysed to know the variation in the amino acid profiles of these cultivars (Table 33). Protein hydrolysate of these cultivars were analysed using the amino acid analyser. Tryptophan was determined by the method of alkaline hydrolysis followed by analysis in an amino acid analyser. Amino acid coposition of these cultivars is presented in Table 33. Lysine content (g/16g N) ranged between 6.75 and 7.63 with a mean value of 7.30 for these cultivars.

Table 33. Amino acid composition (g/16g N) of ten cultivars of pigeonpea[a]

Amino acid	HY-3C	ICP-1	ST-1	No 148	T-7	T-17	T-21	BDN-1	C-11	Gwalior
Lysine	7.63	7.61	7.32	7.25	6.75	7.38	6.76	7.60	7.38	7.42
Histidine	4.55	3.81	3.76	3.78	3.30	3.71	3.44	4.15	3.61	3.93
Arginine	7.27	7.23	6.82	6.86	6.41	7.38	6.16	7.66	6.51	7.49
Aspartic acid	10.91	10.30	10.88	10.19	9.36	11.04	8.73	10.84	10.62	10.88
Threonine	3.99	3.92	4.12	3.74	3.29	4.28	3.05	4.05	3.72	4.06
Serine	5.61	6.39	5.61	4.92	4.39	5.38	3.91	5.30	4.97	5.47
Glutamic acid	24.85	24.11	25.53	23.03	20.59	25.53	19.59	24.58	23.27	24.18
Proline	5.25	4.74	4.81	5.15	4.00	4.86	3.59	4.66	4.95	4.82
Alanine	5.12	4.78	5.05	4.85	4.13	5.23	3.81	5.07	4.56	5.04
Cystine	0.90	1.10	1.20	0.90	0.76	1.04	0.91	0.90	1.04	0.86
Valine	5.03	4.78	4.63	4.73	4.03	4.75	3.80	4.97	4.40	5.02
Methionine	1.18	1.10	1.13	1.01	0.80	1.13	0.78	0.92	1.04	1.17
Isoleucine	4.46	4.01	3.98	3.92	3.49	4.02	3.14	4.15	3.75	4.26
Leucine	9.24	8.33	8.61	7.90	7.14	8.86	6.42	8.48	7.90	8.82
Tyrosine	3.05	3.20	3.14	2.91	2.41	3.40	2.26	3.08	3.03	3.03
Phenylalanine	9.90	10.61	11.43	9.46	8.75	11.47	8.23	10.00	10.24	10.05
Tryptophan	0.87	0.84	0.92	0.90	0.96	0.72	0.88	0.84	0.83	0.80
Protein %	23.3	24.8	23.0	25.3	26.7	24.9	24.2	24.4	24.6	27.0

a, Defatted and moisture free basis; N x 6.25.

Methionine and cystine varied from 1.56 to 2.33 with a mean value of 1.98 g/16g N. Tryptophan content varied between 0.72 and 0.96 g/16g N for these cultivars. This data show a considerable variation in the levels of these essential amino acids among these cultivars.

In order to find out if a wider variability exists for these amino acids, several wild relatives of pigeonpea were analysed for their amino acid composition and the results are presented in Table 34. Some differences were observed in the levels of essential and nonessential amino acids of total seed proteins of the wild relatives. Lysine and phenylalanine levels were higher in Atylosia volubilis than in other wild relatives and Cajanus cajan, whereas the reverse trend was true for aspartic acid, threonine, cystine and tyrosine. Atylosia sericea and A. scarabaeoides contained the highest amounts methionine and cystine which showed an appreciable variation among the species. Atylosia scarabaeoides had the lowest lysine contents of all species. Amino acid analysis of more available wild relatives would be of interest to breeders and other concerned scientists in programmes that are involved in upgrading the nutritional quality of the grain.

Methionine and cystine were estimated in 85 accessions representing several wild species of pigeonpea obtained from

Table 34. Amino acid composition of wild relatives and cultivated species of pigeonpea

Amino acid	A.albicans	A.lineata	A.platycarpa	A.scarabaeoides	A.sericea	A.volubilis	Flemingia grahamiana	Rhynchosia rothii	Mean	Cajanus cajan (cv.T-21)
	(g/16gN)									
Lysine	7.10	6.33	6.95	6.17	6.74	7.48	6.31	6.82	6.74	7.06
Histidine	3.27	4.19	3.19	3.44	4.03	3.18	3.61	3.62	3.57	4.21
Arginine	5.98	6.54	8.32	8.07	7.55	6.55	6.21	7.72	7.12	7.89
Aspartic acid	10.64	10.72	10.75	10.78	10.24	8.82	10.09	10.96	10.38	10.74
Threonine	3.46	3.84	4.83	4.29	4.32	3.42	3.66	4.19	4.00	4.24
Serine	4.83	4.78	5.09	5.73	4.90	4.82	5.06	5.31	5.07	6.30
Glutamic acid	25.08	23.75	24.06	23.84	24.19	23.42	22.75	18.93	23.25	24.71
Proline	4.25	4.20	5.53	4.76	5.11	3.98	4.26	5.10	4.65	3.90
Glycine	3.53	4.65	4.70	4.79	4.58	3.79	5.84	4.48	4.55	4.57
Alanine	3.24	4.96	5.38	3.27	5.17	4.63	3.72	4.21	4.82	5.02
Cystine	0.97	0.90	1.17	1.31	1.15	0.88	1.16	1.58	1.14	1.03
Valanine	4.71	4.80	6.32	5.18	4.96	4.47	4.84	5.71	5.12	5.70
Methionine	1.16	1.17	1.08	1.17	1.34	0.96	1.08	0.75	1.09	1.00
Isoleucine	3.66	4.06	4.02	4.40	4.22	4.01	4.23	4.39	4.12	4.06
Leucine	8.31	8.95	8.73	9.60	7.88	8.56	8.76	8.39	8.65	8.70
Tyrosine	2.75	3.03	3.24	3.27	3.16	2.65	2.75	3.28	3.02	3.18
Phenylalanine	10.02	11.00	10.45	9.26	11.31	13.44	12.19	8.20	10.73	10.01
Total	102.96	107.90	113.85	111.61	110.85	105.11	108.60	103.50	108.05	111.39
Nitrogen recovery (%)	87.23	89.51	96.13	94.38	92.46	86.46	90.10	88.00	90.53	91.53

the Genetic Resources Unit. These amino acids were determined by amino acid analyser after performic acid oxidation. Large variation was observed among and within the species as summarised in Table 35 and detailed in Appendix-10. When considered together, methionine and cystine varied from 2.55 to 3.43 with mean being 2.93 g/16g N for 28 accessions of A. scarabaeoides and from 2.34 to 2.79 with mean being 2.55 g/16g N for 10 accessions of A. albicans. A. scarabaeoides appeared to contain the highest amount of sulphur amino acids (Table 35).

6.2.1. Amino acid profiles of Cajanus, Atylosia species and their hybrids:

Attempts have been made to develop high protein lines by crossing the cultivated species with wild species, previously reported as a high protein source, and this had led to some improvement in the protein content of their derivatives. Eleven samples representing Cajanus and Atylosia species and their derivatives grown during kharif 1977-78 were analysed for their amino acid composition (Table 36), in a Beckman Model 120C amino acid analyser. The protein content of these samples was determined by the microKjeldahl method. Amino acid profiles were similar for

Table 35. Ranges and means of methionine and cystine (g/16g N) of wild species of pigeonpea[a]

Species	No of accessions	Protein %		Methionine		Cystine		Met + Cys	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
A.alibicans	10	26.00-32.45	28.72	1.09-1.34	1.22	1.19-1.44	1.33	2.34-2.79	2.55
A.cajanifolia	4	25.20-33.55	29.16	1.15-1.21	1.19	1.08-1.51	1.31	2.28-2.72	2.50
A.grandifolia	1	-	24.1	-	1.18	-	1.41	-	2.59
A.lineata	6	27.75-34.15	30.23	1.22-1.44	1.33	1.06-1.31	1.19	1.06-1.31	2.52
A.mollis	1	-	33.75	-	1.32	-	1.08	-	2.40
A.platycarpa	9	26.05-31.30	28.74	0.95-1.20	1.11	1.05-1.38	1.20	2.18-2.49	2.31
A.scarabaeoides	28	25.60-30.65	27.72	1.27-1.62	1.44	1.22-1.84	1.49	2.55-3.43	2.93
A.sericea	3	30.50-30.55	30.53	1.26-1.43	1.35	1.28-1.43	1.36	2.69-2.71	2.70
A.volubilis	9	21.75-33.75	27.88	1.19-1.56	1.35	0.99-1.38	1.14	2.24-2.94	2.49
A.albiflora	1	-	24.55	-	1.09	-	1.28	-	2.37
A.bracteata	2	28.20-29.00	28.60	0.99-1.00	1.00	1.39-1.46	1.43	2.38-2.46	2.43
A.densiflora	1	-	26.35	-	0.96	-	1.16	-	2.12
A.minima	1	-	25.95	-	1.06	-	1.40	-	2.46
A.cana	1	-	30.65	-	1.17	-	1.43	-	2.60
R.rothi/ R.viscosa	5	27.30-30.30	29.37	0.75-0.97	0.88	1.50-1.89	1.71	2.42-2.80	2.59
R.rufercens	1	-	25.40	-	1.37	-	1.42	-	2.79
R.suaveolens	1	-	24.85	-	1.50	-	1.39	-	2.89
R.viscida	1	-	24.85	-	1.21	-	1.07	-	2.28

a, Analysis of defatted dhal.

the Atylosia species, Cajanus cultivars and their hybrids (Table 36). The lowest lysine value (6.22 g/16g N) was observed in Baigani, a Cajanus cultivar. The Atylosia species would be considered satisfactory for lysine when compared with the Cajanus cultivars as both A. sericea and A. albicans had relatively higher levels of lysine. Methionine and cystine when considered together were low in cv. Pant A-2 and A. albicans while cv. Baigani had slightly higher amount of these amino acids. This did not appear to be different from A. sericea or A. scarabaeoides. The results also indicated that there is no strong relationship among parents and hybrids for methionine plus cystine content. There appears to be some variation for methionine plus cystine for the two cultivars and three Atylosia species.

6.3. Amino acid score (chemical score) of chickpea and pigeonpea seed:

Amino acid score is considered as one of the indicators of the nutritional quality of protein of a given crop. The amino acid score values are expressed individually in proportion to the content of a corresponding amino acid in a suitable reference protein and FAO/WHO suggested amino acid pattern are taken as standard reference figures. The ratio

ble 36. Amino acid composition (g/16g N) of dhal of *Cajanus, Atylosia* species and their hybrids

erial	Protein %[a]	Lys	His	Arg	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Be	Leu	Tyr	Phe
t A-2	24.38	7.04	4.10	6.43	9.06	3.46	5.18	21.85	4.23	3.82	4.35	0.85	4.22	0.76	3.13	6.64	2.23	8.52
gani	27.17	6.22	3.41	6.35	10.93	4.31	5.84	23.75	4.68	4.44	5.35	0.98	5.12	1.00	4.42	9.47	3.23	10.10
ericea	30.02	7.32	3.59	7.18	10.23	4.36	5.49	23.40	4.74	4.34	4.90	0.96	4.83	1.07	4.20	8.19	3.17	10.47
caraba-oides	28.74	6.32	3.00	7.22	10.82	4.33	5.37	20.87	4.56	3.89	5.01	0.83	5.12	1.14	4.36	9.50	2.93	8.59
albicans	30.16	7.25	3.71	6.74	8.91	3.53	4.20	22.41	3.79	3.68	4.46	0.77	4.25	0.81	3.45	7.61	2.66	9.32
nt A-2 x sericea	30.87	6.85	3.33	7.27	9.99	4.01	4.97	23.54	4.42	4.43	4.69	0.84	4.49	1.10	4.02	7.95	3.05	10.56
nt A-2 x scaraba-oides	28.53	6.92	3.48	6.96	9.71	4.16	5.53	23.78	4.24	4.06	4.89	0.95	4.95	0.99	4.06	8.84	2.75	9.81
nt A-2 x albicans	29.50	7.14	3.78	6.61	9.47	3.99	5.31	23.97	3.94	3.98	5.65	1.03	4.62	1.04	3.95	8.49	2.78	10.85
sericea	33.41	7.04	3.86	7.45	10.06	3.96	5.02	22.94	4.11	3.93	4.77	0.81	4.53	0.91	3.93	8.39	2.72	10.41
igani x scaraba-des	29.03	7.06	3.45	7.03	9.53	4.08	5.54	24.08	4.27	4.11	4.87	0.87	4.81	0.94	4.15	8.90	2.35	9.56
igani albicans	31.25	7.26	4.07	7.17	9.83	4.01	5.41	24.57	4.15	4.14	4.98	1.06	4.63	1.10	4.08	8.75	2.89	12.53

, Moisture free basis (N x 6.25).

for each amino acid is calculated by using the formula given below and the amino acid that shows the lowest score is considered as the first limiting amino acid in a given crop.

Amino acid score :

$$\frac{\text{mg of amino acid per g of test protein}}{\text{mg of amino acid per g of reference protein}} \times 100$$

Table 37. Amino acid score (chemical score) of pigeonpea seed protein

Amino acids	Suggested level (mg/g protein)[a]	Amino acid (mg/g protein)[b]		Chemical score	
		Chick-pea	Pigeon-pea	Chick-pea	Pigeon-pea
Lysine	55	66	73	120	132
Threonine	40	36	38	90	95
Methionine + Cystine	35	23	20	66	57
Tryptophan	10	11	82	110	82
Isoleucine	40	41	39	103	98
Leucine	70	78	82	111	117
Phenylalanine+ Tyrosine	60	83	130	138	216
Valine	50	42	46	84	92

a, FAO/WHO (1973); b, Average value of 10 cultivars.

The essential amino acids of the chickpea and pigeonpea cultivars (average values of 10 cultivars for each crop) were expressed as amino acid score (chemical score) on the basis of FAO/WHO (1973) suggested level to know the relative sufficiency of these amino acids. In case of pigeonpea sulphur amino acids had the lowest value followed by tryptophan as shown in Table 37. This shows that sulphur amino acids are the first limiting amino acids whereas tryptophan is the second limiting amino acid in pigeonpea. Other amino acids that had values below 100 were valine, threonine, and isoleucine in decreasing order of importance. For chickpea, sulphur amino acids appear to be of greater significance from nutrition point of view. It is interesting to note that tryptophan was not observed to be a limiting amino acid of chickpea. Other amino acids which had a chemical score of below 100 were valine and threonine in order of importance in the case of both chickpea and pigeonpea. Valine may not be of great nutritional importance in a cereal/legume diet as cereals are reported as a good source of this amino acid. But threonine should receive some consideration as some of the cereals are also deficient in this amino acid. Moreover, some grain legumes have also been reported to be deficient in this amino acid besides sulphur containing amino acids.

7. Seed protein fractions and amino acid composition of chickpea and pigeonpea:

The proteins present in legume seeds can be broadly classified into metabolic proteins, which are involved in normal cellular activities, and storage proteins, which are synthesised during seed development. The storage protein, globulin, constitutes a major proportion of the legume seed proteins and the limitations of these proteins in the nutrition of humans and other monogastric animals are well known (Millerd, 1975). The amino acid composition of food crops can be altered either by varying the relative proportions of embryo and endosperm or by changing the relative proportions of metabolic and storage proteins as in the case of opaque-2 maize (Mertz et al. 1964). We studied the distribution of seed protein fractions in different anatomical parts of chickpea and pigeonpea and then the amino acid composition of these fractions in order to know if any protein fraction is a richer source of essential limiting amino acids of these pulse crops. For this study, seed material used was as follows. Pigeonpea (cv. Hy-3c) and chickpea (cv. G-130) were grown in rainy and post-rainy seasons of 1978-79, respectively, and were supplied by our breeding program. Seed coat was separated from the whole grain manually after soaking the seed material at 4°-5°C for 4 h. Embryo was separated from the cotyledons by hand

dissection using a needle. The different components were dried in an oven at 65°C and samples were ground to a fine powder in a Udy cyclone mill using a 0.4 mm screen. The samples were defatted in a Soxhlet apparatus using hexane.

7.1. Distribution of seed protein fractions in different anatomical parts of chickpea and pigeonpea:

The separation of different protein fractions was carried out using the procedure described earlier (Singh et al. 1981). The protein extracts containing albumin and globulin in 0.5 M sodium chloride solution in 0.01 M phosphate buffer (pH 7.0) were dialysed against six changes of distilled water at room temperature (25°C) for 72 h and the volume was made to 50 ml. The dialysate was then centrifuged (12,000 g for 15 min) and the pellet and supernatant of the dialysate was referred to as the globulin and albumin, respectively. However, nonprotein nitrogen was lost during the process. These fractions were analysed for nitrogen and then freeze dried. Nonprotein nitrogen (NPN) was estimated by extraction of the samples with 10% trichloroacetic acid (TCA) as described earlier (Singh and Jambunathan, 1981).

The results on distribution of protein fractions in different seed components of chickpea and pigeonpea are summarised in Table 38. Both chickpea and pigeonpea are made of three anatomical structures: the seed coat, the cotyledons and the embryonic tissue. Embryos constitute only a small proportion of the total seed weight whereas the cotyledons constitute 82.9% and 85.3% of total dry weight in chickpea and pigeonpea, respectively (Table 38). These values agree with earlier reported values. Embryo and seed-coat contents were slightly higher in chickpea than in pigeonpea. Protein fractionation of seed coats, embryos, cotyledons, and whole seeds of chickpea and pigeonpea did not reveal large differences between these two legumes (Table 38), but considerable differences in the distribution pattern of protein fractions among the embryo, cotyledons and seed coats of these two legumes were observed. When compared with other components, the embryo was found to be richer in albumin both in chickpea and in pigeonpea. Whole-seed chickpea had a slightly lower concentration of globulin than pigeonpea. Nonprotein nitrogen and glutelin fractions were higher in the seed coat as compared to other components and they had a much smaller proportion of albumin and globulin fractions.

Table 38. Distribution of protein fractions in different components of chickpea and pigeonpea

Crop/ component	Amount (%)[a]	Protein (%)[a] (Nx6.25)	Nonprotein nitrogen (%)	Protein fractions[b]					
				Albumin	Globulin	Glutelin	Prolamin	Residue	Total
Chickpea									
Embryo	1.2	52.1	5.8	22.5	50.0	21.4	3.0	1.5	98.4
Cotyledon	82.9	24.8	10.7	15.9	62.7	17.5	2.3	1.0	99.4
Seed coat	16.4	4.1	21.3	3.5	22.8	33.2	3.4	30.5	93.4
Whole seed	-	21.3	11.2	12.6	56.6	18.1	2.8	4.9	95.0
Pigeonpea									
Embryo	0.7	49.6	6.2	17.0	52.7	21.3	2.7	2.1	95.8
Cotyledon	85.3	22.2	9.5	11.4	64.5	18.2	3.5	1.8	99.4
Seed coat	14.3	4.9	27.4	2.6	26.3	32.8	4.2	23.0	88.9
Whole seed	-	20.5	12.8	10.2	59.9	17.4	3.0	5.3	95.8

a, Dry weight; b, Values are averages of two determinations and expressed as percentage of total protein (N x 6.25).

7.2. Amino acid composition of different protein fractions:

Having observed that the cotyledons accounted for about 80%-85% of the total dry-seed weight, various protein fractions of this component were analysed for amino acid composition and the results are shown in Table 39. When the amino acid profile of different fractions was compared, albumin was noticed to have the largest amount of sulphur amino acids, methionine and cystine, lysine, aspartic acid, glycine and alanine in the case of both chickpea and pigeonpea. By calculation it was observed that this fraction contributed about 36% and 35% of the total sulphur amino acids of the cotyledons of chickpea and pigeonpea, respectively.

Globulin, the major protein fraction, had lower methionine and cystine contents than the glutelin fraction. Since methionine is one of the limiting essential amino acids of these legumes, a larger proportion of protein fractions containing this amino acid would be advantageous from the nutritional viewpoint. The results obtained suggest that the selection of cultivars in which the albumin or glutelin fraction is present in higher proportions would result in improved methionine content in the whole seed.

Table 39. Amino acid composition (g/16g N) of seed protein fractions of chickpea and pigeonpea cotyledons

	Chickpea				Pigeonpea			
Amino acid	Albumin	Globulin	Glutelin	Prolamin	Albumin	Globulin	Glutelin	Prolamin
Lysine	10.8	6.4	6.8	2.3	10.0	6.9	7.1	1.0
Histidine	2.3	2.6	2.9	2.6	3.5	3.4	4.3	1.0
Arginine	5.6	10.7	6.8	4.8	6.4	7.0	7.6	1.3
Aspartic acid	13.8	12.7	10.1	10.3	13.9	10.8	11.8	3.9
Threonine	5.4	3.5	5.7	2.2	6.0	3.6	5.2	0.6
Serine	5.2	5.2	5.6	1.9	6.1	4.7	6.0	1.0
Glutamic acid	18.4	15.2	16.6	17.7	24.3	22.0	25.1	15.9
Proline	4.9	5.2	4.8	7.2	4.8	3.3	7.1	2.3
Glycine	5.4	3.7	4.7	3.1	5.9	4.0	4.8	1.3
Alanine	5.3	4.3	4.9	2.3	7.2	4.1	5.7	1.3
Cystine	3.5	1.0	1.4	0.6	3.2	0.9	1.0	0.4
Valine	4.5	4.2	5.7	2.1	6.2	5.5	5.8	1.8
Methionine	1.8	0.8	1.2	0.9	1.7	0.8	1.3	0.3
Isoleucine	5.1	4.4	5.4	2.3	4.1	3.4	5.1	0.7
Leucine	9.8	7.5	9.1	1.6	7.7	6.7	9.2	0.8
Tyrosine	4.2	2.9	3.7	2.3	4.2	3.9	3.9	0.9
Phenylalanine	5.1	6.1	4.4	3.4	4.7	10.9	8.0	6.5
Total	111.1	96.4	99.8	64.6	119.9	101.9	119.0	41.0
N recovery (%)	95.7	90.5	87.5	54.2	98.8	84.6	96.5	29.3

Chickpea and pigeonpea differed from each other with respect to the amino acid profile of prolamin fraction. In the case of pigeonpea, this fraction had the highest amount of glutamic acid, followed by phenylalanine; whereas aspartic acid and glutamic acid were the predominant amino acids of this fraction in chickpea. Nitrogen recovery values were the lowest for these two prolamin fractions. When expressed on an equal nitrogen recovery basis, they had the poorest lysine of all other fractions.

7.3. Amino acid composition of different seed components:

Amino acid profiles of whole-seed, embryo, cotyledon and seed-coat samples of chickpea and pigeonpea are shown in Table 40. Amino acid composition of cotyledons revealed some noticeable differences between chickpea and pigeonpea. Levels of lysine, glutamic acid, and phenylalanine were higher in pigeonpea than in chickpea. But the reverse was the trend for aspartic acid and sulphur-containing amino acids. Differences in the amino acid composition of the cotyledons will affect the overall nutritional potential of these legumes since cotyledons constitute a major proportion of the whole seed. Amino acid composition of embryos was observed to be nutritionally better than that of the cotyledons in both chickpea and pigeonpea as these contained higher amounts of lysine and sulphur amino acids. Levels of other amino acids of embryos were very similar to those of their respective cotyledons. Seed coats of chickpea and

Table 40. Amino acid composition (g/16g N) of different seed components of chickpea and pigeonpea

Amino acid	Chickpea				Pigeonpea			
	Whole seed	Embryo	Coty-ledons	Seed coat	Whole seed	Embryo	Coty-ledons	Seed coat
Lysine	6.2	7.9	6.7	5.0	6.8	7.0	7.1	3.9
Histidine	2.7	2.6	2.7	2.4	3.6	3.3	3.9	1.0
Arginine	10.9	10.3	10.8	4.2	6.3	8.4	7.1	3.2
Aspartic acid	12.2	10.4	11.8	9.0	10.4	10.1	11.0	5.7
Threonine	4.0	4.5	3.8	3.7	3.8	4.7	4.3	2.5
Serine	5.5	5.0	5.3	4.7	5.0	5.3	5.5	3.5
Glutamic acid	16.3	17.6	16.1	10.7	19.0	16.2	20.6	6.9
Proline	4.0	2.6	3.9	3.9	4.3	4.7	4.3	3.1
Glycine	4.1	4.6	3.9	4.3	3.8	4.5	4.0	4.5
Alanine	4.0	5.1	4.2	3.9	4.6	6.0	4.5	3.0
Cystine	1.3	1.5	1.5	1.1	1.2	1.7	1.3	-
Valine	5.0	5.1	4.8	5.2	4.4	6.6	5.6	3.2
Methionine	1.1	1.5	1.1	1.1	1.0	1.4	1.2	0.7
Isoleucine	4.5	4.1	4.2	3.5	3.9	4.4	4.3	2.9
Leucine	7.6	7.4	7.2	6.3	7.2	7.1	7.8	4.0
Tyrosine	2.8	3.2	2.7	2.4	3.0	3.8	3.2	1.7
Phenylalanine	5.5	4.3	5.5	4.6	9.7	6.8	10.7	2.3
Total	97.7	97.7	96.2	76.0	98.0	102.0	106.4	52.1
N recovery (%)	91.5	88.5	89.1	54.4	92.5	94.5	97.4	46.9

pigeonpea showed amino acid compositions slightly different from those of embryos and cotyledons. The relative proportions of serine, threonine, proline and glycine appeared to be considerably larger in seed coat than that in cotyledons in both chickpea and pigeonpea, when expressed on an equal nitrogen recovery basis.

In conclusion it may be mentioned that the distribution of various anatomical parts of seeds did not reveal large differences between chickpea and pigeonpea. While no noticeable differences between chickpea and pigeonpea are apparent with respect to the levels of various protein fractions, the higher levels of sulphur-containing amino acids in glutelin than in globulins of these pulse crops suggest that cultivars with a higher ratio of glutelin to globulin should be identified to improve their seed protein quality.

REFERENCES

1. Bhatty,R.S., Finlayson,A.J. and Mackenzie,S.L. 1977. Total sulphur as a screening criterion for sulphur amino acids in legumes. Canadian Journal of Plant Sciences. 57:177-183.

2. Chavana,S. and Haque,A. Njaa,R.L., and Patterson,J. 1980. Methods for methionine and cysteine determination. A comparison between two institutes. Journal of the Science Food and Agriculture. 31:1027-1033.

3. Concon,J.M. 1975. Rapid and simple method for the determination of tryptophan in cereal grains. Analytical Biochemistry. 67:206-219.

4. Evans,R.J.,Bauer,D.H., Adams,M.W., and Saettler,A.W. 1978. Methionine and cystine contents of bean (Phaseolus) seeds. Journal of Agriculture and Food Chemistry. 26:1234-1237.

5. Evans,I.M., Ford,J.E. and Boulter,D. 1976. Comparison of chemical and microbiological methods in the estimation of methionine in cowpea seeds. British Journal of Nutrition. 36:289-293.

6. FAO/WHO 1973. (Food and Agriculture Organization/World Health Organization) 1983. Energy and protein requirements. Reports of a Joint FAO/WHO Adhoc Expert Committee, World Health Organization Technical Report Series 522, Geneva, Switzerland, 63 pp.

7. Felker,P., and Waines,G. 1978. Colorimetric screening assay for cystine plus cysteine in legume meals. Analytical Biochemistry 87:641-647.

8. Finlayson,A.J. and Macknzie, 1975. A rapid method for methionine determination in plant materials. Analytical Biochemistry. 70:399-402.

9. Friedman,M. and Finley,J.W. 1971. Methods of tryptophan analysis. Journal of Agricultural and Food Chemistry. 19:626-631.

10. Gehrke,C.H. and Neuner, T.E. 1974. The determination of methionoine. Journal of the Association of Official Analytical Chemists. 57:683-689.

11. Goa, J. 1961. Micro determination of cysteine and cystine in proteins. Acta Chemica Scandinavica. 15:853-855.

12. Hannah,L.C., Rhodes, B.B. and Evans, I.M. 1977. Examination and modification of the use of the Leuconostoc mesenteroides for measurement of sulfur containing amino acids from vigna unguiculata. Journal of Agriculture and Food Chemistry 25:620-623.

13. Hugli,T.E. and Moore,S. 1972. Determination of the tryptophan content of proteins by ion exchange chromatography of alkaline hydrolysis. Journal of Biological Chemistry. 247:2826-2834.

14. Jambunathan,R. and Singh,U. 1981. Relationship between total sulphur and sulphur amino acids in chickpea (C.arietinum L) and pigeonpea (Cajanus cajan L.) Qual Plantanum Plant Foods Human Nutrition. 31:109-117.

15. LaRue,Thomas, 1965. Spectrophotometric determination of methionine with pentacyanoammino ferrate. Analytical Biochemistry. 10:172-174.

16. Lewis,A.J., Holden,P.J., Ewan,R.C. and Zimmerman,D.R. 1976. An automated fluorometric method for the measurement of tryptophan in plasma. Journal of Agricultural and Food Chemistry. 24:1081-1083.

17. Lunder,T.J. 1973. An improved method for the colorimetric determination of methionine in acid hycholysates of biological products. Ind.Aliment (Pinerlo Italy). 12: 94-98.

18. Mackenzie, L. 1977. Analysis of methionine in plant materials. Journal of Chromatography. 130:399-402.

19. McCarthy, T.E. and Sullivan, M.X. 1941. A new and highly specific colorimetric test for methionine. Journal of Biological Chemistry. 141:871-876.

20. Mertz., E.T., Bates, L.S. and Nelson, O.E. 1964. Mutant gene that changes protein and increase lysine content of maize endosperm. Science 145:279-280.

21. Millerd, A. 1975. Biochemistry of legume seed proteins. Annual Review of Plant Physiology 26:53-72.

22. Pieniazek,D., Rakowska,M., Szkilladziowa, W. and Grabarek,Z. 1975. Estimation of available methionine and cysteine in proteins of food products by in vitro methods. British Journal of Nutrition. 34:175-190.

Rama Rao,M.V., Tara,M.R. and Krishnan,C.K. 1974. Colorimetric estimation of tryptophan content of pulses. Journal of Science Food and Technology. 11:213-215.

Singh, U. and Jambunathan, R. 1981. Relationship between total nitrogen and nonprotein nitrogen in chickpea (Cicer arietinum L.) seed. Journal of Agricultural and Food Chemistry 29:423-425.

Singh, U., Jambunathan, R. and Gurtu, S. 1981. Seed protein fractions and amino acid composition of some wild species of pigeonpea. Journal of Food Science and Technology 18:83-85.

Spies, J.R. and Chambers, D.C. 1949. Chemical determination of tryptophan in proteins. Analytical Chemistry. 21:1249-1266.

APPENDIX I

Methionine estimation by amino acid analyser and microbiological assay in chickpea and pigeonpea[a]

Chickpea			Pigeonpea		
Cultivar	Amino acid analyser	Microbiological assay	Cultivar	Amino acid analyser	Microbiological assay
	....Methionine (g/16 g N)...			...Methionine (g/16 g N)....	
N-59	1.04	1.26	No. 148	1.03	1.18
NEC-1196	1.00	1.19	Bhandra Coll	1.00	1.08
NEC-1572	1.06	1.25	Pant A-2	0.85	0.97
NEC-1614	1.11	1.30	UPAS-120	0.84	0.93
NEC-1713	1.07	1.28	Pakanjore Coll	0.87	0.93
T-3	1.12	1.36	4685/1	0.85	0.98
12-0?1-05093	0.93	1.04	NP(WR)-15	0.78	0.90
P-134-1	0.99	1.06	T-17	0.97	1.10
P-416	1.01	1.22	Badal Khedi Coll	0.95	1.08
P-1081	1.05	1.27	Bhedaghat Coll	0.90	1.04
P-1081-1	1.04	1.24	BDN-2	0.86	0.95
P-1181-A	1.12	1.32	Sharda	0.81	0.90
P-1213-2	1.02	1.22	BDN-1	0.81	0.92
P-1231	1.12	1.30	Gwalior-3	0.90	1.02
P-1363-1	1.14	1.36	C-11	1.00	1.12
P-1630	0.97	1.02	Prabhat	0.93	0.98
Annegiri	1.13	1.30	T-21	0.86	0.91
Caina	1.09	1.31	DH-34-1	0.92	0.94
Chaffa	0.97	1.24	Mukta	1.13	1.20
C-104	1.09	1.28	HY-2	0.97	1.04
C-214	0.97	1.18	AS-71-37	0.88	0.93
NEC-34	1.11	1.27	KWR-1	1.05	1.03
BEG-482	0.90	1.18	Hy-3C	0.94	1.04
BR-70	0.98	1.04	T-7	1.15	1.20
GG-24	1.00	1.23			
L-550	0.99	1.16			
K-4	1.14	1.27			
P-3090	0.89	1.09			
NP-34	0.94	1.10			
Kaka	1.07	1.20			
Mean	1.04	1.22	Mean	0.93	1.06

[a]Defatted dhal samples analysed.

Appendix-2

A comparison of methods of sulphur amino acids estimation in chickpea (dhal)

Cultivar/line	Protein (%)	Methionine (g/16g N)		Cystine (g/16g N)		Total Met + Cys (g/16g N)	
P-82	20.5	1.10	1.21	1.25	1.30	2.35	2.51
P-99	23.5	1.13	1.17	1.13	1.10	2.26	2.27
P-149-1	24.4	1.11	1.12	0.97	1.08	2.08	2.20
P-257	25.6	0.93	0.91	0.93	0.97	1.86	2.08
P-317	26.4	0.91	0.94	0.95	0.98	1.86	2.12
P-416	24.2	1.14	1.23	1.15	1.12	2.29	2.45
P-431	23.0	1.12	1.13	1.11	1.18	2.23	2.31
P-436	25.4	0.87	1.11	0.85	0.96	1.72	2.07
P-514	23.8	1.02	1.17	1.09	1.07	2.11	2.24
P-623	23.9	1.05	1.15	1.09	1.10	2.14	2.25
P-726-2	24.2	1.14	1.23	1.15	1.17	2.29	2.40
P-861	21.0	1.24	1.29	1.47	1.48	2.71	2.78
P-1022	23.3	1.16	1.17	1.08	1.16	2.24	2.33
P-1081	21.0	1.13	1.16	0.99	1.05	2.12	2.32
P-1294	21.9	1.12	1.15	1.19	1.24	2.31	2.50
P-1426	21.7	1.25	1.26	1.30	1.16	2.55	2.44
P-1437	22.8	1.16	1.20	1.18	1.20	2.34	2.40
P-1469-2	22.0	1.15	1.15	1.17	1.20	2.32	2.35
P-1489	21.6	1.21	1.18	1.25	1.29	2.46	2.47
P-1610	25.0	0.95	1.02	0.94	1.03	1.89	2.05
P-1630	23.3	1.08	1.18	1.15	1.19	2.23	2.37
P-1774	23.0	1.17	1.14	1.07	1.10	2.24	2.24
P-2170	20.5	1.30	1.36	1.23	1.26	2.53	2.62
P-2173-1	19.8	1.31	1.39	1.22	1.25	2.53	2.64
P-2249	23.5	1.17	1.15	1.14	1.10	2.31	2.25
P-2422	22.8	1.24	1.25	1.27	1.29	2.51	2.54
P-2422-2	21.7	1.25	1.26	1.11	1.13	2.36	2.30
P-2591	17.3	1.33	1.39	1.21	1.24	2.54	2.64
P-2602	18.6	1.30	1.38	1.21	1.20	2.51	2.58

Cultivar/line	Protein (%)	Methionine (g/16g N)		Cystine (g/16g N)		Total Met + Cys (g/16g N)	
		a	b	a	b	a	b
P-2940	21.8	1.23	1.25	1.17	1.19	2.40	2.42
P-4079	23.7	1.07	1.08	1.06	1.16	2.13	2.06
P-4265	20.4	1.40	1.38	1.36	1.40	2.76	2.78
P-4278-2	22.6	1.26	1.27	1.29	1.32	2.54	2.59
P-4341-2	22.4	1.21	1.19	1.19	1.18	2.40	2.37
P-4353-1	23.4	1.05	0.96	0.99	1.04	2.04	2.00
P-4412-1	21.2	1.22	1.33	1.21	1.26	2.43	2.59
P-4446-1	20.8	1.34	1.29	1.22	1.23	2.56	2.53
P-4449-1	21.5	1.21	1.25	1.28	1.30	2.49	2.55
P-4500	22.0	1.29	1.23	1.27	1.30	2.56	2.53
P-4515	23.0	1.13	1.17	1.17	1.19	2.30	2.26
P-4528	22.0	1.23	1.20	1.27	1.25	2.50	2.45
P-4706	22.0	1.24	1.22	1.26	1.30	2.50	2.42
Chala	21.0	1.28	1.30	1.31	1.34	2.59	2.58
G-130	22.2	1.21	1.18	1.17	1.23	2.38	2.41
JG-62	24.2	1.11	1.04	1.20	1.25	2.31	2.22
F_3 WF Gram x 38A 1-NF	22.8	1.23	1.20	1.30	1.29	2.53	2.49
JG-109	25.0	1.07	1.02	0.93	1.08	2.00	2.10
1-8-19	21.5	1.25	1.30	1.28	1.30	2.53	2.60
1-209-15	21.0	1.29	1.27	1.39	1.36	2.68	2.63
2-1-3	23.9	1.05	1.08	0.90	1.01	1.95	2.18
NEC-422	20.5	1.31	1.30	1.24	1.28	2.55	2.58
NEC-444	21.4	1.25	1.34	1.34	1.32	2.49	2.66
NEC-495	19.8	1.34	1.43	1.36	1.35	2.70	2.78
NEC-754	21.0	1.32	1.26	1.38	1.37	2.70	2.63
NEC-760	20.7	1.32	1.30	1.37	1.30	2.69	2.60
Bronz Leaf	25.2	1.08	1.13	0.91	1.12	1.99	2.25
Chaffa 8-16	26.8	0.85	0.98	1.05	1.06	1.90	2.14
Mean	22.4	1.17	1.20	1.17	1.19	2.34	2.40

[a]Amino acid analyser;

[b]Colorimetric procedure (nitroprusside reaction for methionine and Goa method for cystine).

Appendix-3

A comparison of methods of sulphur amino acids estimation in pigeonpea (dhal)

Cultivar/line	Protein (%)	Methionine (g/16g N)		Cystine (g/16g N)		Total Met + Cys (g/16g N)	
		a	b	a	b	a	b
P-3768	24.8	1.02	1.05	0.91	0.95	1.93	2.00
P-606-35	23.4	0.92	0.90	0.99	0.97	1.91	1.87
P-606-35	22.4	0.96	0.96	1.10	1.13	2.06	2.09
P-4600-1	24.0	1.10	1.12	1.16	1.16	2.26	2.28
P-1794-2	23.3	1.17	1.16	1.06	1.09	2.23	2.25
AS-71-37	22.2	0.99	1.04	1.02	1.03	2.01	2.07
Baigani	24.4	1.17	1.18	1.18	1.19	2.35	2.37
ANM-25	18.8	1.05	1.01	1.24	1.26	2.29	2.27
DSL-55	19.3	1.01	0.96	1.10	1.12	2.11	2.08
P-2805	23.1	1.20	1.18	1.06	1.02	2.26	2.20
P-4038	23.8	1.20	1.17	1.16	1.20	2.36	2.37
ANM-365	20.1	1.00	0.90	1.15	1.18	2.15	2.08
ANM-54-55	22.4	0.94	0.99	1.06	1.05	2.00	2.04
ANM-55-56	23.7	1.10	1.08	0.92	1.01	2.02	2.09
ANM-56	25.1	0.98	0.96	1.09	1.10	2.07	2.06
ANM-60	23.9	0.90	0.89	0.88	0.95	1.78	1.84
ANM-84	27.8	0.91	0.93	1.05	1.10	1.95	2.03
ANM-539	23.1	1.15	1.13	1.20	1.22	2.35	2.35
ANM-543	23.5	1.04	1.07	1.29	1.31	2.33	2.37
ANM-575	22.4	0.86	0.93	0.88	1.01	1.74	1.94
ANM-579	24.4	0.83	0.94	0.82	1.03	1.65	1.97
P-4040	22.7	0.83	0.93	1.05	1.02	1.88	1.95
P-2627	25.2	0.96	1.02	1.02	1.06	1.98	2.08
P-85	25.2	1.00	1.05	1.00	0.96	2.00	2.01
P-738-2	24.3	0.90	0.93	0.83	0.92	1.73	1.85
P-2448	22.7	0.98	1.03	0.98	0.99	1.96	2.02
P-2479	27.0	0.86	0.90	0.78	0.86	1.64	1.76
P-1555	26.9	0.96	1.08	0.76	0.89	1.72	1.97
JA-275	24.0	0.99	1.05	1.01	1.02	2.00	2.07
P-2514	25.3	0.84	0.90	0.75	0.90	1.59	1.80
P-4663	22.8	1.10	1.15	0.99	0.99	2.09	2.14
P-4523	22.8	1.07	1.10	0.92	0.96	1.99	2.06
P-4640	23.1	1.00	1.06	0.84	0.91	1.84	1.97
P-4681	26.0	1.00	1.01	0.85	0.96	1.85	1.97
P-4687	23.7	1.09	1.16	0.98	1.07	2.07	2.23
DSL-125	24.1	1.06	1.11	0.85	0.99	1.91	2.10
T-28	21.5	1.11	1.23	1.00	1.04	2.11	2.27
P-3558	24.8	0.99	1.03	0.73	0.95	1.72	1.98
P-4126	21.0	1.20	1.26	1.30	1.28	2.50	2.54
P-4219-1	24.3	0.97	1.03	0.87	0.91	1.84	1.94

Cultivar/line	Protein (%)	Methionine (g/16g N)		Cystine (g/16g N)		Total Met + Cys (g/16g N)	
		a	b	a	b	a	b
P-3889	22.0	1.21	1.28	1.12	1.16	2.33	2.44
P-157	23.7	1.04	1.03	0.95	1.05	1.99	2.08
FC-100467	24.5	1.14	1.19	0.96	0.94	2.10	2.13
JA-278-1	22.4	1.05	1.10	1.06	1.18	2.11	2.28
MP-59	22.6	1.30	1.28	1.12	1.15	2.42	2.43
P-305	24.7	0.93	0.97	0.79	0.94	1.72	1.91
NP-WR-15	23.7	1.04	1.07	0.95	1.08	1.99	2.15
P-3219	25.0	0.89	0.98	0.77	0.89	1.66	1.87
Code # 14	24.1	1.18	1.13	0.91	1.05	2.09	2.18
DSL-77	21.4	1.06	1.09	1.03	1.07	2.09	2.16
JA-275	21.4	1.04	1.08	1.03	1.09	2.07	2.17
HY-3C	21.6	1.24	1.26	1.20	1.30	2.44	2.56
AS-44	23.5	1.27	1.20	1.08	1.18	2.35	2.38
UP-34	21.0	1.06	1.03	0.86	1.03	1.92	2.06
P-497	24.1	0.92	0.95	0.70	0.95	1.62	1.90
P-507	23.8	0.96	0.99	0.86	1.06	1.82	2.05
P-520	23.6	1.23	1.28	0.96	1.12	2.19	2.40
P-522	23.4	1.05	1.10	0.87	0.95	1.92	2.05
P-1295-5	22.5	1.19	1.18	1.11	1.18	2.30	2.36
ICC-8858	21.0	0.95	1.02	0.94	1.05	1.89	2.07
T-17	24.0	0.91	0.98	0.97	0.98	1.88	1.96
Pusa Ageti	23.0	1.11	1.14	1.02	1.06	2.13	2.20
BDN-1	21.5	1.05	1.08	0.87	0.99	1.92	2.07
HY-3A	21.0	1.03	1.06	0.89	0.99	1.92	2.05
HY-2	21.4	1.05	1.07	0.93	1.01	1.98	2.08
Mean	23.2	1.03	1.06	0.98	1.04	2.01	2.11

[a]Amino acid analyser;

[b]Colorimetric procedure (nitroprusside reaction for methionine and Goa method for cystine).

Appendix-4

Protein content, total sulphur (TS), methionine (Met) and Cystine (Cys) in chickpea (dhal)

Cultivar	Protein (%) (N x 6.25)	Total sulphur[a] (% of sample)	Methionine[b] (g/16g N)	Cystine[b] (g/16g N)	S in Met as % of TS	S in Cys as % of TS	S in Cys+ Met as % of TS
No. 59	18.1	0.25	1.04	1.16	17.78	23.26	41.04
NEC 1196	18.3	0.21	1.00	1.22	20.53	45.25	65.78
NEC 1572	20.9	0.24	1.06	1.37	21.44	34.73	56.17
NEC 1614	16.6	0.22	1.11	1.51	19.63	33.27	52.90
NEC 1713	17.2	0.22	1.07	1.29	20.00	24.25	44.25
T-3	16.2	0.17	1.12	1.23	24.56	33.74	58.30
12-071-05093	21.5	0.24	0.93	1.20	19.45	31.50	50.95
P-134-1	22.7	0.27	0.99	1.32	19.62	32.69	52.31
P-416	21.8	0.22	1.01	1.24	23.13	35.47	58.60
P-1081	23.0	0.25	1.05	1.39	22.72	37.48	60.20
P-1081-1	21.1	0.22	1.04	1.32	23.47	37.30	60.77
P-1181-A	21.0	0.27	1.12	1.37	20.48	31.37	51.85
P-1213-2	18.8	0.19	1.02	1.26	23.73	35.48	59.20
P-1231	17.1	0.19	1.12	1.30	19.56	35.90	55.46
P-1363-1	20.5	0.23	1.14	1.49	23.77	38.65	62.42
P-1630	21.9	0.23	0.97	1.22	21.60	34.00	55.60
Annegiri	16.4	0.18	1.13	1.37	23.85	36.20	60.05
China	21.6	0.22	1.07	1.27	24.30	36.01	60.31
Chafa	20.0	0.23	0.97	1.25	19.70	31.68	51.30
C-104	18.5	0.22	1.09	1.35	21.26	32.68	53.94
C-214	19.5	0.22	0.97	1.23	19.86	32.39	51.25
NEC-34	16.6	0.25	1.11	1.29	17.28	24.00	41.20
BEG-482	25.2	0.25	0.90	1.13	22.04	37.04	59.08
BR-70	22.8	0.22	0.98	1.24	23.88	37.71	61.59
G-24	20.1	0.23	1.00	1.13	20.61	28.77	49.38
L-550	19.5	0.21	0.99	1.16	21.17	30.93	52.10
K-4	16.2	0.21	1.14	1.34	20.47	30.14	50.61
P-3090	23.9	0.19	0.89	1.15	25.91	41.70	67.61
NP-34	18.7	0.20	0.94	1.18	20.65	32.35	53.00
Kaka	21.2	0.26	1.07	1.15	20.50	27.50	48.00
Mean	19.99	0.22	1.04	1.27	21.49	33.35	54.84
SE ±	0.18	0.01	0.04	0.04	0.41	0.66	1.07

[a]Wet digestion method; [b]Amino acid analyser.

Appendix-5

Protein content, total sulphur (TS), Methionine (Met) and Cystine (Cys) in pigeonpea (dhal)

Cultivar	Protein (%) (N x 6.25)	Total sulphur[a] (% of sample)	Methionine[b] (g/16 g N)	Cystine[b] (g/16g N)	S in Met as % of TS	S in Cys as % of TS	S in Cys+ Met as % of TS
No. 148	23.3	0.18	1.03	1.40	30.76	52.16	82.95
Bhandra coll	24.1	0.17	1.00	1.25	33.05	51.57	84.62
Pant A-2	23.2	0.15	0.85	0.94	31.01	42.88	73.89
UPAS-120	21.7	0.17	0.84	1.05	25.57	39.99	65.56
Pakhanjore coll	22.2	0.16	0.87	1.16	27.62	45.91	73.53
4685/1	23.6	0.17	0.85	1.13	27.28	43.35	70.63
NP (WR)-15	24.0	0.16	0.78	0.98	27.43	43.00	70.43
T-17	22.0	0.16	0.97	1.27	31.25	51.06	82.31
Badalkhadi coll	21.0	0.16	0.95	1.23	29.56	47.87	77.40
Bhedaghat coll	20.9	0.15	0.90	1.16	29.61	47.05	76.66
BDN-2	22.4	0.16	0.86	1.16	28.67	48.03	76.70
Sharda	21.9	0.17	0.81	1.16	24.41	43.76	68.17
BDN-1	22.9	0.16	0.81	1.11	28.00	48.00	76.00
Gwalior-3	25.9	0.14	0.90	0.96	38.25	40.42	78.67
C-11	23.9	0.18	1.00	1.27	31.34	47.91	79.25
Prabhat	21.4	0.16	0.93	1.16	29.19	45.37	74.56
T-21	20.0	0.15	0.86	1.27	26.93	49.60	76.53
DH-74-1	28.0	0.16	0.92	1.17	33.13	49.77	82.90
Mukta	21.3	0.19	1.13	1.42	30.54	47.83	78.37
Hy-2	20.4	0.17	0.97	1.24	28.19	44.71	72.90
As-71-37	19.4	0.17	0.88	1.07	24.58	35.65	59.23
KWR-1	21.1	0.18	1.05	1.37	29.77	48.34	78.11
T-7	22.7	0.19	1.15	1.30	32.90	46.45	79.35
Hy-3C	22.0	0.17	0.94	1.18	28.76	44.96	73.72
Mean	22.4	0.165	0.93	1.18	29.45	46.07	75.52
SE ±	0.23	0.006	0.04	0.03	0.54	0.70	1.24

[a]Wet digestion method; [b]Amino acid analyser.

Appendix 6

A comparison of methods of tryptophan estimation in chickpea (dhal)

Cultivar/line	Protein (%)	Method 1	Method 2	Method 3
		g/16g N		
P-82	20.5	1.25	1.14	1.20
P-99	23.5	1.20	1.31	1.21
P-149-1	24.4	1.16	1.24	1.23
P-257	29.9	1.21	1.28	1.23
P-317	29.9	1.18	1.31	1.22
P-416	24.2	1.23	1.31	1.19
P-431	23.0	1.12	1.36	1.24
P-436	22.4	1.15	1.15	1.08
P-514	23.8	1.18	1.11	1.22
P-623	23.9	1.33	1.37	1.28
P-726-2	24.2	1.26	1.34	1.37
P-1022	23.3	1.27	1.22	1.32
P-1294	21.9	1.32	1.38	1.33
P-1387	21.4	1.29	1.28	1.28
P-1426	23.6	1.17	1.08	1.12
P-1437	22.8	1.46	1.40	1.45
P-1469-2	22.0	1.03	1.19	1.05
P-1610	22.9	1.25	1.24	1.24
P-1630	23.3	1.08	1.21	1.12
P-1774	23.0	1.17	1.23	1.11
P-1781	21.9	1.25	1.35	1.26
P-2170	20.5	1.30	1.32	1.27
P-2173-1	19.8	1.34	1.35	1.28
P-2249	23.5	1.23	1.29	1.23
P-2422-2	21.7	1.64	1.67	1.53
USA-613	26.8	0.91	0.89	0.94
853-3/27	27.2	0.89	0.86	0.95
Pant G-114	29.3	0.92	0.84	0.89
CPS-1	26.4	1.13	0.93	1.07
T-3	23.5	1.02	0.96	0.96
Annegiri	24.2	1.10	1.02	1.03
K-4	23.9	1.05	0.95	0.95
C-104	27.3	0.85	0.80	0.78
BG-203	26.5	1.05	0.94	0.97
P-5462	24.4	1.14	1.03	0.98
Rabat	24.1	0.97	0.85	0.88
L-550	21.8	1.19	1.00	1.00
GL-629	23.1	1.22	1.00	1.02
Giza	25.8	1.14	1.02	1.09
No. 501	26.1	1.07	0.91	0.94
Mean	24.0	1.17	1.16	1.14

Method 1: Hugli & Moore (1972); Method 2: Concon (1975);
Method 3: Spies & Chambers (1949).

Appendix-7

A comparison of methods of tryptophan estimation in pigeonpea (dhal)

Cultivar/line	Protein (%)	Method I	Method 2	Method 3
P-3768	24.8	0.71	0.68	0.73
P-606-35-2	23.4	0.71	0.70	0.70
T-10	22.4	0.77	0.83	0.77
P-4600/1	24.0	0.64	0.67	0.72
AS-71-37	22.0	0.81	0.85	0.80
Baigani	24.4	0.73	0.74	0.77
ANM-25	18.8	0.80	0.80	0.82
DSL-55	19.3	0.89	0.87	0.88
P-2805	23.1	0.71	0.69	0.71
P-4038	23.3	0.69	0.68	0.67
ANM-36D	21.6	0.84	0.78	0.83
ANM-36F	20.1	0.80	0.80	0.81
ANM-54	22.4	0.80	0.77	0.76
ANM-56	25.1	0.74	0.69	0.74
ANM-84	27.8	0.68	0.69	0.69
ANM-539	23.1	0.93	0.84	0.90
ANM-543	23.0	0.80	0.79	0.83
ANM-575	22.4	0.76	0.72	0.73
ANM-579	24.9	0.75	0.69	0.72
P-4040	22.7	0.82	0.81	0.80
P-2627	25.2	0.93	0.89	0.95
P-85	25.5	0.74	0.79	0.76
P-738-2	24.3	0.75	0.73	0.76
P-2448	22.7	0.69	0.68	0.70
P-2479	24.2	0.75	0.77	0.76
P-1555	26.9	0.68	0.68	0.67
JA-275	21.1	0.76	0.74	0.72
P-2514	24.5	0.73	0.70	0.71
P-4663	22.8	0.76	0.75	0.74
P-4523	22.8	0.76	0.80	0.76
P-4640	23.1	0.75	0.77	0.74
P-4681	26.0	0.70	0.71	0.65

Cultivar/line	Protein (%)	Method I	Method 2	Method 3
P-4687	23.7	0.66	0.74	0.68
DSL-125	24.1	0.76	0.67	0.77
T-28	21.5	0.74	0.82	0.77
P-3558	24.9	0.65	0.65	0.68
P-4126	23.3	0.68	0.70	0.68
P-4219/1	24.3	0.72	0.69	0.70
P-3889	24.2	0.70	0.68	0.64
P-157	24.7	0.68	0.78	0.72
EC-100467	24.5	0.70	0.72	0.70
JA-278-1	22.4	0.74	0.77	0.79
MP-69	22.6	0.76	0.78	0.75
P.3219	23.9	0.65	0.71	0.70
Code # 14	24.1	0.67	0.74	0.68
DSL-77	23.8	0.64	0.73	0.63
PS 41	21.6	0.65	0.74	0.67
P-1234	23.3	0.85	0.88	0.82
JA-275	21.6	0.64	0.72	0.65
HY-3C	21.6	0.71	0.71	0.70
AS-44	23.6	0.79	0.79	0.75
UD-34	21.0	0.87	0.90	0.89
P-497	24.1	0.65	0.72	0.66
ANM-60	23.9	0.73	0.71	0.74
Mean	23.3	0.74	0.75	0.74

Method I: Hoogli & Moore (1972); Method 2: Concon (1975);

Method 3: Spies & Chamber (1949).

Appendix - 8

Analysis of chickpea (dhal) cultivars/lines grown during rabi 1977-78 for protein, methionine and total sulphur content.

Cultivar	Protein (%)	Methionine		Total Sulphur		Met-S as % of Total Sulphur
		g/100g sample	g/16g N	g/16g N	g/100g sample	
P-274	16.7	0.21	1.26	1.08	0.18	25.0
JG-62	17.3	0.23	1.31	1.15	0.20	24.5
P-3719	16.4	0.23	1.42	1.16	0.19	26.3
P-3942	17.6	0.24	1.39	1.14	0.20	26.0
P-4081	16.9	0.22	1.28	1.36	0.23	20.4
P-4275	25.4	0.31	1.20	0.71	0.18	36.0
P-4279	24.0	0.30	1.25	1.04	0.25	26.0
P-4306-2	26.5	0.31	1.15	1.03	0.28	24.0
P-4321	19.0	0.23	1.20	1.26	0.24	20.4
P-4332-1	21.2	0.26	1.21	1.13	0.24	22.9
P-454301	25.8	0.31	1.18	0.78	0.20	32.5
NEC-582	24.9	0.27	1.10	0.76	0.19	31.0
NEC-741	23.5	0.28	1.17	0.89	0.21	28.1
NEC-750	24.3	0.30	1.25	0.78	0.19	34.2
NEC-892	24.6	0.30	1.22	1.06	0.26	24.6
NEC-902	26.1	0.30	1.15	0.93	0.23	27.8
NEC-961	24.6	0.30	1.20	0.77	0.19	33.2
NEC-962	25.8	0.32	1.23	0.81	0.21	32.4
NEC-1036	17.0	0.22	1.29	1.41	0.24	19.6
NEC-2205	15.2	0.21	1.36	1.58	0.24	18.3
NEC-2287	16.5	0.21	1.29	1.45	0.24	19.2
NEC-513	16.3	0.21	1.25	1.35	0.22	20.0
NEC-2607	17.4	0.24	1.35	1.09	0.19	26.3
NEC-2610	19.2	0.24	1.23	1.09	0.21	24.3
NEC-2617	18.4	0.23	1.26	1.03	0.19	26.3
NEC-2649	21.6	0.26	1.20	1.15	0.25	22.4
NEC-2675	20.2	0.26	1.29	0.94	0.19	29.5
NEC-2678	25.4	0.30	1.18	0.94	0.22	29.0
NEC-2691	20.6	0.24	1.17	0.87	0.18	28.9
NEC-2629-1	27.3	0.30	1.11	0.84	0.23	28.7
NEC-2632-2	27.1	0.32	1.19	0.77	0.21	32.9
NEC-2734	24.9	0.41	1.63	0.80	0.20	43.5
P-9733	20.8	0.28	1.34	1.11	0.23	26.1
P-9789	17.0	0.26	1.51	1.23	0.21	26.2
P-9800	19.8	0.28	1.41	1.11	0.22	27.3
JM-969	18.7	0.27	1.44	1.02	0.19	30.0
JM-975B	18.3	0.22	1.21	1.09	0.20	24.0
JM-981	24.2	0.29	1.18	0.62	0.15	40.7
JM-982	15.9	0.22	1.37	1.19	0.19	24.7
NEC-2021	23.2	0.29	1.20	0.86	0.20	31.0

Cultivar	Protein (%)	Methionine		Total Sulphur		Met-S as % of Total Sulphur
		g/100g sample	g/16g N	g/16g N	g/100g sample	
P-9710	18.5	0.28	1.50	1.08	0.20	30.0
SL-133B	24.7	0.32	1.31	0.89	0.22	31.8
SL-971B	18.4	0.28	1.50	1.57	0.29	20.3
SL-1227A	19.0	0.27	1.43	1.10	0.21	27.6
SL-1227B	17.2	0.26	1.50	1.16	0.20	27.5
SL-1476B	20.8	0.30	1.43	0.91	0.19	33.7
JM-517	24.7	0.32	1.31	1.01	0.25	28.0
310479	21.6	0.25	1.16	0.79	0.17	31.8
P-9629	26.9	0.36	1.35	0.71	0.19	41.1
NEC-2300	24.1	0.34	1.40	0.83	0.20	36.0
P-3225	23.9	0.31	1.28	0.79	0.19	34.7
P-853-1	24.1	0.31	1.28	0.83	0.20	33.0
Galbron	22.7	0.29	1.29	0.75	0.17	37.1
P-1798-1	23.7	0.31	1.31	0.76	0.18	37.2
P-4252	23.1	0.32	1.37	0.74	0.17	40.0
N-8	23.7	0.35	1.48	0.72	0.17	44.7
NEC-179	23.6	0.31	1.31	0.72	0.17	38.8
NEC-197	23.6	0.31	1.33	0.68	0.16	41.9
NEC-2332	24.4	0.31	1.26	0.65	0.16	41.3
G-130	23.7	0.34	1.43	0.72	0.17	42.9
JM-482-4	25.7	0.30	1.16	0.84	0.22	29.8
P-1710	24.9	0.33	1.31	0.90	0.23	31.1
P-1713	22.2	0.31	1.37	0.86	0.19	34.7
P-1716	27.2	0.32	1.17	0.63	0.17	40.0
P-720	27.1	0.32	1.17	0.63	0.17	40.0
P-1723	26.2	0.23	1.24	0.76	0.20	35.0
P-1728	25.6	0.31	1.23	0.78	0.20	33.5
C-161	25.4	0.34	1.34	0.75	0.19	38.4
C-309	27.6	0.35	1.25	0.69	0.19	38.9
T-39-1	29.6	0.39	1.30	0.67	0.20	41.5
P-1761	26.2	0.32	1.21	0.61	0.16	42.5
P-1761-2	24.4	0.30	1.22	0.70	0.17	37.6
P-1782-1	22.9	0.28	1.21	0.70	0.16	36.9
P-1789-2	21.5	0.27	1.27	0.79	0.17	34.1
P-1790	21.5	0.27	1.27	0.84	0.18	32.8
P-4237	18.3	0.26	1.44	1.04	0.19	29.5
T-1-A	28.6	0.34	1.19	0.70	0.20	36.5
P-3318	29.3	0.34	1.16	0.68	0.19	38.4
P-1137	17.5	0.25	1.42	0.96	0.16	30.8
Mean	22.4	0.29	1.29	0.93	0.20	31.3

Appendix - 9

Protein and amino acids of chickpea (dhal) cultivars/lines grown during 1979-80 and 1980-81

Cultivar/line	1979-80				1980-81			
	Protein (%)	Cystine	Methionine	Tryptophan	Protein (%)	Cystine	Methionine	Tryptophan
		.....g/16g N.....				.....g/16g N.....		
P-799	23.5	1.14	1.19	0.98	19.5	1.16	1.13	1.12
P-799-11	23.8	1.21	1.23	1.02	18.2	1.20	1.17	0.95
P-801	21.3	1.03	1.20	1.12	17.0	1.24	1.26	1.03
P-803	24.9	1.18	1.08	0.93	20.7	1.10	1.09	0.86
P-807	24.0	1.04	1.16	0.95	20.0	1.26	1.16	0.82
P-808	21.1	1.30	1.18	1.06	18.4	1.30	1.16	0.95
P-809	23.3	1.24	1.25	1.04	20.0	1.10	1.10	0.80
P-810	23.0	0.86	1.20	1.03	19.0	1.10	1.17	0.76
P-812-M	27.3	0.97	0.99	0.96	22.8	1.04	1.11	0.86
P-813	24.5	1.28	1.22	0.97	20.8	1.18	1.21	0.85
P-823	25.7	1.20	1.10	1.15	23.0	0.98	0.91	1.02
P-827	25.4	1.05	0.97	1.11	20.4	1.28	1.33	1.11
P-827-1	26.0	1.20	0.90	1.00	22.9	1.30	1.33	1.15
P-831	26.8	1.36	1.21	1.11	22.5	1.16	0.97	1.12
P-834	22.5	1.12	0.90	1.20	19.0	1.28	1.41	1.10
P-843	24.8	1.35	1.21	1.03	22.5	1.10	0.86	1.01
P-843-1	23.2	0.96	1.15	1.00	22.0	1.30	1.48	1.04
P-1675	24.0	1.04	1.07	0.98	21.0	1.10	1.07	0.86
P-1681	23.8	1.01	0.91	0.98	20.6	1.15	1.08	0.76
P-1683-1	25.1	1.16	1.36	1.10	20.9	1.14	1.21	0.89
P-1683-2	24.8	1.04	0.94	1.10	18.8	1.18	1.18	0.83
P-1688	25.2	0.78	0.93	1.00	24.0	1.24	1.33	0.90
P-1688-1	23.7	0.91	1.08	1.19	21.3	1.20	1.17	0.98
P-1688-2	25.2	0.78	1.00	1.17	20.8	1.20	1.19	1.05
P-1689	24.9	0.94	1.07	1.10	19.0	1.34	1.27	1.21
P-1693-1	25.0	1.04	0.93	1.13	21.6	1.26	1.18	1.33
P-1693-1	26.2	0.92	1.14	1.18	22.7	1.30	1.23	1.23
P-1694	19.9	1.17	1.17	1.07	17.0	1.32	1.36	1.11
P-1694-1	21.0	1.14	1.19	1.14	19.4	1.20	1.13	1.05
P-1696-2	26.1	1.30	1.13	1.03	23.0	1.14	1.06	1.06

Cultivar/line	1979-80				1980-81			
	Protein (%)	Cystine	Methionine	Tryptophan	Protein (%)	Cystine	Methionine	Tryptophan
		.....g/16g N.....				.....g/16g N.....		
P-1697-1	22.6	0.96	1.18	1.10	18.5	1.18	1.04	1.15
P-1698-1	26.9	0.84	0.99	0.91	22.8	1.24	1.23	1.25
P-1699	25.0	0.99	1.07	1.09	21.6	1.10	1.00	1.05
P-1699-1	24.6	1.17	1.13	1.08	19.9	1.18	1.29	1.07
P-1701	26.7	1.04	1.13	0.95	24.5	1.10	1.11	1.08
P-1707-3	27.1	0.91	1.05	0.92	25.0	1.40	1.36	1.14
P-1710	26.9	1.00	1.05	0.92	24.3	1.28	1.24	1.09
P-1712	27.2	1.04	1.04	0.80	22.9	1.18	0.97	1.26
P-1712-1	28.0	1.30	1.19	0.86	24.0	1.20	1.16	1.11
P-1714-1	28.3	0.93	0.94	0.80	23.8	1.30	1.14	1.27
P-1714-2	29.2	0.85	0.91	0.77	25.6	1.26	1.24	1.27
P-1716-1	24.2	1.06	1.03	0.92	22.0	1.10	0.97	1.11
P-1717-1	25.9	1.16	1.13	0.90	22.7	1.15	1.05	1.10
P-1720	29.1	1.04	0.94	0.72	24.9	1.20	1.09	1.05
P-1723	26.6	0.96	1.00	0.81	21.0	0.98	0.81	1.20
P-1725	26.5	1.18	1.21	0.94	23.5	1.10	1.06	1.06
P-1726	26.9	1.10	0.93	0.83	23.8	1.12	1.04	1.11
P-1734	23.2	1.14	1.01	1.00	19.5	1.19	1.08	1.15
P-1741-1	25.5	1.30	1.28	1.00	22.0	0.90	0.97	1.04
P-1745	29.1	1.18	0.94	0.81	25.0	1.25	1.23	1.22
P-1747	25.8	1.30	1.20	1.10	21.8	1.30	1.11	1.05
P-1748	27.4	1.20	1.09	1.10	22.9	1.20	1.16	1.10
P-1749-1	27.7	1.24	1.20	0.80	23.4	1.24	1.33	1.20
P-1751-1	23.4	0.98	1.06	1.12	20.4	0.98	0.93	0.88
P-1752-1	26.7	0.99	0.97	1.10	25.0	0.96	1.15	0.97
P-1759	26.1	0.94	1.09	0.89	20.7	1.18	1.17	0.95
P-1787	26.7	1.10	1.21	0.97	21.0	1.16	1.09	0.89
P-4101-4	25.4	1.24	1.23	0.85	22.0	1.20	1.23	1.00
P-46615	25.7	1.16	1.12	0.87	21.5	1.20	1.24	0.97
P-9693	24.3	1.28	1.33	0.95	20.6	1.28	1.34	0.85
P-9733	26.5	1.14	1.01	0.95	22.0	1.30	1.31	1.21
P-9746	25.1	1.00	1.06	1.08	20.8	1.04	1.13	1.24

Cultivar/line	1979-80				1980-81			
	Protein (%)	Cystine	Methionine	Tryptophan	Protein (%)	Cystine	Methionine	Tryptophan
		.....g/16g N.....				.....g/16g N.....		
P-9757	23.9	0.98	1.02	1.00	19.6	1.16	1.24	1.08
P-9777	24.5	1.20	1.14	1.15	22.0	1.10	0.89	1.19
P-9781	23.5	1.18	1.13	0.89	21.6	1.30	1.41	1.19
P-9790	23.0	1.40	1.41	0.94	19.0	1.24	1.21	1.26
P-9792	23.2	1.28	1.39	0.92	19.5	1.30	1.49	1.25
P-9800	21.4	1.10	1.09	0.94	18.0	1.42	1.52	1.34
P-9815	23.3	1.23	1.27	0.87	19.5	1.14	1.05	1.23
V3	26.5	1.38	1.46	0.74	24.6	1.18	1.15	1.08
V12	25.5	1.20	1.07	0.76	22.6	1.10	0.93	1.04
NEC-20	19.8	1.19	1.26	0.87	18.6	1.24	1.22	0.91
NEC-24	21.5	0.90	0.91	0.84	18.8	1.30	1.26	1.00
NEC-27	20.5	1.26	1.24	0.90	18.9	1.10	1.15	1.07
NEC-120	20.6	1.10	1.02	0.95	19.0	0.96	0.81	1.31
NEC-124	23.2	0.86	0.92	0.87	18.7	0.96	1.01	0.92
NEC-125	22.5	1.30	1.33	0.95	18.7	1.32	1.50	0.90
NEC-212	20.4	1.10	1.14	1.04	15.7	1.10	1.21	----
NEC-1609	19.6	1.28	1.39	1.03	17.6	1.28	1.14	1.15
NEC-1610	20.4	1.26	1.24	1.02	17.7	1.16	1.34	1.18
NEC-1611	19.7	1.35	1.44	1.02	17.1	1.14	1.01	1.18
NEC-1612	21.2	1.18	1.26	0.89	17.8	1.06	0.97	1.18
NEC-1614	21.3	0.98	1.10	0.91	17.3	1.16	1.00	1.09
NEC-1616	20.5	1.10	0.98	1.05	16.4	1.10	1.01	1.16
NEC-1619	21.4	1.24	1.34	0.97	16.6	1.00	1.00	1.13
NEC-1657	19.9	1.30	1.40	0.96	19.8	1.30	1.55	1.22
NEC-1663	19.6	1.24	1.22	1.08	19.5	1.24	1.26	1.24
NEC-1664	19.9	1.18	1.44	1.03	18.7	1.03	1.02	1.24
NEC-1671	20.7	1.26	1.28	0.98	17.9	1.20	1.17	1.21
P-9687	20.0	1.14	1.09	1.00	19.4	1.10	0.95	0.95
P-9731	26.0	1.05	0.90	0.83	21.8	1.30	1.22	0.97
P-9756	21.8	1.13	0.92	0.87	16.2	1.24	1.28	1.11
Nasic Bulk	21.6	1.06	1.08	0.84	17.5	1.26	1.20	-

Cultivar/line	1979-80				1980-81			
	Protein (%)	Cystine	Methionine	Tryptophan	Protein (%)	Cystine	Methionine	Tryptophan
		.....g/16g N.....				.....g/16g N.....		
JM-460	23.5	1.14	1.09	0.91	18.9	1.18	1.13	1.33
JM-465	25.9	1.25	1.29	0.81	22.5	1.19	1.27	1.02
JM-508	21.2	1.30	1.26	1.06	19.0	1.20	1.19	1.00
JM-551	24.4	1.10	0.96	0.86	22.4	1.16	1.13	0.80
JM-552	27.0	1.12	1.23	0.87	24.6	1.18	1.15	0.92
JM-556	25.6	1.10	0.99	0.89	23.5	1.09	1.14	0.95
NEC-1760	23.2	1.18	1.29	0.99	20.4	1.24	1.32	1.11
NEC-1860	26.1	1.13	0.91	0.88	21.8	1.16	1.20	1.16
NEC-1892	29.7	1.10	1.07	0.75	23.0	1.15	1.10	0.98
NEC-1896	23.7	1.30	1.27	0.94	21.1	1.24	1.30	1.16
NEC-1907	25.4	1.30	1.23	0.90	21.0	1.23	1.28	1.10
NEC-2086	28.3	1.20	1.18	0.84	24.5	1.30	1.36	1.13
NEC-2232	27.9	1.24	1.25	0.84	23.0	1.28	1.36	1.07
P-830	27.5	1.16	1.09	0.77	21.6	1.20	1.27	1.09

Appendix-10

Sulphur amino acids (g/16g N) in dhal samples of wild species of pigeonpea[a].

Coll No.	Species	Protein (%)	Cystine	Methionine	Cys + Met
			....g.....		
JM-2337	A.*Albicans*	31.4	1.32	1.14	2.46
JM-2356	A.*Albicans*	27.1	1.22	1.19	2.41
JM-3023	A.*Albicans*	29.8	1.23	1.11	2.34
JM-3360	A.*Albicans*	29.0	1.45	1.34	2.79
JM-3472	A.*Albicans*	27.8	1.44	1.23	2.67
JM-4023	A.*Albicans*	32.5	1.19	1.34	2.53
PR-4816	A.*Albicans*	30.2	1.20	1.18	2.38
NKR-138	A.*Albicans*	27.1	1.41	1.31	2.72
NKR-177	A.*Albicans*	26.0	1.44	1.09	2.53
NKR-185	A.*Albicans*	26.3	1.41	1.27	2.68
JM-2739	A.*Cajanifolia*	29.4	1.08	1.20	2.28
PR-4868	A.*Cajanifolia*	28.5	1.40	1.19	2.59
PR-4876	A.*Cajanifolia*	33.6	1.24	1.15	2.39
NKR-193	A.*Cajanifolia*	25.2	1.51	1.21	2.72
EC-124363	A.*Grandifolia*	24.1	1.41	1.18	2.59
JM-2639	A.*Lineata*	29.9	1.06	1.22	2.28
JM-3366	A.*Lineata*	27.8	1.31	1.44	2.75
NKR-76	A.*Lineata*	29.7	1.16	1.34	2.50
NKR-126	A.*Lineata*	30.2	1.23	1.32	2.55
NKR-150	A.*Lineata*	29.8	1.19	1.33	2.52
Bole's coll	A.*Lineata*	34.2	1.20	1.35	2.55
JM-2943	A.*Mollis*	33.4	1.08	1.32	2.40
JM-2873	A.*Platycarpa*	30.1	1.13	1.14	2.27
JM-2987	A.*Platycorpa*	26.4	1.22	1.20	2.42
JM-3310	A.*Platycorpa*	27.1	1.24	1.18	2.42
JM-4351	A.*Platycorpa*	26.5	1.15	1.09	2.24
PR-4550	A.*Platycorpa*	33.3	1.05	1.13	2.18
PR-4557	A.*Platycorpa*	26.8	1.18	1.03	2.21

Coll No.	Species	Protein (%)	Cystine	Methionine	Cys+Met
			.....g.....		
PR-4572	A.*Platycorpa*	31.3	1.17	1.09	2.26
LJR coll	A.*Platycorpa*	26.1	1.38	0.95	2.33
Kosbad coll	A.*Platycorpa*	31.3	1.31	1.18	2.49
JM-1965	A.*Scarabaeoides*	28.1	1.46	1.42	2.88
JM-1967	A.*Scarabaeoides*	29.3	1.28	1.27	2.55
JM-1985	A.*Scarabaeoides*	29.4	1.22	1.39	2.61
JM-1988	A.*Scarabaeoides*	27.7	1.35	1.42	2.77
JM-2289	A.*Scarabaeoides*	26.4	1.73	1.42	3.15
JM-2323	A.*Scarabaeoides*	27.3	1.37	1.32	2.69
JM-2367	A.*Scarabaeoides*	29.6	1.36	1.40	2.76
JM-2865	A.*Scarabaeoides*	29.4	1.26	1.31	2.57
JM-2939	A.*Scarabaeoides*	27.4	1.45	1.42	2.87
JM-2958	A.*Scarabaeoides*	30.7	1.22	1.38	2.60
JM-4147	A.*Scarabaeoides*	27.5	1.84	1.59	3.43
PR-4516	A.*Scarabaeoides*	25.6	1.54	1.54	3.08
PR-4739	A.*Scarabaeoides*	28.0	1.44	1.30	2.74
PR-4771	A.*Scarabaeoides*	27.2	1.55	1.53	3.08
PR-4814	A.*Scarabaeoides*	28.2	1.43	1.40	2.83
PR-4879A	A.*Scarabaeoides*	26.4	1.68	1.48	3.16
NKR-112	A.*Scarabaeoides*	27.5	1.70	1.61	3.31
ANM-557	A.*Scarabaeoides*	30.2	1.52	1.49	3.01
NO 3463	A.*Scarabaeoides*	26.5	1.69	1.62	3.31
ARKS-12347	A.*Scarabaeoides*	26.9	1.81	1.59	3.40
EC-121206	A.*Scarabaeoides*	26.4	1.60	1.46	3.06
EC-121207	A.*Scarabaeoides*	28.0	1.55	1.47	3.02
EC-122342	A.*Scarabaeoides*	27.7	1.56	1.39	2.95
EC-122344	A.*Scarabaeoides*	28.9	1.27	1.39	2.66
Site coll	A.*Scarabaeoides*	27.7	1.36	1.30	2.66
RJW coll	A.*Scarabaeoides*	25.9	1.58	1.37	2.95

Coll No.	Species	Protein (%)	Cystine	Methionine	Cys+Met
			.....g.....		
Jangalpalli coll	A.*Scarabaeoides*	26.4	1.53	1.57	3.10
LJR coll	A.*Scarabaeoides*	26.6	1.36	1.37	2.73
JM-1961	A.*Sericea*	30.6	1.43	1.26	2.69
LJR coll	A.*Sericea*	30.5	1.28	1.43	2.71
EC-121208	A.*Sericea*	24.8	1.30	1.38	2.68
JM-1984	A.*Volubilis*	28.2	1.38	1.56	2.94
JM-4208	A.*Volubilis*	33.8	1.06	1.25	2.31
JM-4220	A.*Volubilis*	31.8	1.15	1.26	2.41
PR-4877	A.*Volubilis*	29.0	0.94	1.10	2.04
RPSP-685	A.*Volubilis*	28.4	0.99	1.32	2.31
NKR-73	A.*Volubilis*	27.3	1.05	1.19	2.24
NKR-154	A.*Volubilis*	27.8	1.25	1.52	2.77
NKR-184	A.*Volubilis*	28.7	1.13	1.32	2.45
NKR-187	A.*Volubilis*	27.2	1.13	1.40	2.53
NKR-143	R.*Albifloria*	24.6	1.28	1.09	2.37
JM-3952	R.*Bracteata*	28.2	1.39	0.99	2.38
JM-4219	R.*Bracteata*	29.0	1.46	1.00	2.46
JM-3438	R.*Densiflora*	26.4	1.16	0.96	2.12
JM-2855	R.*Minima*	26.0	1.40	1.06	2.46
JM-3556	R.*Cana*	30.7	1.43	1.17	2.60
JM-2296	R.*Rothi/viscosa*	29.5	1.67	0.75	2.42
JM-3364	R.*Rothi/viscosa*	29.8	1.89	0.91	2.80
JM-3410	R.*Rothi/viscosa*	27.3	1.83	0.81	2.64
JM-4547	R.*Rothi/viscosa*	30.3	1.65	0.94	2.59
JM-4557	R.*Rothi/viscosa*	30.0	1.50	0.97	2.47
JM-2366	R.*Rothi/viscosa*	25.4	1.42	1.37	2.79
JM-3312	R.*Suaveolens*	24.9	1.39	1.50	2.89
EC-12204	R.*Viscida*	24.9	1.07	1.21	2.28
	Mean	28.4	1.36	1.28	2.64

[a]Seed material was obtained from Genetic Resources Unit during 1982.