GRAIN QUALITY AND BIOCHEMISTRY

Chickpea and Pigeonpea

Protein Content

Prepared by:

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FOREWORD

This detailed report describes the work that has been carried out on chickpea and pigeonpea protein content in the Grain Quality and Biochemistry Support Program during 1976-1982. In addition to this report, reports of research results have appeared in the ICRISAT ANNUAL REPORTS. Our program has closely collaborated with the Genetics Resources Unit, Pigeonpea Breeding, Chickpea Breeding and Pulse Physiology programs at ICRISAT and their contributions and assistance are gratefully acknowledged.

I sincerely 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

This report describes the results on the following three aspects.

1. Methods of protein estimation
2. Variability for protein content in the germplasm accessions
3. Protein content as influenced by environments

Many reliable rapid methods are now available for the analysis of protein content in seed. In this report, four methods were compared for chickpea. Results obtained with a Technicon auto analyser (TAA) were precise and were highly correlated with microKjeldahl (MKJ) values. It is possible to carry out accurate determinations on large numbers of samples within a relatively short time. Therefore, the TAA procedure would be the most suitable method to be used in a breeding programme. As an alternative, where the TAA facility is not available, the dye binding capacity (DBC) procedure can be adapted for the estimation of protein content. The biuret method, due to poor protein extractability, was not as accurate as the TAA or DBC method, but the method may still find use in some programmes, depending mainly on their objectives.

Also, rapid procedure of TAA for protein analysis could be used for both the whole grain and dhal samples, while the DBC procedure seems to be better-suited to analyse dhal samples only in case of pigeonpea. Considering the cost and simplicity of the DBC method in relation to the TAA method, analysis of whole-grain samples by the DBC method is suggested where large number of samples (germplasm) are involved and where ranking of cultivars for their protein content is more important rather than the absolute amount. Small grains gave a lower correlation between whole-grain and dhal protein content and overall only 76% of the variation in dhal protein could be attributed to the variation in whole-grain protein content in pigeonpea.

Based on the analysis of 12653 samples, large variations appear to exist for protein content in chickpea germplasm collections. Protein content of these whole seed samples of chickpea ranged between 14.2 and 31.5 percent with an average value of 19.3 percent. Interestingly, there was no correlation between seed weight and seed protein percent and this indicated that it would be possible to increase both the seed weight and protein content in chickpea.

The protein content of 6215 whole seed samples of pigeonpea germplasm accessions ranged between 15.4 and 27.6 percent whereas of 2832 dhal samples between 16.3 and 28.6 percent. Several wild species of pigeonpea were identified as sources of high protein (28.4-30.5%).

Seed protein content was determined in several genotypes of chickpea grown at different locations in India in different years. Statistical analysis showed that locations had the greatest influence on seed protein content. The effects due to cultivars although significant were of low magnitude. Cultivars x location interactions were found to be nonsignificant and there were good correlations among locations suggesting that breeding for improved seed protein content in chickpea could be effectively carried out at a single location.

1. INTRODUCTION

Legume seeds are primarily important for their supply of protein in the diets of people in many parts of the world. Chickpea and pigeonpea are important grain legumes in several developing countries of SAT regions. Improvement of protein quality of these pulses through effective breeding program is one of the objectives of ICRISAT. The success of such a breeding program will depend on the availability of rapid and accurate analytical procedures for estimating the desired constituents. Therefore, attempts were made to identify accurate, rapid and reliable procedures for the estimation of protein. After identifying a suitable method, protein content was estimated in the available germplasm collections of these crops in order to know the variation for this character. Efforts were also made to study the effects of environments on protein content. The present report summarizes the results on these aspects under the following three main headings.

I. Methods of protein estimation

II. Variability for protein content in the germplasm accessions

III. Protein content as influenced by environments

2. Methods of protein estimation:

Several methods have been reported for the protein estimation in cereals and grain legumes. Every method has its advantages and disadvantages. Since our efforts have been to develop a rapid and

reliable procedure for protein estimation in chickpea and pigeonpea, we investigated the usefulness of the following methods for screening large number of samples for protein content.

2.1 Kjeldahl method:

In 1883, the publication of this method was made by John Kjeldahl and subsequently the method has been named after him. The principle involved in this procedure is well known. The sample is digested in the presence of concentrated sulphuric acid until the nitrogen is transformed into ammonium sulphate. By distilling in the presence of concentrated alkali, the liberated ammonia is collected and measured by a suitable method. The nitrogen content in the sample is calculated from the amount of ammonia liberated. For the estimation of protein content in chickpea and pigeonpea, a standard microKjeldahl (MKJ) procedure (AOAC, 1975) was followed as described below.

A portion of the sample (30-40 mg) was weighed into a microKjeldahl flask and 2 g of digestion mixture consisting of mercuric oxide and potassium sulphate properly mixed in the ratio of 4:190 was added. Then 2 ml of conc. sulphuric acid was added and digested for 1 hr. The digested sample was dissolved in minimum amount of water and transferred to the distillation set. After giving one more washing, 10 ml of 60% sodium hydroxide containing 5% sodium thiosulphate was added. The distillate was collected in 5 ml of 4% boric acid containing 2 drops of mixed indicator (0.2% methyl red and 0.2% bromo cresol green in the ratio of 1:5) for 5 minutes and then titrated against standard hydrochloric acid.

2.2 Technicon auto analyser (TAA) method:

The colorimetric method using the TAA is frequently used in research program where large numbers of samples have to be analysed for protein estimation. In this method, NH_4^+ is estimated colorimetrically in an alkaline medium after reaction with phenol in sodium hypochlorite, (Mitcheson and Stowell, 1969). We have slightly modified the TAA procedure for nitrogen estimation in chickpea and pigeonpea samples. For chickpea and pigeonpea samples, a suitable amount of the sample (60-70 mg) was weighed and placed in a specially made digestion tube of 75 ml capacity. One Kjel-tab (auto tablet) and 3 ml of sulphuric acid-phosphoric acid mixture (95 parts concentrated sulphuric acid, 5 parts of 85% phosphoric acid, v/v) were added to the digestion tube and a set of 40 tubes was digested in a block digestor maintained at 370°C for 1 hr. After cooling, distilled water was added to bring the volume upto the etched mark in the same tube representing a total volume of 75 ml. A suitable aliquot was used for nitrogen estimation using the TAA which is capable of analysing 40 samples per hr with a sample to wash ratio of 9:1. The nitrogen value thus obtained was converted into crude protein content by multiplying with a factor of 6.25. Using this procedure, two persons can analyse about 100 samples a day, which includes the time taken for calculations, preparing the reagents, and washing of glasswares.

2.3 Dye binding capacity (DBC) method:

This method operates on the principle in which the basic amino acids react with the mono-sulphonic azo dye in an acid medium to form an insoluble complex with proteins and results in a decreased intensity of the dye. Thus, the unbound dye concentration is measured colorimetrically as percent of transmission. The estimates of protein from a conversion table are based on colorimetric measurement of unbound dye through its relationship to total nitrogen as determined by the microKjeldahl procedure.

Procedure:

Using the dye, acid orange-12 (obtained from Boulder, Colorado, USA), the following procedure was standardised to estimate the protein content in chickpea and pigeonpea samples. A finely ground sample (320 mg) was weighed and transferred into a plastic bottle and 40 ml of reaction dye solution (acid orange-12, 1.3 mg/ml) was added. The bottles were stoppered and shaken in a reciprocating shaker for 1 hr. The suspension was then filtered using a glass fiber filter and % transmission was recorded against the reference dye solution (obtained from Boulder, Colorado, USA), using a Udy flow through colorimeter. Two persons can analyse about 150 samples a day using this procedure including the preparation of reagents and washing of filters and bottles.

2.4 The biuret method:

The principle involved in this procedure is related to the development of purple colour when substances containing two - $CONH_2$ groups joined either directly or through a carbon or nitrogen atom are treated with copper sulfate in the presence of a strong alkaline solution. The peptide structure as found in proteins and their linkages also give a positive reaction to the biuret test. Two or more peptide linkages are required to give a positive test. Proteins give purplish violet colour while proteases and peptones give a pink colour and peptides give a very light pink colour. This test has been utilized for the estimation of proteins in cereals and grain legumes (Johnson and Craney, 1971, Sodek et al. 1976).

Procedure:

Two modifications of the biuret procedure were used for the estimation of protein and these will be referred as B1 (biuret procedure 1) and B2 (biuret procedure 2) in this report. The biuret reagent for procedure B1 was prepared by mixing 10 ml of 10 N KOH and 20 ml of 25% sodium potassium tartarate. To this was added 40 ml of 4% cupric sulphate pentahydrate while stirring vigorously and the volume was made upto 500 ml. This solution was mixed in equal proportion with propan-2-ol and used. Two hundred mg of sample were weighed and dispersed in 2 ml of propan-2-ol in a conical flask and 50 ml of biuret reagent was added. The flask was stoppered and shaken for 15 min. The extract was classified by centrifugation and read in a spectrophotometer at 550 nm.

For procedure B2, the biuret reagent was the same as described above except that it did not contain $CuSo_4 \cdot 5H_2o$ (Johnson and Craney, 1971). As in the case of B1 method, 200 mg of the sample were taken in a conical flask. Then 200 mg cupric carbonate were added and the contents were dispersed in 2 ml propan-2-ol followed by the addition of 50 ml biuret reagent. The rest of the procedure was same as in the case of B1.

3. Results obtained with chickpea:

3.1 A comparison of different methods of protein estimation in chickpea:

From our germplasm collection, 150 accessions that exhibited a wide range in their protein content from our previous analysis were selected for this study (Singh & Jambunathan, 1980). Whole-seed samples were ground in a Udy mill to pass through a 0.4 mm sieve and were dried overnight at 70°C. The analyses were carried out on these dried samples. Samples were divided into low, medium- and high-protein groups based on crude protein values obtained by the MKJ method. To study the effect of flour particle size on protein estimation, samples of one cultivar (P-1137) were ground in a Wiley mill using 10, 20, 40, 60, 80, and 100-mesh sieves till all the material passed through the sieve. In order to test the influence of seed coat pigment in the biuret and DBC methods, protein content was determined in 'dhal' (decorticated split cotyledon) and whole seed samples. For the preparation of dhal, whole seed were soaked in distilled water overnight at 5-6°C. Excess water was decanted and

seed coats were removed manually. Dhal samples were dried at 70°C overnight in an oven before processed in a similar way for the estimation of protein content.

The results of protein analysis of germplasm accessions by four different methods are given in Appendix I.

Results of correlation coefficients, standard errors of estimation, and regression equations obtained between the MKJ method and other rapid methods evaluated are shown in Table 1. The TAA method was significantly correlated with the MKJ method (r=0.99) and DBC method (r=0.98). Correlation of MKJ method with the biuret method was 0.96 and with the biuret method B2 was 0.95. It was observed that both the procedures gave higher standard errors of estimation in comparison with the DBC and TAA methods.

In order to find out the usefulness of these methods in analysing samples with a wide range of protein content, the correlation coefficients and standard errors of estimation among DBC, TAA and MKJ methods were compared for the low-, medium- and high-protein lines (Table 2). The MKJ values of medium-protein lines had a significantly higher correlation with DBC and TAA procedures as compared to the low- and high-protein lines. On the other hand, correlation between MKJ method and biuret procedures B1 and B2 was higher for the low-protein lines as compared to the medium and high-protein lines (Table 2). This table also shows that both the biuret procedures had higher standard errors of estimation for the high-protein lines when compared to the low- and medium-protein lines. The protein values obtained

Table 1. Statistics for comparing the degree of correlation between TAA, DBC, and biuret methods (B1 & B2) respectively with MKJ method for the estimation of crude protein content (N x 6.25) in chickpea

Method	Correlation coefficient[a]	Standard error of estimate	Regression Equation
MKJ vs TAA	0.99	0.55	y = 0.29 + 1.001x
MKJ vs DBC	0.98	0.69	y = 7.43 + 0.35x
MKJ vs DBC	0.98	0.69	y = 1.05 + 0.67x - 0.00376x2
MKJ vs Log DBC	0.98	0.69	y = 130.95 + 33.01x
MKJ vs B1	0.96	0.99	y = 6.57 + 101.22x
MKJ vs B2	0.95	0.95	y = -11.81 + 102.02x

a Significant at 1% level. B1 Modified biuret method of Pinckney (1961). B2 Modified biuret method of Johnson and Craney (1971).

by Biuret B1 and B2 in comparison with MKJ on 134 chickpea whole seed samples are listed in Appendix 2.

Correlation studies (Tables 1 and 2) indicated that the MKJ and other methods examined in the present investigation did not exhibit significant differences in the mean protein content values. However, it was osberved that the mean protein content for low-protein lines obtained by the DBC method was slightly higher than the MKJ mean protein content (Table 3). This was also apparent from the relationship between the MKJ and DBC methods. The use of a linear regression equation between DBC and MKJ protein values over estimated the MKJ protein content in the low-protein lines. However, the use of

Table 2. Correlation coefficients and standard errors of estimate of different methods of protein (N x 6.25) estimation in comparison with MKJ method for low-, medium-, and high protein chickpea lines

Method	Correlation coefficient[c]			Standard error of estimate		
	Low	Medium	High	Low	Medium	High
MKJ vs TAA	0.84	0.96	0.86	0.56	0.47	0.56
MKJ vs DBC[a]	0.77	0.95	0.80	0.59	0.50	0.57
MKJ vs DBC[b]	0.78	0.95	0.81	0.59	0.50	0.57
MKJ vs B1	0.83	0.79	0.68	0.71	0.65	0.81
MKJ vs B2	0.80	0.78	0.73	0.54	0.73	1.02

a Linear regression equation. b Curvilinear regression equation.

c All values significant at 1% level.

new conversion table based on a curvilinear regresssion equation between DBC and MKJ protein values slightly improved the results (Table 3). A regression equation between log DBC reading and MKJ protein values was calculated and there was no significant difference between the protein values obtained by using this equation and those obtained by using the curvilinear regression equation.

Considerable variations in the protein values, particularly in high-protein lines, were observed when the samples were analysed by biuret methods B1 and B2 (Table 4). This was also reflected in the lower correlation obtained between these methods and the MKJ method (Table 2). One reason for the observed low correlation between the two methods may be due to the poor extraction of protein as a result of using propan-2-ol in the biuret reagent as described below.

Table 3. Mean protein content (N x 6.25) of different groups of chickpea lines as determined by TAA, DBC, and MKJ methods

Method	Mean protein conent (%)			
	Low n=56	Medium n=49	High n=45	Total n=150
MKJ	17.81 (14.9-19.8)	23.11 (20.2-25.0)	26.47 (25.2-29.6)	22.18 (14.9-29.6)
TAA	17.58 (14.7-19.5)	22.90 (19.4-25.5)	26.03 (24.9-29.5)	21.86 (14.7-29.5)
DBC[a]	18.13 (15.8-20.0)	22.89 (19.0-25.8)	26.44 (24.3-30.6)	22.18 (15.8-30.6)
DBC[b]	17.98 (15.0-20.3)	23.20 (19.0-25.9)	26.27 (24.4-28.9)	22.18 (15.0-28.9)
L.S.D. (5%)	0.43	0.61	0.51	0.85

Figures within the parenthesis indicate the range of protein content in the samples analyzed; a Linear regression equation; b Curvilinear regression equation.

3.1.1 Effect of different concentrations of propan-2-ol on protein extraction in chickpea:

In order to study the effect of different concentrations of propan-2-ol on protein extraction, 10 ml of 1M KOH was taken in each of the 100-ml volumetric flasks and, after adding 10, 20, 30, 40, 50, and 60 ml of propan-2-ol to the respective flasks, the final volume was made to 100 ml. Fourteen sub samples of 200 mg each were dispersed in 1 ml of propan-2-ol. To each of the two sub samples,

Table 4. Mean protein content (N x 6.25) of different groups of chickpea lines as estimated by biuret methods (B1 and B2) and MKJ method

Medium	Mean Protein Content (%)			
	Low n=42	Medium n=49	High n=43	Total n=134
MKJ	17.82 (15.2-20.8)	23.07 (21.5-25.0)	26.87 (25.3-29.6)	22.64 (15.2-30.0)
B1	18.12 (14.3-22.2)	23.26 (19.7-25.6)	26.43 (24.4-30.4)	22.67 (14.3-30.4)
B2	18.30 (14.5-21.4)	23.03 (19.3-26.4)	26.45 (25.2-30.5)	22.65 (14.5-30.5)
L.S.D. (5%)	0.70	0.66	0.61	0.90

Figures within the parenthesis indicate range of protein content in the samples analyzed. B1 Modified method of Pinckney (1961). B2 Modified method of Johnson & Craney (1971).

40 ml KOH solutions containing a different concentration of propan-2-ol was added. Flasks were shaken for 15 min using a mechanical shaker. After centrifugation at 3000 x g for 10 min, the protein contents in the supernatants were determined by the MKJ method. The amount of N extracted decreased as the concentration of propan-2-ol increased (Table 5) but at a concentration of 40% or less, the extracts obtained after centrifugation were not clear, indicating the interference of pigments in the extraction procedure.

Table 5. Effect of different concentrations of propan-2-ol on nitrogen extraction from chickpea meal[a]

Concentration of 2-propan-ol (v/v)	% nitrogen extracted
0	84.9
10	80.0
20	74.3
30	70.2
40	61.4
50	56.7
60	49.3

a Mean of two independent determinations

Earlier workers have reported that the use of 50% propan-2-ol in biuret reagents promoted the extraction of all proteins from beans (Sodek et al. 1976). Higher concentrations of propan-2-ol favor the solubility of cereal seed proteins which contain large amounts of alcohol-soluble protein (Concon, 1973). This is not the case with the grain legumes which contain mostly salt-soluble proteins and have very little alcohol-soluble protein. In the present study, although the use of 50% propan-2-ol extracted only 57% of nitrogen (the results were comparble with MKJ values), this may be a fortuitous coincidence. It would seem that incomplete protein extraction and interference of tannins and other pigments, in colorimetric assays are the two main reasons for the unsuitablllity of the biuret method for protein estimation in chickpea.

3.1.2 Effect of shaking and particle size on protein estimation in chickpea:

Some factors were investigated in establishing the conditions for the biuret (B1) and DBC methods for protein estimation in chickpeas. Increasing the shaking time (> 15 min) at room temperature had no measurable effect on the absorbance of clarified extract for biuret method B1. With the DBC method, readings increased considerably upto 1 hr of shaking, and further mixing had no measurable effect on the dye binding reading (Table 6).

Table 6. Effect of shaking on protein estimation by DBC and biuret methods in chickpea

Shaking time (min)	Protein (%)[a]	
	DBC	Biuret
15	15.8	16.2
30	16.5	16.4
60	16.7	16.5
90	16.7	16.4
120	16.7	16.6

a P-1137; Average of two determinations.

Flour of finer particles of chickpea was found to give higher protein values by all the procedures tested (Table 7). Differences in protein values estimated by the biuret (B1) and DBC methods were greater than MKJ and TAA values. DBC results obtained between 20 and 60-mesh screen samples were in good agreement with the MKJ method. But in the case of the modified biuret method, 40 and 60-mesh samples produced results in good agreement with MKJ values. As it would be impracticable to grind all samples to a very fine particle size, it would be convenient from the point of energy and time consideration to use a particle size of 40-60 mesh for routine screening of large numbers of samples.

Table 7. Effect of particle size on protein estimation in chickpea by four methods[a]

	Particle size (mesh)					
Method	10	20	40	60	80	100
	Protein (%)					
MKJ	16.2	16.3	16.1	16.4	17.3	17.3
TAA	16.3	16.8	16.7	16.5	17.2	17.7
DBC	14.1	16.3	16.2	16.3	18.3	18.7
B1	8.0	11.9	15.7	16.8	20.7	21.3

a Mean of two independent determinations. B1 Modified biuret method of Pinckney (1961).

3.1.3 Interference of seed coat pigments in protein estimation in chickpea:

To study the influence of seed coat pigments on protein estimation, whole seed and dhal samples from ten cultivars each having different seed coat colours were analysed by the biuret (B1) procedure and DBC method. The values were compared with MKJ values (Table 8). Results of protein analyses did not show any interferences due to seed

Table 8. Effect of seed-coat pigments on protein estimation in chickpea by DBC, Biuret (B1) and MKJ methods[a]

Cultivar	100-seed wt(g)	Color	Seed coat (%)	Protein (%)						
				MKJ			DBC		Biuret (B1)	
				Seed coat	Whole-seed	Dhal	Whole-seed	Dhal	Whole-seed	Dhal
NP-34	12.5	White	15.1	3.1	16.3	18.6	16.8	18.9	16.1	18.5
P-3090	21.9	"	14.4	4.0	19.7	22.8	19.7	23.1	19.8	23.4
L-550	20.1	Salmon white	4.5	5.5	18.8	19.5	19.6	20.3	18.8	19.6
K-4	18.1	"	5.8	5.2	15.6	16.5	16.0	17.0	15.4	16.0
G-130	13.7	Yellow brown	14.5	4.3	20.9	24.6	20.7	25.0	20.7	24.0
BEG-482	12.6	"	17.5	3.8	21.0	26.1	21.8	27.2	20.7	25.8
BR-170	12.6	Brown	15.2	3.8	19.7	23.3	20.1	23.3	20.0	24.0
G-24	10.4	"	16.1	3.4	16.7	19.6	16.4	19.7	17.1	20.0
Kaka	10.7	Black	16.0	3.7	16.9	20.5	16.4	20.0	16.9	20.1
L-345	10.5	Green	16.0	3.6	22.0	25.2	21.5	24.2	21.6	24.9

a Mean of two determinations

coat pigments. The differences in the protein content of whole-seed and dhal samples seemed to be related to differences in seed coat of the sample. This observation was confirmed by comparing the results of these two methods with the MKJ method in which seed coat pigment did not interfere in the estimation of protein content. For example, in the case of BEG-482 (yellow-brown) cultivars, whole-seed and dhal samples differed significantly in their protein contents (5.1%) and the seed coat content of BEG-482 was 17.5%. In L-550 (salmon white) cultivar, the difference between whole seed and dhal protein was small (0.7%) and L-550 had only 4.5% of seed coat. This indicates that the seed coat, which is inversely related to seed weight affects the protein content of whole chickpea samples.

To conclude, it is suggested that TAA procedure for the determination of protein should be used in a breeding program for screening purpose as the results obtained with the TAA procedure were precise and highly correlated with MKJ values. As an alternative, where TAA facility is not available, the DBC procedure can be adapted for the estimation of protein content. The incomplete protein extraction and the interference of tannins and other pigments in colorimetric assays are the two main reasons for the unsuitability of biuret methods for protein estimation in chickpea.

3.2 Dhal protein content as influenced by methods of seed coat removal in chickpea:

Most of the laboratories determine protein content in either whole seed or dhal sample depending on the priority, accuracy and rapidity of the analysis. Although we have carried out the protein analysis using whole seed samples of chickpea, we determined the effect of methods of seed coat removal on the protein values of dhal samples. Seed coat is generally removed from the seed by following wet and dry methods. In case of wet method seeds are soaked in water

Table 9. Effect of the methods of seed coat removal on dhal protein content in chickpea

Cultivar	Dry method		Wet method[c] (soaking temp.)	
	Control[a]	Barley Pearler[b]	5°C	25°C
	Dhal Protein (%)			
G-130	21.45	19.89	21.40 (0.012)	21.13 (0.24)
Annigeri	18.54	17.69	18.52 (0.014)	18.30 (0.25)
L-550	17.83	16.01	17.82 (0.013)	17.54 (0.22)
850-3/27	20.62	19.04	20.54 (0.012)	20.40 (0.21)

Values within parenthesis are protein percentages lost in soaking water. a Without soaking seed coat was removed manually using forceps; b Without soaking seed coat was removed using Barley Pearler; c After soaking for 16 hr seed coat was removed manually using forceps.

prior to seed coat removal whereas in dry method this step is not followed. Seed coat was removed manually by soaking the seeds at cold temperature (5°C) and at room temperature (25°C). Seed coat was also removed manually and by Barley Pearler without soaking the seed. A comparison of protein values obtained on dhal prepared by different methods is given in Table 9. The negligible amount of nitrogen was lost when seeds were soaked at 5°C. More nitrogen was lost in case of soaking at room temperature and this could be due to an increased solubility of proteins at higher temperature. The analysis of soaking water for nitrogen content also revealed such differences. Seed coat removal by Barley Pearler was not found satisfactory as it resulted in a noticeable reduction in protein values of dhal sample. This might have been due to the removal of protein rich peripheral layers of cotyledons by the abrasive action of the roller in Barley Pearler. However, the results suggest that soaking of seed at low temperature may be followed for protein analysis on dhal samples in chickpea.

3.3 Nonprotein nitrogen (NPN) and total nitrogen in chickpea:

In the normal procedure for estimating protein intake, nitrogen content is obtained by the standard micro-Kjeldahl method and a factor is used to convert the figure into protein percentage.

In this process, it is tacitly assumed that all the nitrogen is associated with the protein. But in fact, this is not true. Therefore any large variation in NPN content would affect the estimated protein of the sample and would consequently affect the

estimated protein intake in the diet. However, some of the NPN probably consists of amino acids and peptides which would be utilized. Experiments were conducted to determine the variation, if any, that might exist in chickpea samples and to identify the relationship between NPN and crude protein nitrogen in chickpea.

From the chickpea germplasm lines grown at ICRISAT Center during 1975-76 and analysed for protein content in our laboratory, 98 accessions with a wide range in crude protein were selected for this study. Whole-seed samples were ground to a fine meal (60-mesh sieve) and oven dried at 70°C overnight. Direct extraction of meal NPN using different trichloroacetic acid (TCA) concentrations (1,5,10,15 and 20%) and 80% ethanol, was carried out on the sample in order to determine the variability in the amount of nitrogen extracted.

Table 10. Effect of ethanol and TCA on N solubility of chickpea meal[a]

Solvent	Concn %	Nonprotein nitrogen as % of Meal	Nonprotein nitrogen as % of Total nitrogen
Ethanol (v/v)	80	0.12	3.69 ± 0.22
TCA (w/v)	1	0.57	16.92 ± 0.44
	5	0.33	9.58 ± 0.38
	10	0.23	6.86 ± 0.11
	15	0.27	7.97 ± 0.26
	20	0.29	8.58 ± 0.18

a Defatted whole-seed sample of chickpea (cv. G-130). Mean of eight determinations.

Five-hundred mg of the sample dispersed in 15 ml solvent were shaken in a centrifuge bottle, using a reciprocating shaker for 1 hr at room temperature. The insoluble material was sedimented by centrifugation (12,000 g for 15 min). Residue was washed twice with the solvent with 1/2 hr shaking each time and then centrifuged to separate the insoluble material. The supernatants were combined and final volume was made up to 25 ml. Nitrogen content in the meal sample and in the supernatants was determined by the standard micro-Kjeldahl procedure. To determine the levels of NPN in different germplasm lines, TCA concentration of 10% (w/v) was used. Extraction procedure was same as described above. It was observed that further extraction of the residue with TCA did not yield any additional soluble nitrogen. Attempts were also made to find out the amount of protein nitrogen solubilized by 10% TCA using the biuret procedure.

TCA extracted more meal nitrogen than ethanol (Table 10). However, it is also apparent from Table 10 that concentrations of 1 and 5% TCA extracted higher meal nitrogen than did 10% TCA. Presumably, lower concentrations of TCA extracted proteins in addition to NPN from the meal. In order to find out whether the lower concentrations of TCA had extracted proteins, the aliquots of 1 and 5% TCA extracts were adjusted to a final TCA concentration of about 10% (w/v). As a result, proteins were precipitated from the extracts, indicating that the lower concentration of TCA extracted proteins as well as NPN from the flour meal. In this study, extraction of the meal with TCA concentrations up to 10% did not cause protein hydrolysis, as shown by decreasing solubility of meal nitrogen.

Slightly higher values for NPN were observed when 20% TCA concentration was used (Table 10). Extraction of more nitrogen by 20% TCA does not necessarily mean that hydrolysis occurred. This may also indicate that perhaps the proteins are soluble at TCA concentrations above 10%.

Having examined the effects of different concentrations of TCA on nitrogen solubility of chickpea meal, we made further attempts to determine the amount of protein nitrogen solubilized by 10% TCA. As mentioned earlier, the residue and supernatant obtained after 10% TCA treatment were analysed for their protein content by the biuret procedure and for total nitrogen by the micro-Kjeldahl procedure. It was observed that only 3% of the total protein was solubilized by 10% TCA while 10.5% of total nitrogen of the meal was found in the supernatant. A positive reaction is obtained with the biuret reagent even with small peptides. Therefore, small peptides may be present as such in mature chickpea seeds. As a negligible amount of protein (peptides) was dissolved by 10% TCA, it can be concluded that the values obtained by direct extraction using 10% TCA represent the NPN of the meal.

Based on the results of this investigation, a TCA concentration of 10% (w/v), at which nitrogen solubility was observed to be minimal, was employed for the extraction of NPN in germplasm samples. The means and ranges of total meal nitrogen and NPN in 98 germplasm lines of chickpea are presented in Table 11. Total meal nitrogen in these lines varied between 2.43 and 4.85%, whereas NPN as percentage of the

Table 11. Correlation coefficients (r) between total nitrogen and nonprotein nitrogen in ninety-eight germplasm accessions of chickpea

Component	Range	Mean	r (of % total N)
Total N as % of meal	2.43 - 4.85	3.58	-
NPN as % of meal	0.16 - 0.73	0.36	0.802[a]
NPN as % of total N	5.84 - 16.48	9.84	0.468[a]

a Significant at the 1% level.

sample varied between 0.16 and 0.73. A positive and highly significant correlation (r=0.80) was obtained between percentage of the total meal nitrogen and percentage of the NPN of the meal. On the other hand, when expressed as percentage of the meal nitrogen NPN varied between 5.84 and 16.48 and showed a lower but appreciable correlation (r=0.47) with the percentage of the total nitrogen in the meal. Therefore, whether expressed either as percentage of the meal or as percentage of the total nitrogen NPN increased when the total nitrogen of the meal increased. It is evident from these results that all nitrogen present in chickpea is not associated with seed protein, suggesting that NPN has to be taken into account if total protein content is to be measured accurately.

4. Results obtained with pigeonpea

4.1 A comparison of different methods of protein estimation for pigeonpea:

For this study, the seed samples from a breeders' trial comprising 7 early, 14 medium and 22 late cultivars in a randomized block design with four replicates and samples from 83 germplasm lines were used. They were grown at ICRISAT Center, Patancheru (near Hyderabad) during the 1977-78 and 1978-79 rainy seasons, respectively. The weight of 100 seeds was determined for each cultivar and whole grain and dhal samples were analysed. Dhal samples were prepared by soaking the whole seeds in distilled water overnight at 5°C. Excess water was decanted and seed coats were removed from the seeds manually. The whole grain, seed coat, and dhal fractions were dried at 70°C overnight in an oven and then weighed. Samples were ground in a Udy cyclone mill to pass through a 0.4 mm sieve.

The ranges and means of the protein content of the different seed components of the 43 cultivars from the breeders' trial determined by the MKJ method are shown in Table 12. Seed coat content ranged between 13.2 and 18.9% and 100-grain weight varied from 6.3 to 13.9 in these cultivars. A negative and highly significant correlation ($r = -0.80^{**}$) was obtained between the grain weight and seed-coat content. Individual results of analysis of each of the cultivar are given in Appendix 3. Protein content varied between 17.9 and 24.3% for whole grain and between 21.1 and 28.1% for dhal samples. On an average, dhal protein was found to be 3.1 units higher than the whole

Table 12. Ranges and means of components of pigeonpea[a]

Constituent	Min	Max	Mean	Correlation with 100-grain wt
100-grain wt (g)	6.3	13.9	9.9	
Seed coat (%)	13.2	18.9	15.5	-0.80**
Protein content[b] (%):				
Seed coat	4.5	6.4	5.4	0.20
Whole grain	17.9	24.3	21.2	0.16
Dhal:				
Determined[b]	21.1	28.1	24.3	0.13
Calculated[c]	19.9	27.6	23.6	
Calculated[d]	20.8	28.5	24.2	

a Based on an analysis of 43 cultivars; b MKJ values;

c Using the equation: Pd = Pwx100-PscxSc/100-Sc;

d Using a linear multiple regression equation (see text);

** Significant at 1% level.

grain protein content. Although the differences between calculated and observed dhal protein values existed, they were not statistically significant. The calculated mean values for dhal protein content were

less than the observed values. The protein values of whole seed might have been underestimated because of the presence of seed coat. No significant correlation between protein content and grain weight for these cultivars was osberved. This was also confirmed when 83 germplasm lines with a wide range in 100-grain weight (4.9 to 21.1 g) were analysed for protein content by the TAA method. Protein content and 100-grain weight of these lines are shown in Appendix 4.

The protein values obtained by the TAA and DBC methods were compared with those of the MKJ method using the results obtained for the 43 cultivars from the breeders' trial. Table 13 illustrates the correlation coefficents and standard errors of the estimates between MKJ, TAA and DBC methods. The MKJ procedure was found to be positively and significantly correlated with TAA procedure for the whole grain (r=0.95) and dhal (r=0.97) protein.

Correlation of the values of MKJ method with those of the DBC method was 0.87 for whole grain and 0.94 for dhal samples. Also, the standard error of estimate was higher for whole-grain (r=0.83) as compared to dhal samples (r=0.70). This difference could be due to the interference of seed coat pigments in DBC method. When the whole-grain and dhal samples each containing about equal protein content were analysed it was observed that the seed coat absorbed some of the dye resulting in higher DBC values reading (percent transmission) in the case of whole grain samples.

Table 13. Comparison of methods of protein estimation for whole-grain and dhal samples of pigeonpea

Method	Correlation coefficient	Standard error of estimate (% protein)	Regression equation
1. Whole-grain protein:			
MKJ vs TAA	0.95**	0.53	Y = 0.94 + 0.95X
MKJ vs DBC	0.87**	0.83	Y = 0.99 + 0.97X
2. Dhal protein:			
MKJ vs TAA	0.97**	0.61	Y = 3.41 + 0.87X
MKJ vs DBC	0.94**	0.70	Y = 2.20 + 0.92X

** Significant at 1% level.

4.1.1 Factors that affect the protein estimation by the DBC method in pigeonpea:

The effects of duration of mixing, flour particle size, and temperature on protein values of whole grain and dhal samples of two cultivars estimated by the DBC method were investigated. It was found that the smaller size flour particle (40-mesh) sample had a higher protein content compared to a 20-mesh sample (Table 14), indicating the effect of interaction of finely ground materials. Different durations of mixing did not significantly affect the protein values although the protein percentage increased with longer mixing time (Table 14). Such variation among the cultivars might also affect the

Table 14. Effect of flour particle size and time of mixing on protein estimation by DBC method in pigeonpea[a]

Cultivar	Particle mesh size[b]			Time of mixing (min)[c]				
	20	40	60	15	30	60	90	120
				Protein %				
Whole grain:								
HY-3C	18.5	19.9	20.3	19.8	20.1	19.7	19.9	19.9
Gwalior-3	19.2	22.7	23.1	22.5	22.8	23.1	23.4	23.4
Dhal:								
HY-3C	22.0	24.0	24.2	23.7	23.8	23.8	24.0	24.1
Gwalior-3	23.6	27.0	27.4	26.5	26.8	27.1	27.5	27.6

a Average of two estimations; b Mixed for 60 minutes; c 60-mesh samples.

correlation between the MKJ and the DBC methods. However, for routine screening it was osberved that the DBC results of 40 and 60 mesh samples were similar to the MKJ values.

To test the effect of heating on protein estimation, whole grain and dhal samples of three cultivars each were dried at 70, 100, and 130°C for 24, 15, and 2 hr, respectively, and DBC values were obtained on these samples. Moisture percentages lost due to various treatments were determined, and protein values obtained on undried samples were appropriately corrected to obtain estimated values (Table 15).

Table 15. Effect of heating on protein estimation by DBC method in pigoenpea

Cultivar	Component	Sample treatment			
		Fresh wt basis	70°C for 24 hr	100°C for 15 hr	130°C for 2 hr
		Protein (%)			
HY-3C	Whole grain	21.8	23.0 (23.2)	23.0 (23.3)	23.0 (23.5)
	Dhal	24.0	25.2 (25.4)	25.1 (25.7)	25.5 (25.9)
ST-1	Whole grain	23.0	25.4 (24.6)	25.0 (24.8)	25.0 (25.0)
	Dhal	25.5	27.2 (27.1)	26.8 (27.3)	27.2 (27.5)
Sharda	Whole grain	22.8	24.1 (24.3)	23.9 (24.5)	24.2 (24.6)
	Dhal	24.8	26.6 (26.3)	26.2 (26.6)	26.3 (26.9)

Values within parenthesis are the estimated values obtained by applying the moisture correction to protein values obtained on undried fresh samples.

When determined by DBC method, only a slight variation in protein values was observed due to heating. This suggests that heat treatments as described above may have no significant effect on protein estimation by DBC method.

4.1.2 Relationship between whole grain and dhal protein contents in pigeonpea:

A positive and significant correlation (r=0.87) was observed between the whole-grain and dhal protein contents determined by the MKJ method (Table 16), while the TAA and DBC methods exhibited correlation coefficients of 0.89 and 0.77, respectively. The relatively lower correlation coefficient obtained by the DBC method could be due to the interference of seed coat pigments in the whole grain samples.

Table 16. Correlation coefficient and standard error of estimate between whole-grain and dhal protein content obtained by MKJ, TAA and DBC methods[a]

Method	Correlation between whole-grain and dhal protein	Standard error of estimate (% protein)	Regresssion Equation
MKJ	0.87**	0.78	Y = 5.81 + 0.87X
TAA	0.89**	0.65	Y = 5.59 + 0.88X
DBC	0.77**	1.03	Y = 7.30 + 0.80X

a Based on 43 cultivars

The relationship between whole-seed and dhal protein content can be affected by the percentage of seed coat, its protein content, and grain weight. The effect of seed coat percentage and its protein content were examined for the 43 cultivars by calculating the expected protein content of dhal according to the following equation: Pd = Pwx100-PscxSc/100-Sc where Pd, Pw, and Psc are percentages of

dhal, whole-grain, and seed-coat protein, respectively, and Sc represents the percentage of seed coat in the whole grain samples. The minimum, maximum, and mean values are reported in Table 9. The calculated dhal protein percentages differed from the observed values by 0.5 to 8.7 percentage units.

Further, whole grain and dhal samples of a different lot of 83 germplasm accessions with a wide range in grain weight were analysed for protein content by the Technicon auto analyser (Table 14). The results of protein analysis of these lines are given in Appendix 4. The difference in the protein content of whole grain and dhal samples of these lines varied between 2.9 and 3.7 percentage units. Whole grain and dhal protein values showed a higher correlation coefficient ($r=0.93$) for the medium group as compared to that of low and high groups thus indicating a variability in relationship among the different groups (Table 14). Also the correlation coefficient of all the three groups together was 0.87 indicating that about 76% variation in dhal protein content may be related to the whole seed protein content. In the case of low group, only 63% of variation in dhal protein was associated with the variation in the whole grain protein, and this might be due to the observed negative correlation between the grain weight and percentage of seed coat.

In an attempt to find out if the correlations could be improved by the use of variables like percentage of seed coat and protein percentage in seed coat, the following linear multiple regression

Table 17. Relationship between the protein content of whole-grain and dhal samples in 83 germplasm accessions analysed by the Technicon Auto Analyser

Group	100 grain wt (g)	Protein (%)		Unit difference between whole seed and dhal protein	Correlation coefficient[a]
		Whole grain	Dhal		
Low (n=28)	7.0	21.3	25.0	3.7	0.79**
Medium (n=27)	9.6	21.5	24.9	3.4	0.93**
High (n=28)	14.2	20.8	23.7	2.9	0.88**
Total (n=83)	10.3	21.2	24.6	3.3	0.87**

Mean values and ranges are shown in parenthesis. a Between whole grain and dhal protein contents. **Significant at 1% level.

equation was obtained : Y = 0.92 + 1.14x1 - 0.22x2 + 0.19x3, where x1, and x2 and x3 represent the percentages of whole grain protein, seed coat content and seed coat protein, respectively. A correlation coefficient of 0.92 was obtained between the whole grain and dhal protein content. As expected, a slight improvement in the coefficient between these variables was achieved. Using this equation, dhal protein content was calculated for 43 cultivars, and the minimum, maximum, and mean values are reported in Table 9. The calculated dhal protein percentages varied from -1.4 to 3.7 from the observed values. But this equation will find little use in a screening program as it involves the estimation of other components also.

To conclude, it may be mentioned that rapid procedure of TAA could be used for the analysis of protein content in pigeonpea whole grain and dhal samples, while the DBC procedure seems to be better suited to analyse dhal samples only. Considering the cost and simplicity of the DBC method in relation to the TAA method, analysis of whole grain samples by the DBC method is suggested where large numbers of samples (eg. germplasm) are involved and where ranking of cultivars for their protein content is more important rather than the absolute amount. However, in a selection procedure for high protein lines involving smaller number of samples, analysis of dhal samples is preferable. Small grains gave a lower correlation between whole grain and dhal protein content and overall only 76% of the variation in dhal protein could be attributed to the variation in whole grain protein content.

4.2 Estimation of error of protein determination in pigeonpea:

An experiment was conducted in cooperation with breeders and statistician for estimating the relative importance of error of laboratory determination, sampling error, and field sampling of genotypes for protein determination. Materials consisted of 10 cultivars grown in 3 replicates test with maize intercrop on black soil. Two sub-samples were taken from seed from each plot, and each of these was subdivided in the lab for two determinations of protein

Table 18. Analyses of variance of results with 10 cultivars analysed for protein in a test to estimate relative error due to determination, sampling and genotype x environment interaction

		5.4.77		14.4.77	
Source of variation	D.F.	M.S.	F.	M.S.	F.
Among replicates	2	1.85	1.65	1.10	0.80
Among genotypes	9	3.53	3.10*	4.11	2.98*
Reps. x genotypes	18	1.14	-	1.38	-
Sampling error	30	0.34	-	0.30	-
Determination error	60	0.43	-	0.22	-

* Significant at 5% level.

by the Technicon auto analyser. The test was repeated on two different days. Combined analysis showed virtually no effect of days. Analyses of variance for the two days are presented in Table 18.

It is apparent that error of determination in the laboratory and sampling within the lot of seed were both insignificant sources of error in relation to the genotype x replicate interaction. Differences among replicates were not significant, and differences among genotypes were significant at the 5% level. Three important indications from this study are: (1) Single determinations on single samples should be sufficient to measure differences among seed lots; (2) The genotype x environment interaction is important enough to raise questions of the validity of estimates on single plants or unreplicated plots; and (3) with three replicates, relatively small differences in protein level among genotypes can be detected.

4.3 Dhal protein content as influenced by methods of seed coat removal in pigeonpea:

As it was done in case of chickpea, the effect of methods of seed coat removal on dhal protein content in pigeonpea was determined. Similarly, wet and dry methods of seed coat removal were compared. Higher protein values were obtained for dhal samples prepared by dry method but the differences were not large enough to question the validity of analysis of dhal samples prepared by wet methods (Table 19). However, it should be noted that seed soaking at higher temperature would yield lower protein value on dhal sample. The nitrogen content of soaking water was more in case of soaking at room temperature as compared to the soaking at low temperature. Since the dry method of seed coat removal is tedious and time consuming, wet method should be preferred and further soaking at low temperature is desirable. Unlike chickpea, seed coat removal by Barley Pearler was found satisfactory as no greater losses in dhal protein values were obtained. Barley Pearler fitted with a wooden roller was tried and suitable modifications introduced. This will be discussed in detail in a separate progress report of our department.

5. Genetic variability for protein content in the germplasm accessions:

As stated earlier, in a crop improvement program with an objective to improve the nutritional quality of the grain, one of the task should be to screen the available germplasm accessions for protein content and limiting essential amino acids in order to

Table 19. Effect of the methods of seed coat removal on dhal protein content in pigeonpea.

Cultivar	Dry method		Wet method[c] (Soaking temp.)	
	Control[a]	Barley Pearler[b]	5 °C	25 °C
	Dhal Protein (%)			
C-11	22.84	22.40	22.75 (0.025)	22.18 (0.46)
BDN-1	23.06	22.73	22.93 (0.056)	22.75 (0.38)
HY-3C	21.85	21.52	21.78 (0.043)	21.28 (0.40)
LRG-36	22.63	22.07	22.45 (0.030)	22.18 (0.31)

Values within parenthesis are protein percentages lost in soaking water. a Without soaking seed coat was removed manually using forceps; b Without soaking seed coat was removed using Barley Pearler; c After soaking in distilled water for 16 hr seed coat was removed manually using forceps.

identify the lines having the desirable amino acid profiles and protein content. So the analyses of chickpea and pigeonpea germplasm accessions for their protein content was undertaken to know the variability for this character.

5.1 Protein analysis of germplasm accessions of chickpea:

The world collections of chickpea germplasm accessions available in our Genetic Resources Unit were analysed for protein content by the dye binding capacity (DBC) procedure as described and discussed earlier. A brief acccount of the source of these lines is given in Table 20.

Table 20. Accession details of world chickpea germplasm collection

Country	Total Number	Country	Total Number
India	4983	Jordan	24
Iran	4091	Cyprus	21
Afghanistan	675	Iraq	18
Turkey	432	Algeria	18
Mexico	264	Italy	18
Ethiopia	159	Lebanon	18
Pakistan	151	Syria	12
U.S.A.	108	Chile	9
U.S.S.R.	89	Czechoslovakia	8
Spain	77	Burma	6
Morocco	53	Bulgaria	5
Egypt	50	Hungary	4
Isreal	48	Portugal	4
Tunisia	30	Sudan	4
Greece	24	Others	80

Several accessions have been added to our collection since these analyses were made. A complete catalogue of these lines is available with our Genetic Resources Unit. In order to ensure the accuracy of the DBC method, every twentieth or so sample from each lot that was analysed by the DBC procedure was again analysed by the standard microKjeldahl method. The correlations between the DBC and microKjeldahl method were found ranging between 0.95 and 0.99 for different lots of samples analysed during that period. For checking the reproducibility of the procedure, protein estimation of different lots of bulk check samples were carried out during the analysis and the results were tablulated and standard errors and coefficients of variation were worked out as shown in Table 21. The coefficients of variation of estimation ranged between 2.01 and 5.48% during the entire period of analysis.

Table 21. Standard error and coefficient of variation of DBC method used for protein estimation in chickpea[a]

Year	Cultivar	n	Range	Mean	SE	CV
			... Protein (%) ...			
1976-77	P-1137	40	17.4 - 18.6	18.0	0.44	2.45
1977-78	P-1137	21	17.6 - 18.5	18.1	0.42	2.37
1978-79	L-550	16	20.4 - 21.8	21.1	0.50	2.83
1978-79	L-550	16	20.2 - 22.1	21.2	1.16	5.48
1979-80	L-550	29	19.2 - 20.3	19.8	0.48	2.41
1980-81	G-130	8	23.5 - 24.4	24.0	0.48	2.01

a Analysis of whole seed sample.

Large variations appear to exist for protein content in chickpea germplasm collections. Percent protein in whole seed chickpea ranged between 14.2 and 31.5 percent with an average value of 19.3 percent as presented in Table 22. The analysis of check samples showed large variation (Table 23). As the entries were grown over different

Table 22. Variability of protein content in germplasm accessions of chickpea[a]

Year	No of samples	Protein (%)	
		Range	Mean
1975-76	761	14.2 - 24.5	18.4
1976-77	3656	14.3 - 30.9	19.0
1977-78	3360	14.8 - 31.5	19.5
1978-79	1874	17.3 - 28.3	20.6
1979-80	1609	15.4 - 29.6	18.8
1980-81	1393	14.8 - 27.4	20.8
1981-82[b]	640	17.4 - 29.3	22.7

a Whole seed samples analysed by dye binding capacity (DBC) method.

b Dhal samples were analysed by Technicon auto analyser.

years, some differences in the results are expected to be due to environmental interactions. Analysis of a limited number of cultivars grown at 4 different locations was carried out to study the environmental interactions due to location. The results indicated that while location effect was nonsignificant the varietal differences with regard to protein content were significant this has been discussed in more detail in the following sections:

Table 23. Variation in protein values of check sample of chickpeas (cv.G-130,L-550 and JG-62) analysed during different years[a]

Year	Cultivar	n	Range	Mean	SD	CV
			... Protein (%) ...			
1975-76	G-130	37	16.7 - 23.3	19.7	1.63	8.28
	L-550	29	18.5 - 22.8	20.1	1.32	6.56
	JG-62	30	16.4 - 23.4	19.2	1.65	8.63
1976-77	G-130	70	18.3 - 26.6	21.8	1.83	8.39
	L-550	71	18.2 - 26.3	21.9	1.78	8.13
	JG-62	67	17.6 - 25.7	21.1	1.84	8.70
1977-78	G-130	33	19.1 - 25.6	21.8	1.63	7.50
	L-550	32	18.2 - 25.4	21.2	1.48	6.99
	JG-62	31	18.4 - 23.6	20.5	1.19	5.81
1978-79	G-130	47	16.8 - 24.5	19.9	2.20	11.05
	L-550	3	17.3 - 23.2	19.4	3.32	17.16
	JG-62	17	15.7 - 20.6	17.9	1.16	6.48
1979-80	G-130	11	19.5 - 27.2	24.8	2.30	9.27
	L-550	9	15.7 - 22.1	19.8	2.18	10.97
	JG-62	9	20.2 - 27.1	24.9	2.04	8.22
1980-81	G-130	57	13.7 - 17.5	15.5	0.78	5.02
	L-550	61	13.8 - 19.5	15.4	0.94	6.12
	JG-62	51	14.1 - 20.6	17.0	1.36	7.96
1981-82	G-130	22	16.8 - 27.0	19.7	1.97	10.02
	L-550	12	14.0 - 20.5	17.5	1.37	7.83
	JG-62	11	16.6 - 19.8	18.3	1.11	6.10

a Protein analysis of whole seed by dye binding capacity (DBC) method.

5.1.1 Relationship between seed size and protein content in chickpea:

A limited amount of information is available on the relationship between seed size and protein content in grain legumes. To obtain information on this aspect in chickpea, 150 germplasm accessions varying in seed size were analysed for protein content (Appendix I). There was a very wide range in 100-seed weight (Table 24) among the germplasm accessions. Negligible correlation was obtained between the 100-seed weight and seed protein content. In order to know whether such a correlation exist even in the lots of chickpeas having smaller variations in seed weight, these germplasm accessions were grouped

Table 24. Relationship between seed size and protein content in chickpea

Protein Group	Protein (%)		100-seed wt (g)		Correlation coefficient[a]
	Range	Mean	Range	Mean	
Low (n=56)	14.9 - 19.8	17.8	10.0 - 37.8	14.8	0.09
Medium (n=49)	20.2 - 25.0	23.1	9.5 - 34.4	17.4	-0.06
High (n=45)	25.2 - 29.6	26.5	11.1 - 36.7	17.2	-0.07
Total (n=150)	14.9 - 29.6	22.2	9.5 - 37.8	16.4	0.16

a Between 100-seed weight and protein content

into low, medium and high based on their 100-seed weight as shown in Table 24. Interestingly there was no correlation between the seed weight and seed protein content for lines belonging to any of these groups. This shows that it is possible to increase both the seed weight and protein content in chickpea.

5.2 Protein analysis of germplasm accessions of pigeonpea:

At ICRISAT, we have several thousands of pigeonpea germplasm accessions originating from different countries (Table 25). Protein analysis of germplasm accessions of pigeonpea was carried out by using the Technicon auto analyser procedure because this procedure was found to be suitable as described earlier. Initially, we analysed dhal samples for protein content. After establishing the correlation between whole grain and dhal protein contents, the analysis of whole grain samples was undertaken. The analysis revealed that protein content ranged between 15.4 and 27.6 percent for whole grain samples and between 16.3 and 28.6 for dhal samples indicating the possibility of some high protein sources (Table 26). In order to know the accuracy of this rapid procedure, every twentieth sample or so was analysed by the standard MKJ procedure and the values were compared. Bulk check samples were also included during routine analysis and error involved during the analysis for different years is given in Table 27. Coefficients of variation of protein analysis ranged between 1.35 and 2.62 percent. However, the results include the analyses of samples that were obtained from unreplicated trials and no attempt was made to study the influence of environmental or seasonal efects on protein.

Another source of high protein was identified in the wild species. Some of the species of Atylosia, a related gene, were found to have higher protein levels. Intergeneric lines from crosses of T-21 and Atylosia species showed that a few lines had more than 30%

Table 25. Accession details of world pigoenpea germplasm collection

S.#	Country	Accessions	S.#	Country	Accessions
1	Australia	47	18	Pakistan	15
2	Bangla Desh	54	19	Peru	5
3	Brazil	7	20	Puerto Rico	45
4	British Guyana	7	21	The Philippines	13
5	Burma	66	22	Senegal	10
6	Columbia	5	23	Sri Lanka	66
7	Dominican Republic	6	24	Taiwan	3
8	French Antilles	23	25	Tanzania	5
9	Ghana	1	26	Thailand	7
10	India	9001	27	Trinidad	22
11	Indonesia	4	28	Uganda	1
12	Jamaica	18	29	USSR	2
13	Kenya	64	30	USA	3
14	Madagascar	1	31	Venezuela	16
15	Malawi	17	32	Zambia	14
16	Nepal	116	33	Mexico	2
17	Nigeria	30	34	Unknown (Source Newzealand)	1

Total = 9697

Table 26. Analysis of pigeonpea germplasm accessions for protein content

Year	No of samples	Protein (%)[a]	
		Range	Mean
1975-76	1745	16.3 - 28.0[b]	21.0
1976-77	1087	19.1 - 28.6[b]	22.8
1977-78	1867	15.5 - 26.8	19.6
1978-79	964	16.8 - 25.9	20.3
1979-80	2369	15.4 - 27.6	20.2
1980-81	1015	16.0 - 25.9	19.8
Total a	6215	15.4 - 27.6	19.9
b	2832	16.3 - 28.6	22.4

a Whole seed, N x 6.25; b Dhal sample.

Table 27. Error involved during routine protein analysis by TAA procedure[a]

Year	No of samples	Cultivar	Range	Mean	SE	CV
			-- Protein (%) --			
1975-76	34	ST-1[b]	25.4 - 26.8	25.9	0.68	2.62
1976-77	52	Sharda	19.8 - 21.0	20.4	0.48	1.86
1977-78	66	ST-1	21.8 - 22.5	22.2	0.30	1.35
1978-79	103	ST-1	22.0 - 23.2	22.6	0.43	1.90
1979-80	98	HY-3C	21.5 - 22.8	22.4	0.38	1.69
1980-81	87	C-11	19.8 - 21.2	20.7	0.39	1.88

a Bulk defatted whole seed samples were analysed; b Defatted dhal samples were analysed.

protein (Reddy et al. 1978). Protein percent of some of the wild relatives of pigeonpea is shown in Table 28. In wild relatives the protein percent values were higher than the cultivated species. But the values of protein per seed were lower in wild relatives and this is because of their smaller seed sizes.

Table 28. Protein content of some wild relatives of pigeonpea[a]

Species	100-Seed wt (g)	Protein (%)	Protein/seed (mg)
1. *A. scarbaeoides*	2.55	28.4	7.24
2. *A. sericea*	4.47	29.4	13.14
3. *A. albicans*	2.54	30.5	7.74
4. *A. volubilis*	4.75	28.3	13.44
5. *A. platycarpa*	4.63	29.2	13.51
6. *A. lineata*	2.71	29.1	7.88
7. *Flemingia grahamiana*	3.03	29.3	8.87
8. *R. rothi*	3.20	28.7	9.18
9. *Cajanus cajan* (T-21)	8.1	24.2	19.60

a Dhal sample, moisture free (N x 6.25)

5.2.1 Relationship between seed size and protein content in pigeonpea:

From breeding point of view, increasing the yield at constant protein content or the selection of genotypes of superior protein content with average yield capability would be advantageous. In order

to harvest more yield of protein per unit area per unit of time, it would be desireable to have pigeonpea lines with higher protein content with normal seed size and good yield potential. It remains to be seen if this could be achieved in a breeding program which aims at developing high protein cultivars.

Keeping this in mind, the relationship between 100-grain weight and protein percent was worked out in pigeonpea. For this purpose, a lot of 43 cultivars representing different maturity groups were analysed and variations for seed weight and protein percent for these cultivars are shown in Table 29. Correlation coefficients between

Table 29. Relationship between 100-grain weight and protein percent in pigeonpea

Cultivar	100-grain weight (g)		Protein (%)[a]		Correlation coefficient[b]
	Range	Mean	Range	Mean	(r)
Early (n=7)	6.3 - 9.5	7.4	18.3 - 22.4	20.7	0.551**
Medium (n=14)	8.0 - 12.5	9.7	17.9 - 23.1	20.6	-0.266
Late (n=22)	7.9 - 10.8	10.8	19.4 - 24.3	22.8	-0.483**
Total (n=43)	6.3 - 13.9	9.9	17.9 - 24.3	21.2	0.189

a Analysis of whole grain oven dried sample. b Between 100-grain weight and protein %. ** Significant at 1% level.

these two characters varied for different groups. A positive and significant correlation was obtained for early cultivars whereas a negative and significant correlation was noticed for late maturing

cultivars. No significant correlation existed for medium cultivars. However, no correlation was noticed when the data from all the cultivars were analysed (Table 29). In view of the widespread cultivation of late maturing cultivars in India, the negative correlation between protein content and seed size for these cultivars may have some implications in a breeding program. Our results indicate that an increase in protein content results in a reduction in seed size in case of late maturing cultivars. This observation needs further confirmation by analysing more number of cultivars obtained from different locations.

6. Protein content as influenced by environments:

The effect of environments on protein quantity in cereals have been extensively investigated by several workers. Information concerning environmental effects on protein content in legumes is scanty. It is with this background that some efforts were made to study the effect of different environments on protein content of chickpea. Such experiments were planned and carried out in collaboration with the breeders and physiologists. More data have been obtained on chickpea from different locations.

6.1 Effect of environments on protein content in chickpea:

6.1.1 The protein content of chickpea grown at different locations:

In order to study the effect of location on protein content, 47 cultivars of chickpea were grown at Patancheru (ICRISAT Center), Hissar, Pantnagar and Jabalpur during the post-rainy season of

1975-76. Although, these locations slightly differ in their agroclimatic conditions the major chickpea growing areas of the country are represented by these locations except Rajasthan. Protein content of whole seed samples was determined by the TAA procedure. Results indicated that mean protein content of these cultivars was the highest when grown at Pantnagar and was the lowest when grown at Hissar (Table 30). The protein data of these cultivars were analysed

Table 30. Means and ranges of whole seed protein contents of chickpea cultivars grown at different locations during 1975-76 and 1977-78.

Year	Location	Protein (%)		S.D.[a]
		Range	Mean	
1975-76	ICRISAT Center	16.1 - 22.1	19.5	1.04
(n=47)	Hissar	16.1 - 19.4	18.2	0.64
	Pantnagar	20.7 - 24.4	22.4	1.35
	Jabalpur	19.3 - 23.0	21.4	1.28
1977-78	Hissar	21.3 - 25.5	23.2	1.04
(Desi)	Ludhiana	24.4 - 28.5	26.6	1.70
(n=25)	New Delhi	20.5 - 23.8	21.6	0.58
	Berhampore	20.4 - 27.1	21.9	1.27
1977-78	Hissar	20.1 - 24.7	22.6	0.84
(Kabuli)	Ludhiana	24.9 - 30.3	27.7	1.68
(n=15)	New Delhi	19.3 - 22.9	21.6	1.25

a Standard deviation of the location mean

statistically and the results are shown in Tables 31 & 32. This data clearly showed that protein content of genotypes was greatly influenced by the location as significant differences in protein values were obtained when cultivars were grown at different locations. The differences due to replications were not significant, the varietal differences were significant with respect to protein content.

This experiment was repeated during 1977-78 and 25 cultivars belonging to desi-late group and 15 cultivars belonging to kabuli group grown at Berhampore, Hissar, Ludhiana and New Delhi (Table 30). These cultivars were also grown at ICRISAT Center, but data from this location were not included in this study as the protein content was found to be extremely low because of saline field conditions and this effect has been discussed under a separate section. Large variations

Table 31. Mean squares from analysis of variance of seed protein contents of chickpea cultivars grown at different locations in 1975-76.

	1975-76		
Source	d.f.	Mean squares	% Total SS
Locations	3	172.12**	3.4
Cultivars	46	2.01*	5.6
Locations x cultivars	138	0.53	4.4
Error	561	1.72	58.6

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

Table 32. Mean squares from analysis of variance of seed protein contents of chickpea cultivars grown at different locations in 1977-78.

	ICCT-Desi			ICCT-Kabuli		
	d.f.	Mean squares	% Total SS	d.f.	Mean squares	% Total SS
Locations	3	518.71**	59.1	2	186.07**	61.4
Cultivars	24	15.00**	13.7	14	3.46**	8.0
Location x cultivars	72	2.55**	6.9	28	1.44	6.7
Error	297	1.79	20.2	84	1.73	24.0

ICCT : International Coordinated Chickpea Trials. ** Significant at 1% level.

in protein content were observed when cultivars were grown at different locations. Mean protein content (26.6%) was the highest for these cultivars when grown at Ludhiana and was found to be the lowest (21.6%) when grown at New Delhi for both desi-late and kabuli cultivars. Analysis of variance, as reported in Table 31, also confirmed the earlier observation that location effects are significant. Differences among the cultivars were significant but small compared with those among location differences. This is also indicated by the very high percent of total sum of squares as compared to those obtained for cultivars and cultivar x location. More important was the observation that genotype-environment interaction

was not significant. This shows that cultivar x location interactions is nonsignificant and suggests that breeding for improved seed protein content in chickpea could be effectively carried out at a single location.

6.1.2 Effect of crop years on protein content in chickpea:

As part of this study, an experiment was planned to study the effect of different crop years on the protein content and amino acids in chickpea. A total of 126 cultivars were selected and planted during 1975-76 and 1976-77 on black soil at ICRISAT Center (Appendix-5). The whole seed samples of these cultivars were analysed for protein content by the DBC procedure.

It is very interesting to note that the protein content of cultivars did not exhibit remarkable differences when the data of two years were compared. On an average, protein content of cultivars grown during 1975-76 was slightly higher than the cultivars grown during 1976-77.

6.1.3 Salinity and protein content in chickpea:

Chickpea is considered to be sensitive to salinity, alkalinity, poor soil drainage and related nutrient disorders (Gupta, 1977). Salinity not only reduces the crop growth severely but in extreme conditions can also lead to complete failure of the crop. In collaboration with breeders, we conducted experiments to study the effect of saline field conditions on the protein content. In 1977/78, the breeding materials examined included nine short duration desi and

Table 33. Soil analyses of experimental plots of chickpea grown at ICRISAT Center, near Hyderabad, India.

Year	Breeding material tested	Soil pH+	EC+(mmho/cm)
1977/78	Desi culivars	S+ 8.0	1.20 to 3.40
1977/78	Kabuli cultivars	N+ 8.2	< 0.15
1977/78	Desi F5 and F6 bulked lines	S 8.2	0.55 to 0.60
		N 8.2	< 0.15
1979/80	Desi cultivars	S 8.75	1.50 to 3.40
		N 8.2	0.20

+ pH and EC (Electrical Conductivity) were measured on a soil to water ratio of 1:2. + S = Saline; N = Non-saline.

nine kabuli cultivars in International Chickpea Cooperative Trials (ICCTS) and 46 F5 and F6 bulked breeding lines. In the saline fields the ICCTs were sown in randomized blocks with four replicates in plots of four rows, 3 m long and 30 cm apart. In 1979/80, 15 elite cultivars were grown in saline and non saline conditions to examine the effects of soil salinity. Results of soil analyses of experimental plots of chickpea grown at ICRISAT Center are shown in Table 33. Protein percent and 100 seed weight data for the four chickpea cultivars grown during 1977-78 and 1979-80 are shown in Table 34. It was observed that seed weight and seed protein percent are considerably reduced when chickpeas are grown in saline fields. These observations are important to consider in a quality breeding

Table 34. Weight of 100-seed and percentage of seed protein of four cultivars grown in 1977/78 (1) and 1979/80 (2) on saline and non-saline soils.

Cultivar	100-seed weight (g)				Percent seed protein			
	Saline		Non-saline		Saline		Non-saline	
	1	2	1	2	1	2	1	2
Annigeri	15.7	13.6	19.4	15.5	11.1	12.6	19.3	17.8
JG-62	10.5	11.3	17.8	16.2	11.9	16.7	20.3	20.9
850-3/27	19.5	18.6	30.8	26.8	12.3	16.2	20.3	23.4
L-550	15.8	14.7	24.7	21.6	11.9	16.2	20.4	22.4

program where salinity may cause unwanted variations in seed weight and protein content and thereby interfere with the selection. These findings indicate clearly that field conditions are important and should be kept in mind when screening breeding and germplasm accessions for protein content.

6.1.4 Influence of fertilizer and irrigation on protein content in chickpea:

In collaboration with physiology program, an experiment was conducted and seed samples of chickpea (CPS-1) grown during 1980-81 seasons were analysed for protein content. This cultivar with 3 replications was grown in completely randomized fashion. Three irrigations (vegetative, flowering and pod filling stages) were given. Fertilizers were applied at a depth of 45 cm at the rate of 20 kg N/ha. (urea) and 40 kg P_2O_5/ha. (single super phosphate).

Table 35. Influence of irrigation and fertilizer application on protein content in chickpea seed (cv CPS-1)

Field	20 cm depth			45 cm depth			75 cm depth		
	None	SSP	SSP+U	None	SSP	SSP+U	None	SSP	SSP+U
	..... Protein (%)								
Irrigated									
Rep 1	17.3	19.3	20.3	18.6	18.8	20.4	19.4	19.3	20.7
Rep 2	20.1	19.0	20.1	20.3	20.7	21.9	18.0	19.4	21.6
Rep 3	18.3	17.9	18.5	19.5	19.9	21.3	20.7	17.5	20.4
Mean	18.6	18.6	19.6	19.5	19.8	21.2	19.4	18.7	20.9
Unirrigated									
Rep 1	12.8	12.4	14.8	12.4	12.7	13.8	11.1	12.5	16.3
Rep 2	12.9	11.4	15.3	14.0	12.8	14.0	10.2	10.5	12.5
Rep 3	12.6	12.8	13.6	11.9	12.5	14.6	13.2	12.5	15.6
Mean	12.8	11.9	14.6	12.8	12.7	14.1	11.5	11.8	14.8

SSP:Single super phosphate; U:Urea. Exptl design:Completely randomized. Irrigation: 3 times (vegetative, flowering and pod filling stage).

Irrigation had striking effects on the protein content of chickpea. Protein content of chickpea seed increased by about 40 percent as a result of irrigation (Table 35). The application of nitrogen fertilizer resulted in a noticeable increase in the protein content of chickpea seed and this increase in protein content was

consistent in the case of both irrigated and unirrigated fields. No large differences in seed protein content was observed when N was placed at three different depths (Table 35).

6.2 Effect of environments on protein content in pigeonpea:

6.2.1 The protein content of pigeonpea grown at different locations:

In an attempt to study the effect of locations on protein content in pigeonpea eleven cultivars grown at ICRISAT Center, Gulbarga, Sehore and Coimbatore during 1979-80 were analysed. Whole grain pooled samples of cultivars from each location were analysed for protein content by the TAA procedure. No large variation in protein content was noticed among the cultivars when protein data from different locations were compared (Tables 36 & 37). No attempt was made to analyse the data statistically to find out location x cultivar interaction.

Mean protein content of cultivars grown at ICRISAT Center was higher than those grown at other locations, but the differences were not large enough to indicate any effect of location on protein content. However, further studies are required to know location x cultivar interaction and the influence of different environments on protein content in pigeonpea.

Table 36. Effect of location on seed protein content of pigeonpea cultivars grown during kharif 1979-80.

S.#	Cultivar	Protein (%)			
		ICRISAT-Center	Gulbarga	Sehore	Coimbatore
1	ICPL-42	19.2	--	18.6	20.2
2	C-11	20.0	19.1	17.9	20.0
3	ICPL-100	20.0	20.1	18.8	20.4
4	ICPL-96	19.6	19.5	18.2	19.8
5	ICPH-4	19.7	20.9	18.3	19.8
6	ICPL-97	19.5	19.1	17.3	18.8
7	ICPH-2	20.5	20.1	18.8	20.2
8	ICPL-98	20.1	19.8	18.0	20.8
9	ICPL-43	21.5	19.3	17.4	19.3
10	ICPL-99	19.8	19.4	19.3	-
11	ICPL-101	21.0	19.5	-	18.7
	Mean	20.1	19.7	18.3	19.8
	SE ±	0.65	0.51	0.58	0.62

6.2.2 Influence of irrigation and fertilizer on protein content:

From the experiment conducted by pulse physiology programme, seed samples of pigeonpea cultivar BDN-1 were analysed for protein content. Fertilizers were applied at different depths in irrigated and unirrigated fields and experiment was conducted in three replications.

Protein content in whole grain samples was determined by the TAA procedure and results are presented in Table 38. Nitrogen in the form of urea at the rate of 20 kg/hectare and P_2O_5 at the rate of 40 kg/ha. were applied at three different depths.

Table 37. Mean protein percentage of pigeonpea entries in EACT and ACT-2 grown at indicated locations in India during 1980-81 rainy season.

EACT				ACT-2			
	Locations				Locations		
Cultivar	Berham-pore	Naya-garh	S.K. Nagar	Cultivar	Gulbarga	S.K. Nagar	Kanpur
	... Protein (%) ...				... Protein (%) ...		
ICPL-1	19.8	16.5	19.5	BDN-2	18.9	20.2	19.7
ICPL-81	19.8	16.5	21.1	ICPL-227	18.8	18.4	18.3
ICPL-86	18.6	16.8	22.0	20 (105)	19.4	18.6	17.9
ICPL-87	20.0	16.8	19.6	ICPL-42	19.1	19.4	19.6
DL-78-2	20.4	18.5	21.0	ICPH-2	19.7	21.0	20.4
ICPL-85	22.5	19.9	22.5	ICPH-5	17.9	17.9	19.6
H77-208	19.9	18.4	21.0	ICPL-192	18.9	19.9	20.1
Pant A-10	20.6	16.9	20.2				
SE ±	0.42	0.22	0.40		0.24	0.58	0.30
CV (%)	3.6	2.2	3.3		2.5	6.0	3.0

Table 38. Influence of irrigation and fertilizer applications on protein content in pigeonpea seed (cv BDN-1)

Field	20 cm depth			45 cm depth			75 cm depth		
	None	SSP	SSP+U	None	SSP	SSP+U	None	SSP	SSP+U
	..... Protein percent (N x 6.25)								
Irrigated									
Rep 1	17.1	19.0	19.9	19.3	20.3	20.3	18.4	18.1	19.2
Rep 2	18.7	18.6	19.4	18.7	18.9	19.3	19.4	19.3	19.4
Rep 3	17.8	18.9	19.9	19.1	18.2	18.2	19.8	19.4	18.4
Mean	17.8	18.8	19.7	19.0	19.1	19.2	19.2	18.9	18.9
Unirrigated									
Rep 1	18.9	18.6	18.8	18.6	17.3	17.9	17.8	17.2	16.9
Rep 2	17.3	18.4	18.6	17.8	17.9	18.2	17.8	16.6	16.5
Rep 3	17.1	17.1	16.9	16.6	17.4	16.5	18.2	17.6	16.2
Mean	17.8	18.0	18.1	17.7	17.4	17.5	17.9	17.1	16.5

SSP:Single super phosphate; U:Urea. Exp design:Completely randomized Irrigation: 3 times (vegetative, flowering and pod filling stage).

Interestingly, it was observed that the use of different fertilizers did not show any effect on the protein content of pigeonpea. Application of fertilizers at a depth of 20 cm slightly increased the protein content of the seed as compared to the control. Such an increase was not noticed when the fertilizers were placed at 45 cm and 75 cm depths. These responses were observed in case of irrigated field but not in the case of unirrigated field. At this stage, results obtained are inconclusive and further investigations are needed to draw any conclusions.

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Appendix I

A comparison of methods of protein estimation in chickpea

Row No.	ICC #	Cultivar/line	100-seed wt (g)	Protein (%)			
				MKJ	TAA	DBC	Biuret (B_1)
1	6656	NEC-755	15.6	16.0	15.0	17.0	14.9
2	8074	NEC-2205	20.0	15.8	15.4	16.9	14.6
3	8554	JM-982	13.4	16.9	17.0	17.5	16.3
4	9031	NEC-513	10.0	16.5	17.1	17.3	15.7
5	3324	P-3965-1	11.7	17.8	16.6	18.1	17.6
6	3174	P-3719	22.0	16.9	16.5	17.5	16.3
7	8141	NEC-2287	18.8	16.9	16.1	17.5	16.3
8	371	P-274	20.5	17.2	17.5	17.8	16.8
9	3393	P-4081	11.0	16.9	17.7	17.5	16.3
10	4902	P-9789	11.8	17.4	18.4	17.9	17.1
11	4948	G-130	12.7	16.9	18.1	17.5	16.3
12	3685	P-4323	10.8	17.6	17.0	18.0	17.3
13	6401	NEC-374	12.6	19.0	17.7	19.0	19.2
14	6679	NEC-802	22.1	17.6	17.9	18.0	17.3
15	6660	NEC-759A	12.3	17.6	16.9	18.0	17.3
16	7337	PI-310479	16.5	17.2	18.1	17.8	16.8
17	8352	(GULLABx963)x6-1-5-15	16.6	16.9	17.0	17.5	16.3
18	6670	NEC-787	16.7	18.5	18.7	18.6	18.5
19	3689	P-4325-1	12.9	17.6	17.2	18.0	17.3
20	6443	NEC-436	12.0	18.1	17.1	18.3	18.0
21	8600	SL-1227B	11.7	17.6	19.9	18.0	17.3
22	6753	NEC-911	12.4	18.1	19.1	18.3	18.0
23	6389	NEC-356	13.5	18.5	17.7	18.6	18.5
24	JG 62		14.6	17.4	19.1	17.9	17.1
25	8761	NEC-2607	18.3	17.9	19.2	18.2	17.8
26	3185	P-3739-1	13.5	16.5	18.1	17.3	15.7
27	6671	NEC-790	19.7	19.0	19.3	19.0	19.2
28	6672	NEC-791	13.8	19.0	18.7	19.0	19.2
29	8325	CHRYSANTHI-FOLIA BLACK	13.9	17.8	17.6	18.1	17.6
30	3310	P-3942	22.5	17.8	18.7	18.1	17.6
31	8331	DOHAD-YELLOW	12.4	18.8	17.9	18.9	18.9
32	6628	NEC-716	15.2	18.1	18.4	18.3	18.0
33	8549	JM-975B	11.3	18.5	20.6	18.6	18.5
34	8396	SHIND KHEDA	14.4	18.6	19.8	18.7	18.7
35	6444	NEC-440	11.9	18.8	18.8	18.9	18.9
36	8588	SL-971B	10.0	16.7	20.8	17.4	16.0
37	6666	NEC-770	10.9	19.0	17.2	19.0	19.2
38	8330	DOHAD-15-17-1	13.2	19.3	18.7	19.3	19.6
39	8770	NEC-2617	14.3	18.3	20.8	18.5	18.6
40	8351	GULAB	11.1	18.1	17.9	18.3	18.0
41	2227	P-1792	12.4	18.5	18.4	18.6	18.5
42	7585	P-9710	13.9	18.3	18.5	18.5	18.6
43	8545	JM-969	14.0	19.2	19.0	19.1	19.4
44	6428	NEC-410	19.0	18.1	18.1	18.3	18.0
45	6474	NEC-488	12.9	19.3	18.9	19.3	19.6

46	6386	NEC-351	14.4	19.7	19.5	19.5	20.0
47	2792	P-2989-1	10.5	19.5	19.5	19.4	19.8
48	4009	P-4710-1	10.5	19.0	18.8	19.0	19.2
49	3673	P-4313	12.8	18.6	19.6	18.7	18.7
50	8764	NEC-2610	23.5	19.9	20.8	19.7	20.2
51	2791	P-2989	10.8	19.0	17.4	19.0	19.2
52	8326	Chrysantha-folia x BN-11-9-1-753	13.1	19.5	19.8	19.4	19.8
53	8589	SL-972-A	11.5	18.3	19.6	18.5	18.3
54	6388	NEC-355	13.7	19.7	19.5	19.5	20.0
55	9340	NEC-1875	21.7	20.0	20.3	19.8	20.4
56	4907	P-9800	37.8	19.7	20.5	19.5	20.0
57	8825	NEC-2675	17.4	19.0	19.8	19.0	19.2
58	8841	NEC-2691	15.9	20.4	20.6	20.1	20.8
59	2298	P-1953	28.4	20.2	20.3	20.0	20.6
60	8614	SL-1476-B	9.5	20.4	20.9	20.1	20.8
61	4889	P-9733	11.6	20.2	20.9	20.0	20.6
62	8109	NEC-2248	31.3	19.7	20.1	19.5	20.0
63	3700	P-4332-1	19.0	20.2	21.6	20.0	20.6
64	2226	P-1790	13.2	19.7	19.9	19.5	20.0
65	2225	P-1789-2	12.3	20.9	21.1	20.6	21.4
66	8800	NEC-2649	13.2	21.3	21.6	21.9	21.7
67	9282	NEC-1763	26.8	21.4	23.5	21.0	21.9
68	2133	P-1713	13.6	22.0	23.4	21.5	22.4
69	5214	GALBRON	18.1	21.8	22.5	21.4	22.3
70	2213	P-1783	17.0	22.1	22.0	21.7	22.6
71	8317	Chambalpur F-8	17.0	23.2	23.9	22.8	23.6
72	3572	P-4252	11.7	22.1	23.5	21.7	22.6
73	9171	NEC-1134	15.7	23.9	23.0	23.5	24.2
74	8335	F3 Parner 4-14-1x H. Sagar 30-9	12.1	23.6	24.2	23.1	23.9
75	9061	NEC-581	14.7	23.6	24.0	23.1	23.9
76	6644	NEC-741	12.4	24.1	24.6	23.7	24.4
77	6287	NEC-179	21.5	23.2	24.3	22.8	23.6
78	6299	NEC-197	25.4	22.1	23.4	21.7	22.6
79	9130	NEC-930	12.5	23.6	24.8	23.1	23.9
80	8183	NEC-2332	23.2	23.2	24.6	22.8	23.6
81	5334	N-8	12.6	23.2	24.7	22.8	23.6
82	4948	G-130	13.1	23.0	23.6	22.6	23.4
83	9202	NEC-1410	17.9	23.9	23.9	23.5	24.2
84	2231	P-1798-1	12.4	22.9	24.7	22.4	23.3
85	2872	P-3225	13.0	24.6	25.1	24.3	24.8
86	1024	P-853-1	11.8	23.2	25.3	22.8	23.6
87	8103	NEC-2238	34.5	24.4	24.7	24.1	24.7
88	8484	JM-517	29.7	23.9	23.1	23.5	24.2
89	3617	P-4279	10.7	23.2	24.5	22.8	23.2
90	9137	NEC-995	34.4	23.7	23.0	23.3	24.1
91	9341	NEC-876	23.5	24.3	24.1	23.9	24.5
92	9523	NEC-2021	19.6	23.9	24.7	23.5	24.2
93	8553	JM-981	12.8	24.3	26.5	23.9	24.5
94	9192	NEC-1387	13.3	23.9	22.0	23.5	24.2
95	2148	P-1721-1	13.3	24.3	23.4	24.0	24.6

96	6652	NEC-750	18.4	24.6	23.9	24.3	24.8
97	2189	P-1765	19.4	24.0	23.0	23.5	24.2
98	6796	NEC-961	10.3	24.6	25.7	24.3	24.8
99	6739	NEC-958	18.3	24.1	24.7	23.7	24.4
100	8484	JM-517	29.7	22.9	23.1	22.4	23.3
101	8570	SL-133B	10.0	23.9	25.6	23.5	24.2
102	9205	NEC-1417	17.5	25.8	24.9	25.7	25.8
103	9203	NEC-1415	16.9	25.3	24.1	25.1	25.4
104	6522	NEC-582	11.8	25.1	21.3	24.1	25.3
105	2129	P-1710	16.5	23.2	24.7	22.8	23.6
106	739	P-585	11.1	25.3	26.3	25.1	25.4
107	9303	NEC-1787	19.0	25.0	24.2	24.7	25.1
108	9874	NEC-2728-2	17.6	24.3	25.9	23.9	24.5
109	8828	NEC-2678	17.1	25.0	25.9	24.7	25.1
110	5046	C-161	15.9	25.7	25.1	25.5	25.7
111	9275	NEC-1747	29.3	25.7	25.4	25.5	25.7
112	3607	P-4275	12.5	24.9	25.8	24.7	25.1
113	9246	NEC-1681	36.7	25.0	24.4	24.7	25.1
114	2134	P-1714	20.3	26.7	26.0	26.8	26.5
115	2154	P-1729	19.9	25.3	25.9	25.1	25.4
116	4848	P-7048	13.4	25.3	23.8	25.1	25.4
117	9196	NEC-1395	12.4	25.3	25.2	25.1	25.4
118	8463	JM-482-4	17.3	24.1	25.5	23.7	25.4
119	537	P-422	12.4	27.1	25.5	27.2	26.7
120	3875	P-4543-1	13.0	25.7	24.8	25.5	25.7
121	6797	NEC-962	12.9	26.2	27.1	26.1	26.1
122	5035	C-8	24.9	26.9	26.3	27.0	26.6
123	5038	C-46	16.0	25.1	24.5	24.9	25.3
124	6745	NEC-902	17.0	25.5	26.4	25.9	25.5
125	9264	NEC-1711	19.5	26.7	23.9	26.8	26.5
126	2180	P-1761	13.3	25.7	24.3	25.8	25.7
127	2140	P-1717-1	11.5	25.5	25.4	25.3	25.5
128	9260	NEC-1705	19.1	26.9	24.7	27.0	26.6
129	9264	NEC-1711	19.5	27.2	24.6	27.4	26.9
130	7848	NEC-1831	15.1	29.3	25.6	30.3	28.3
131	3659	P-4306-2	18.6	24.4	25.8	24.1	24.7
132	5363	NP-62	11.7	26.7	26.3	26.8	26.5
133	5453	TEHRAN-24	10.8	27.2	25.6	27.4	26.9
134	9250	NEC-1694	23.7	24.1	24.6	23.7	24.4
135	9825	NEC-2630-2	15.1	25.3	26.0	25.1	25.4
136	9319	NEC-1806	18.6	28.6	29.2	29.3	27.9
137	5354	NP-17-1	11.2	26.7	25.4	26.8	26.5
138	7563	P-9629	23.2	28.3	27.8	28.9	27.6
139	9826	NEC-2632-2	14.4	25.8	27.4	25.7	25.8
140	2145	P-1720	11.5	26.0	25.9	25.9	26.0
141	2137	P-1716	20.2	27.4	25.9	27.7	27.0
142	9257	NEC-1702	22.4	27.2	26.8	27.4	26.9
143	6969	NEC-1223	16.3	27.4	27.5	27.7	27.0
144	9252	NEC-1696	19.6	27.9	27.4	28.4	27.4
145	5053	C-309	18.3	26.0	27.0	25.9	26.0
146	9320	NEC-1808	18.9	27.9	25.9	28.4	27.4
147	9320	NEC-1808	18.9	27.9	28.0	28.4	27.4
148	8397	T-1-A	11.5	29.5	27.0	30.6	28.4
149	2918	P-3318	15.6	29.5	30.0	30.6	28.4
150	5912	T-39-1	16.4	30.6	30.5	32.1	29.1

Appendix 2

A comparison of microKjeldahl (MKJ) and biuret (BIU) methods of protein estimation in chickpea

Row #	ICC #	Pedigree	100 seed wt (g)	MKJ	Biuret method B_1	Biuret method B_2
1	371	P-274	20.5	16.7	16.8	16.1
2	4951	JG-62	14.6	17.3	17.2	17.5
3	3174	P-3719	22.0	16.4	16.8	15.6
4	3310	P-3942	22.5	17.8	17.7	16.2
5	3393	P-4081	11.0	16.9	16.0	18.8
6	3185	P.3739-1	12.2	17.5	16.5	16.2
7	6948	NEC-1189	11.0	17.0	16.3	16.9
8	8074	NEC-2205	20.0	15.2	14.6	13.3
9	8141	NEC-2287	18.8	16.5	16.5	15.8
10	9031	NEC-513	10.0	16.3	17.4	17.7
11	8761	NEC-2607	16.2	17.4	18.5	19.4
12	8770	NEC-2617	14.5	18.4	18.7	19.4
13	4902	P-9789	11.8	17.0	16.5	16.4
14	8545	JM-969	18.3	18.7	19.1	20.9
15	8549	JM-975-B	11.3	18.3	18.5	19.6
16	8554	JM-982	13.4	16.0	17.3	15.5
17	7585	P-9710	13.9	18.5	19.4	19.9
18	8588	SL-9718B	10.0	18.4	18.6	19.2
19	8600	SL-1227B	11.7	17.2	18.8	19.4
20	7337	PI-310479	16.5	17.1	17.9	19.3
21	3539	P-4237	16.5	18.3	17.8	19.6
22	8325	CHRYNTHIFOLIA BLACK	13.9	17.8	16.0	19.6
23	8330	DOHAD-15-17-1	13.2	18.4	18.4	18.7
24	6444	NEC-440	11.9	18.3	17.4	18.9
25	6628	NEC-716	15.2	18.2	17.5	19.3
26	6660	NEC-759 A	11.0	17.0	17.2	17.6
27	6679	NEC-802	22.1	17.0	17.5	17.0
28	6753	NEC-911	12.4	17.2	17.7	16.6
29	2792	P-2989-1	10.5	19.0	20.4	19.9
30	4009	P-4710-1	10.5	19.0	19.4	20.6
31	3682	P-4321	11.4	19.2	19.5	19.0
32	3700	P-4332-1	15.0	21.2	21.4	21.5
33	8764	NEC-2610	23.5	19.2	20.6	19.5
34	8800	NEC-2649	15.6	21.6	21.3	20.5
35	8825	NEC-2675	17.4	20.2	20.2	19.5
36	6644	NEC-741	12.4	23.5	24.8	23.0
37	8841	NEC-2681	15.9	20.6	21.4	20.3
38	4889	P-9733	11.6	20.8	20.9	20.5
39	4907	P-9800	37.8	19.8	19.8	19.1
40	8589	SL-972B	11.5	19.4	20.8	20.3
41	8614	SL-1476B	9.5	20.8	22.5	21.3
42	8484	JM-517	29.7	23.9	25.2	23.6
43	6644	NEC-741	12.4	22.7	23.5	23.4
44	2231	P-1798-1	12.4	23.7	25.3	23.9
45	3572	P-4252	11.7	23.1	24.0	22.9

46	5334	N-8	12.6	23.7	24.9	25.7
47	6287	NEC-179	21.5	23.6	23.7	23.6
48	6299	NEC-197	25.4	23.6	23.5	22.8
49	4948	G-130	13.1	23.7	24.0	25.7
50	5203	GRAN-88-3/27	12.1	23.7	24.0	24.8
51	2133	P-1713	13.6	22.2	22.4	21.2
52	2189	P-1765	19.4	24.4	25.2	26.0
53	5214	GAL BRON	18.1	22.9	23.7	22.7
54	2225	P-1789-2	12.3	21.5	20.8	20.7
55	2226	P-1790	13.2	21.5	19.8	20.7
56	9202	NEC-1410	17.9	23.7	24.6	24.7
57	9283	NEC-1415	16.9	24.8	25.4	25.2
58	9282	NEC-1763	31.4	22.1	22.9	22.8
59	9317	NEC-1803	15.5	22.9	23.3	23.1
60	9344	NEC-1879	15.3	24.0	24.5	23.4
61	2872	P-3225	13.0	23.9	23.7	23.2
62	2148	P-1721-1	13.3	24.3	24.0	23.3
63	3607	P-4275	12.5	24.4	24.9	24.1
64	3659	P-4306-2	18.6	26.5	25.2	27.5
65	3875	P-4543-1	13.0	25.8	24.5	24.2
66	6522	NEC-582	11.8	24.9	22.8	22.4
67	6652	NEC-750	18.7	24.3	23.8	21.9
68	6739	NEC-892	18.3	24.6	24.6	23.6
69	6745	NEC-902	17.0	26.1	25.4	27.0
70	6796	NEC-961	10.3	24.6	22.2	23.9
71	6797	NEC-962	12.9	25.8	25.1	25.1
72	8828	NEC-2678	17.1	25.4	24.2	25.3
73	9824	NEC-2629-1	14.6	25.3	25.0	24.2
74	9825	NEC-2630-2	15.1	26.6	27.1	26.6
75	9826	NEC-2632-2	14.4	27.1	27.3	26.7
76	9878	NEC-2634-2	16.5	25.0	30.5	24.7
77	8570	SL-1333B	10.0	25.7	24.7	23.8
78	8484	JM-517	29.7	24.7	23.6	22.8
79	7563	P-9629	23.2	26.9	26.7	26.7
80	8463	JM-482-4	17.3	25.7	25.5	25.7
81	804	P-636	10.1	25.7	25.5	25.0
82	2129	P-1710	16.5	24.9	24.0	23.9
83	2137	P-1716	20.2	27.2	27.4	27.1
84	2145	P-1720	11.5	27.1	27.4	26.9
85	2149	P-1723	22.3	26.2	26.5	25.7
86	2153	P-1728	19.2	25.6	25.9	26.4
87	5046	C-161	15.9	25.4	30.5	25.8
88	5053	C-309	18.3	27.6	28.0	27.6
89	5912	T-40	16.4	29.6	29.9	30.4
90	2180	P-1761	13.3	26.2	26.4	26.6
91	8397	T-1-A	11.5	28.6	27.9	28.7
92	2998	P-3467-1	18.0	28.0	28.3	28.0
93	537	P-422	12.4	25.8	25.2	24.7
94	5453	TEHRAN-24	10.8	26.5	26.7	27.2
95	9319	NEC-1806	16.6	26.7	25.5	25.7
96	9320	NEC-1808	18.9	27.7	26.6	27.3
97	9245	NEC-1678	26.1	25.4	24.7	24.4

98	9250	NEC-1694	23.7	26.5	26.1	25.7
99	9252	NEC-1696	19.6	27.6	26.3	26.3
100	9275	NEC-1747	29.3	25.4	25.9	25.1
101	5350	NP-14	13.3	20.3	21.4	19.2
102	10465	RPSP-198	13.7	17.5	18.0	18.1
103	10565	RPSP-296	14.0	20.8	20.5	20.5
104	10585	RPSP-316	13.9	20.7	20.9	20.5
105	10388	RPSP-123	13.7	22.6	21.4	21.8
106	10460	RPSP-194	14.6	25.0	23.0	25.2
107	10376	JM-2325	13.6	22.9	22.4	22.4
108	10636	NO-94	13.2	24.2	24.9	24.1
109	10669	78-2	14.4	24.9	25.2	27.4
110	2327	P-2003-1	24.5	30.0	25.6	28.3
111	2332	P-2010	18.5	29.7	27.8	27.5
112	2373	P-2123-1	13.0	28.5	25.5	26.5
113	2410	P-2181	12.2	21.7	21.4	21.5
114	2608	P-2619	16.0	27.5	25.5	24.9
115	2610	P-2619-4	17.1	30.0	27.5	27.7
116	2635	P-2650	17.0	28.6	26.2	25.3
117	2873	P-3229	13.4	24.2	24.6	23.9
118	2927	P-3327	13.4	28.1	26.7	29.7
119	3440	P-4117	16.2	25.5	25.1	25.3
120	4122	P-4926	20.7	28.0	26.7	27.2
121	4755	P-6369	24.9	24.4	22.2	22.3
122	4839	P-6612-1	20.4	23.9	24.0	25.7
123	4951	TG-62	14.6	17.3	16.5	17.6
124	8105	NEC-2242	21.9	25.5	24.2	24.7
125	8671	NEC-2513	17.7	28.6	28.3	28.0
126	10825	BURMA-31-19-R	11.3	22.3	22.0	21.0
127	10614	BG-10	26.8	19.9	20.7	19.2
128	8390	PUSA-28	15.2	22.4	23.5	22.8
129	10813	1-24-2	21.5	16.0	17.7	14.5
130	10580	RPSP-311	14.6	19.9	22.7	20.1
131	7901	NEC-1971	21.9	19.0	21.3	18.2
132	2860	P-3167	15.0	19.6	22.2	20.7
133	8488	JM-522A-1	11.0	17.0	18.5	18.7
134	5024	BN 31/8	11.2	23.7	22.9	27.4

A comparison of different methods of protein estimation in pigeonpea

Breeder No.	Cultivar	100 Seed wt (g)	Seed coat (%)	Whole seed			Dhal		
				MKJ	TAA	DBC	MKJ	TAA	DBC
Early	(ACT-1)								
1169	BR-172 (R_1)	8.1	15.3	21.8	22.2	22.8	23.2	24.0	24.1
1170	T-21	6.5	17.8	19.9	19.7	20.1	22.7	22.8	22.6
1171	Hy-1	10.8	15.1	22.4	22.4	22.5	24.9	24.3	24.7
1172	Pusa 4-84	6.9	18.7	20.1	19.9	20.1	22.1	22.6	22.2
1173	DL-74-1	8.2	17.3	20.8	20.6	21.1	22.9	21.5	22.9
1174	HPA-1	6.7	17.3	20.7	20.1	20.6	23.6	24.0	23.6
1175	BS-1	6.1	18.7	18.3	18.5	19.1	21.5	21.6	21.7
1176	BR-172 (R_2)	7.7	16.3	20.9	20.7	21.1	23.4	24.1	24.5
1177	T-21	6.8	18.4	21.1	20.7	21.1	24.5	24.3	24.3
1178	HY-1	8.6	16.3	19.2	19.3	19.7	22.1	22.2	22.2
1179	Pusa 4-84	6.9	18.1	20.9	20.6	21.1	24.0	24.2	24.5
1180	DL-74-1	7.9	18.3	20.2	20.2	20.9	23.3	23.2	24.1
1181	HPA-1	6.5	18.5	20.7	20.2	20.3	22.9	23.0	22.6
1182	BS-1	6.4	17.8	19.3	19.0	19.7	22.2	22.1	22.4
1183	BR-172 (R_3)	7.1	17.6	19.9	19.1	19.7	22.0	22.2	21.7
1184	T-21	7.8	19.1	19.9	19.9	20.6	22.9	22.9	22.9
1185	HY-1	8.4	13.0	19.1	19.0	18.8	22.4	21.8	22.0
1186	Pusa 4-84	6.7	17.8	20.0	20.4	20.6	23.0	23.1	23.5
1187	DL-74-1	7.7	17.4	18.3	20.2	21.7	23.6	23.3	24.1
1188	HPA-1	6.6	18.0	19.4	19.6	19.3	23.4	22.7	22.5
1189	BS-1	6.6	17.5	19.2	18.7	19.9	22.7	23.0	22.8
1190	BR-172 (R_4)	7.6	16.5	21.4	21.2	21.9	23.6	23.7	23.9
1191	T-21	6.1	19.0	18.9	18.6	12.3	21.1	21.1	20.9
1192	HY-1	10.4	13.5	22.3	22.1	23.0	24.0	24.5	24.5
1193	Pusa 4-84	7.3	18.3	21.5	22.0	22.3	25.8	25.0	25.7
1194	DL-74-1	8.6	17.5	20.4	20.2	21.4	24.1	24.1	24.8
1195	HPA-1	6.5	18.3	19.2	19.7	19.7	23.8	23.6	23.4
1196	BS-1	7.1	17.4	21.7	22.2	23.0	26.0	25.5	24.7
Medium	ACT-2								
1197	SA-1 (R_1)	8.2	16.6	23.1	22.4	22.5	24.3	24.7	24.1
1198	PS-11	8.2	17.6	19.7	20.8	19.7	22.6	23.6	22.4

.199	ST-1	9.0	5.5	20.8	21 0	20.1	22.5	23.4	22.2
.200	C-11	10.6	4.9	20.3	21 4	20.1	23.4	23.7	22.6
.201	JA-3	9.3	6.1	19.2	19 2	18.5	22.4	23.1	22.3
.202	ICP-	10.0	4.7	19.2	19 2	18.5	22.8	22.9	22.1
.203	EB-38-70	11.0	5.1	21.1	21 6	20.1	25.1	24.8	24.2
.204	PM-1	8.1	6.0	17.9	19 0	18.0	22.2	22.4	22.0
.205	Mukta	8.1	6.5	19.6	20 5	20.1	22.1	22.7	21.5
.206	Hy-2	12.9	2.8	19.5	20 1	20.1	22.8	22.9	21.4
.207	BDN-1	10.8	5.3	20.0	21 1	19.1	22.1	23.0	21.5
.208	Hy-4	10.5	5.7	19.6	20 4	18.5	22.1	22.7	20.9
.209	No-148	10.6	5.4	20.4	20 4	20.1	22.7	23.0	22.1
.210	As-71-37	10.2	5.2	19.4	20 2	20.1	22.2	22.3	20.7
1211	SA-1 (R_2)	7.2	7.4	18.5	18 9	17.7	21.9	22.8	21.5
1212	PS-11	8.9	6.5	21.7	22 5	22.1	24.9	25.7	24.5
1213	ST-1	8.8	5.6	21.1	21 6	20.5	23.9	24.2	22.9
1214	C-11	10.4	4.8	20.9	21 1	20.9	23.9	24.3	23.1
1215	JA-3	9.0	5.2	19.9	20 4	20.1	23.0	23.0	22.0
1216	ICP-1	9.5	5.6	20.1	20 5	19.3	23.2	24.0	23.5
1217	EB-38-70	10.1	4.6	19.3	19 6	19.1	22.8	23.4	21.8
1218	PM-1	7.8	6.8	19.3	19 0	18.5	22.8	22.9	22.0
1219	Mukta	8.2	7.0	21.5	21 0	20.5	23.6	24.2	22.8
1220	Hy-2	12.7	3.8	19.8	20 6	20.3	23.0	23.5	21.1
1221	BDN-1	10.5	5.4	20.8	21 3	21.1	23.5	23.7	22.6
1222	Hy-4	10.8	6.1	19.9	20.5	19.6	22.9	23.4	22.4
1223	No-148	10.4	6.1	20.1	19.8	19.6	23.6	23.8	22.7
1224	As-71-37	10.3	6.2	19.1	20.1	19.9	24.3	24.5	23.8
1225	SA-1 (R_3	8.0	6.2	21.0	21.5	21.6	25.1	25.2	23.0
1226	PS-11	8.1	6.6	21.2	21.2	22.4	25.5	26.5	24.8
1227	ST-1	8.6	6.0	21.0	20.9	20.5	24.3	24.3	22.9
1228	C-11	10.3	6.4	20.6	21.0	20.1	25.2	25.1	24.5
1229	JA-3	9.5	6.0	19.9	20.4	20.5	23.8	24.1	22.6
1230	ICP-1	10.1	6.1	20.0	20.4	19.9	23.1	22.8	21.8
1231	EB-38-70	11.3	6.1	21.9	22.1	21.1	25.0	24.3	23.8
1232	PM-1	8.0	6.3	20.8	20.8	20.1	23.3	22.9	22.8
1233	Mukta	8.6	6.3	21.7	21.7	21.9	24.3	24.7	23.8
1234	Hy-2	13.1	3.2	19.9	20.3	20.1	23.7	23.4	22.8
1235	BDN-1	10.2	5.1	21.3	22.1	21.6	25.5	24.9	24.5
1236	Hy-4	10.9	6.0	19.6	20.3	20.0	22.5	22.6	21.4
1237	No-148	10.2	6.0	21.5	21.5	21.6	23.2	23.5	22.2
1238	As-71-37	9.6	5.6	19.5	20.3	18.8	22.5	22.6	21.5
1239	SA-1 (R_4		7.7	22.4	22.6	23.0	25.5	24.9	24.8

240	PS-[illegible]	8.6	7.7	22.0	23 3	23.1	25.8	25.9	25.4
241	ST-[illegible]	8.5	7.4	20.4	21 1	20.5	23.6	23.8	23.1
242	C-1[illegible]	10.4	5.1	21.5	22 2	22.1	25.6	24.5	24.2
243	JA-[illegible]	9.7	6.0	21.4	21 4	21.4	24.7	23.9	23.4
244	ICP-1	10.3	5.9	20.3	20 9	21.1	23.1	23.5	21.6
245	EB-38-70	10.3	4.8	21.0	22 2	20.5	25.4	24.6	24.2
246	PM-1	8.1	5.4	20.9	21 5	19.9	25.6	24.4	24.1
247	Mukta	7.9	7.8	20.5	21 1	20.4	24.6	23.8	23.4
248	Hy-2	11.7	4.5	20.2	21 2	20.5	23.1	23.2	22.4
249	BDN-1	10.8	5.2	23.0	23 4	23.2	26.3	25.5	24.9
250	Hy-4	10.2	6.2	19.4	20 3	19.1	23.7	24.0	22.5
251	No-148	10.2	6.2	21.9	22 4	22.5	25.5	25.3	24.6
252	As-71-37	10.1	6.5	21.2	21 6	20.3	25.6	24.4	24.4
Late	ACT-3								
1253	BDN-2 (R_1)	9.6	3.9	20.1	21 0	21.2	23.5	23.3	22.5
254	ICP-7065	7.5	6.7	23.7	24 3	23.4	27.6	26.8	26.7
255	K-16	10.1	5.6	22.8	23 8	22.6	26.7	26.3	25.8
256	NP(WR)-15	7.4	7.0	22.2	23 5	21.6	25.5	25.8	25.5
257	ICP-7086	13.4	2.8	22.7	22 7	22.0	24.8	24.9	24.8
258	AS-44	9.6	5.1	22.6	22 9	21.0	26.0	25.7	25.5
259	1234	10.1	5.1	21.1	21 8	20.3	25.3	25.0	24.9
260	Gwalior-3	8.3	5.0	23.5	24 9	23.0	28.1	27.4	27.3
261	1258	12.2	3.3	21.3	21 0	20.3	24.2	24.4	24.8
262	B-517	11.6	4.4	21.9	22 9	21.8	24.9	25.1	25.0
263	PS-66	13.9	4.4	20.6	21 7	21.0	24.6	24.0	24.5
264	KWR-1	9.1	5.1	21.7	21 9	21.7	24.1	24.6	25.0
265	Gc-6800-67	10.8	4.0	22.8	23 6	22.5	25.9	26.2	26.4
266	Gc-6826-5	9.6	4.8	22.6	22 7	21.6	24.9	25.0	25.1
267	PS-65	12.6	4.1	22.5	22 0	22.5	25.1	25.2	25.4
268	PS-43	12.2	3.5	21.4	21 9	20.0	24.5	24.9	24.7
269	PS-71	12.5	4.4	21.8	21 1	19.6	23.2	24.2	23.8
270	AS-3	8.2	7.2	21.9	21 7	22.2	25.3	25.7	25.1
271	PS-41	13.0	3.2	22.6	22 4	22.5	24.2	24.7	24.3
272	K-23	9.2	5.1	21.3	21 3	21.6	23.7	24.2	24.2
273	Gc-6842-9	10.0	4.3	21.8	21 6	22.1	24.2	25.2	24.5
274	T-7	10.1	4.5	21.6	21 8	20.5	24.4	24.4	24.5
275	I-2 (R_2)	9.7	4.2	20.6	20 8	21.6	23.4	23.9	22.9

1276	ICP-7065	10.7	14.3	23.6	23.3	22.5	26.6	26.8	26.4
1277	K-16	10.4	14.8	23.0	22.8	21.6	25.5	26.1	25.4
1278	NP(WR)-15	7.0	16.7	22.7	22.8	22.2	25.5	26.5	25.8
1279	ICP-7086	12.0	13.5	20.6	21.6	22.5	22.2	24.5	24.8
1280	AS-44	9.6	15.1	22.4	22.7	22.0	25.5	26.0	25.9
1281	1234	10.6	15.2	21.0	21.9	21.8	23.9	25.2	24.3
1282	Gwalior-3	8.1	16.0	23.9	24.0	22.0	27.0	27.4	27.1
1283	1258	13.2	14.3	21.5	21.9	19.8	24.7	24.8	24.8
1284	B-517	11.7	17.3	21.9	22.7	21.5	25.2	25.8	25.2
1285	PS-66	13.1	15.1	22.0	20.7	21.0	24.0	24.2	24.2
1286	KWR-1	8.9	15.1	21.8	23.1	21.8	24.6	26.9	25.4
1287	Gc-6800-67	10.3	14.0	22.8	22.9	23.0	26.0	25.9	25.2
1288	Gc-6826-5	10.0	14.9	21.9	22.2	22.5	25.1	25.8	24.9
1289	PS-65	12.8	14.3	21.7	22.1	22.5	25.5	25.9	25.1
1290	PS-43	12.7	13.0	22.8	23.0	23.6	24.2	25.5	25.3
1291	PS-71	12.8	14.7	21.7	22.0	22.1	24.2	24.7	24.2
1292	AS-3	8.3	16.3	22.4	23.0	22.5	26.5	26.5	26.1
1293	PS-41	12.2	13.8	22.2	22.6	22.0	24.7	25.2	24.8
1294	K-23	13.1	14.8	21.3	22.0	20.8	24.7	24.5	24.8
1295	Gc-6842-9	9.6	15.5	22.4	22.9	21.6	26.1	26.1	26.2
1296	T-7	11.1	14.0	22.0	22.5	22.0	25.2	25.1	25.2
1297	BDN-2 (R3)	10.2	13.4	20.9	21.1	20.7	22.8	23.3	23.2
1298	ICP-7065	7.6	14.0	24.0	24.3	22.7	26.9	27.1	27.0
1299	K-16	10.2	14.4	22.6	23.0	22.5	26.2	26.2	25.5
1300	NP(WR)-15	7.3	16.7	21.8	22.7	22.0	25.6	26.3	25.8
1301	ICP-7086	13.2	12.8	20.4	22.5	20.5	24.5	25.1	25.1
1302	AS-44	10.1	15.1	22.2	22.0	20.5	25.4	26.2	25.1
1303	1234	9.9	15.7	21.0	21.9	21.0	23.8	24.0	24.7
1304	Gwalior-3	9.3	14.7	23.9	24.9	24.2	27.2	27.2	27.3
1305	1258	11.9	15.1	21.9	20.8	21.8	25.5	25.3	25.1
1306	B-517	11.3	14.3	22.0	22.2	21.6	25.0	25.1	25.4
1307	PS-66	12.2	14.6	19.9	21.5	19.6	22.5	23.3	23.9
1308	KWR-1	9.1	15.1	21.1	21.9	22.0	24.0	24.5	24.5
1309	Gc-6800-6	9.9	15.2	22.8	22.8	23.0	26.4	25.5	26.7
1310	Gc-6826-5	10.2	15.0	21.4	21.7	22.2	24.9	25.2	25.2
1311	PS-65	13.0	14.4	21.2	21.4	22.7	25.2	25.0	26.1
1312	PS-43	13.1	13.4	21.6	21.3	22.0	24.2	24.2	24.6
1313	PS-71	13.2	13.8	21.8	22.0	20.5	25.1	25.2	25.7
1314	AS-3	9.2	16.7	23.3	24.1	22.5	26.5	26.6	25.8

315	PS-41	3.5	3.1	21.6	22.6	21.6	25.3	25.3	25.1
316	K-23	9.6	5.6	21.5	22.0	21.8	24.3	24.6	23.1
317	Gc-6842-9	9.9	5.2	22.0	22.2	21.6	25.7	26.1	25.8
318	T-7	0.7	4.1	21.4	21.9	22.0	25.9	25.2	24.8
319	BDN-2 (R_4)	9.9	4.1	20.5	21.7	19.6	25.6	22.9	23.1
320	ICP-7065	7.9	5.2	23.8	23.8	22.7	27.1	27.1	26.8
321	K-16	0.7	4.6	22.1	23.5	22.0	25.2	25.8	25.8
322	NP(WR)-15	8.1	5.2	22.7	23.6	22.9	24.4	25.8	26.1
323	ICP-7086	3.7	1.8	21.1	21.8	21.6	23.2	23.7	22.8
324	AS-44	9.9	4.8	21.9	22.6	21.4	25.0	25.7	24.8
325	1234	1.0	4.5	21.0	21.5	21.4	24.2	25.1	24 8
326	Gwalior-3	9.5	4.6	24.3	25.1	24.9	26.9	27.3	27 0
327	1258	2.4	3.3	21.4	22.2	20.0	25.2	25.1	25 1
328	B-517	2.3	3.2	22.5	23.2	22.9	24.8	25.1	24 9
329	PS-66	3.3	4.2	20.8	21.3	21.4	24.4	24.2	24 2
330	KWR-1	0.1	4.2	21.8	22.7	22.5	25.3	25.5	26 0
331	Gc-6800-67	1.8	5.7	23.5	23.8	22.2	26.2	26.1	26 4
332	Gc-6826-5	0.0	5.3	21.8	22.4	20.5	24.2	24.6	25 6
333	PS-65	3.9	4.9	22.0	22.4	22.5	23.8	25.1	23 2
334	PS-43	3.1	4.0	20.9	21.5	20.5	23.9	24.5	22 7
335	PS-71	3.8	4.0	22.1	22.8	21.8	26.1	25.8	24 3
336	AS-3	9.9	6.5	22.7	23.5	22.7	25.9	26.1	24 8
337	PS-41	3.0	4.0	22.1	22.9	22.2	25.3	25.9	25 9
338	K-23	9.6	5.5	20.8	21.9	21.8	25.8	26.2	24 3
339	Gc-6842-9	9.7	6.1	22.6	22.9	21.8	25.4	25.8	25 8
340	T-7		5.3	21.8	23.0	22.0	24.6	25.8	25 7

Appendix 4

Variation in protein content of whole seed and dhal components among pigeonpea germplasm accessions varying in seed size

ICP #	Cultivar/line	100 seed wt (g)	Seed coat (%)	Protein percent		
				Whole seed	Dhal	Seed coat
677	P-995-1	11.1	14.1	22.1	25.7	5.7
3785	JA-275-2	19.7	12.3	18.3	20.6	6.2
6399	EC-100465	11.0	12.8	19.5	22.0	5.9
6696	2798	12.6	12.8	20.6	23.7	4.9
6393	JA-277	10.1	13.3	20.2	22.5	5.0
7484	ANM-136	11.4	15.4	19.0	22.3	5.6
6407	P-130-4	11.8	13.8	17.2	20.7	4.5
7183	PS-41	11.8	13.0	19.3	22.7	5.1
32	P-230	7.6	15.0	20.0	24.1	4.9
1697	P-1547	13.5	12.6	18.0	21.3	5.2
7259	UQ-46	13.8	14.3	19.6	22.8	6.1
6621	P-2656	7.6	14.1	17.9	20.6	5.2
3545	P-2288-2	7.2	14.5	20.8	24.0	4.9
7362	ANM-36E	6.2	17.3	19.2	23.8	5.6
5800	P-233	7.5	14.6	19.5	24.4	5.0
3534	P-1880	5.8	14.7	19.7	23.1	5.7
2073	P-1685	6.1	14.1	18.1	22.4	4.9
5723	P-23781	6.0	16.0	19.4	23.3	5.2
7390	ANM-90	7.7	14.3	20.0	24.0	5.2
7220	UQ-107	8.6	14.7	19.1	22.5	5.4
4779	NP 69	12.3	13.2	20.4	24.1	4.8
7035	Bhedaghat	18.6	12.3	19.6	23.0	5.5
7395	ANM-65	9.1	13.3	18.5	21.1	4.6
7290	UQ-77	6.5	14.9	19.2	23.0	4.9
7283	UQ-70	6.7	14.9	20.1	23.3	4.9
7375	ANM-45	4.9	15.4	19.7	23.8	5.1
7385	ANM-55	7.0	15.9	20.1	24.5	6.1
6527	P-2299	12.5	12.9	19.7	22.5	5.4
4780	P-207-121-1	11.6	12.7	19.9	22.0	5.4
3769	P-2047	11.2	13.3	20.9	24.1	4.9
6763	P-3075	10.7	14.6	20.8	25.3	4.8
6604	P-2599/1	10.8	13.4	19.7	23.0	5.8
1191	P-4655	10.9	14.7	20.1	23.0	5.2
7579		9.7	14.2	19.3	22.2	5.1
1140	P-4-110-3-1	7.6	16.1	19.7	23.4	5.3
1	Sharda Sel P	10.3	14.9	19.6	23.4	5.2
28	PUSA AGETI	8.7	15.3	20.8	24.3	5.3
6674	P-2746	9.9	14.3	20.3	23.8	5.3
7601	ANM-118	9.5	15.8	19.2	22.6	5.1
7599	BS-5	6.6	17.5	20.0	23.6	4.9
7182	BDN-1	10.0	15.0	20.6	24.2	5.6
3751	Amarkantak-173-1	10.1	15.4	20.2	24.9	5.7

4752	NP-69-119-1	10.7	13.9	19.4	22.6	5.9
5603	P-361	10.1	13.2	21.7	23.9	5.5
4127	P-606-35-1	8.7	14.8	20.6	25.1	5.2
1105	P-4989	8.5	15.1	19.4	22.6	4.8
3341	P-4769-2	9.0	15.8	17.8	20.9	5.2
6020	P-6875	8.2	16.7	19.9	24.2	5.7
7437	ANM-101	9.5	13.8	18.8	21.6	5.6
6555	P-2427	9.3	15.1	22.4	25.6	5.2
7346	ANM-25	21.1	13.5	18.7	19.1	6.2
6394	JA-277-1	8.8	16.0	20.3	24.2	4.9
7201	HY 3-A	17.9	12.3	19.2	22.8	5.7
6893	EC107638 Line-12	15.7	13.4	19.3	21.8	6.0
6896	EC 107641 69-43-1	14.1	13.1	19.4	21.9	5.8
4725	JA-278	17.1	12.5	18.2	21.6	5.4
7403	ANM-73	14.8	13.3	18.1	21.4	5.7
7575	ANM-227	6.4	14.8	18.9	23.0	4.8
7648	H-199	8.1	15.9	18.8	23.8	4.5
6443	NP (WR)-15	7.6	15.3	20.3	23.2	4.9
3670	P-4570-1	7.4	15.2	19.3	23.6	5.2
1822	P-1923	6.9	15.9	19.2	23.3	5.3
828	P-2710	7.0	14.9	19.5	22.2	5.2
5987	P-4113	7.9	12.7	21.1	24.9	4.7
6392	JA-276	10.7	14.6	19.2	22.4	5.2
6519	P-2271	13.5	12.8	18.6	21.4	5.3
2629	Granada-1	12.5	13.1	20.0	22.3	5.8
7593	ANM-245					
		15.2	12.8	20.2	22.5	5.2
7589	ANM-241					
		13.9	12.6	22.2	24.1	5.6
7332	ANM-11	5.2	20.9	20.2	24.2	5.0
7365	ANM-36F	5.8	20.0	20.0	24.5	5.3
7594	ANM-246					
		10.3	13.7	20.7	23.7	5.0
5347	P-3304	7.5	15.8	19.7	22.6	5.2
657	P-672	8.2	14.23	19.4	23.6	5.0
5556	P-4685/1-1	8.0	15.4	20.5	24.5	5.5
4008	P-793/1	9.0	14.0	21.2	24.8	5.4
3651	P-4197	8.5	13.2	20.1	24.8	5.7
2624	ST-1	8.8	13.6	20.3	25.1	5.4
2627	Mukta	8.1	15.0	21.3	26.5	5.3
6889	EC 107634 69-581	16.9	14.1	18.1	24.4	5.9
3783	JA-275	18.0	13.4	18.4	21.8	6.3
6894	EC 107369 142-A	13.1	12.6	19.8	22.5	6.1
6925	Coole # 13	12.2	12.0	21.5	24.8	6.2

Appendix-5

Protein content of chickpea when grown in different years

S.#	Cultivar/line	Protein % 1976[a]	Protein % 1977[b]	S.#	Cultivar/line	Protein % 1976[a]	Protein % 1977[b]
1	Lebanese Local PM	18.7	16.6	46	P-1630	18.3	17.8
2	N59	17.6	15.6	47	P-2386-1	17.8	17.8
3	NP34	16.2	16.6	48	P-2422-1	19.4	18.6
4	NEC-1196	17.9	15.8	49	P-2571	18.1	16.7
5	NEC-1572	18.1	18.0	50	P-2614	16.4	17.0
6	NEC-1604	19.4	16.7	51	P-2774	17.6	18.6
7	NEC-1614	18.2	16.0	52	P-2993	-	17.9
8	NEC-1640	18.7	17.0	53	P-2974	15.3	16.3
9	NEC-1713	17.3	16.3	54	P-3090	18.3	17.2
10	NEC-2438	17.2	15.9	55	P-4203	16.9	17.0
11	PG-72-8	15.9	15.8	56	P-5462	16.0	16.5
12	PG-72-84	16.6	16.6	57	P-9800	18.7	16.5
13	Pyrouz	17.5	15.8	58	P-2718	17.8	17.1
14	PRR-I	19.3	18.0	59	P-2215-1	18.0	17.4
15	RS-11	17.4	16.4	60	P-2236	17.1	16.9
16	T-3	16.4	15.1	61	P-4087	18.5	17.8
17	T-103	18.9	16.5	62	P-9668	16.4	16.6
18	WR-315	18.0	18.1	63	NEC-229	17.1	17.7
19	850-3/27	20.7	16.5	64	P-1845	18.9	18.0
20	12-071-04244	16.6	16.0	65	Dulia III	19.0	18.6
21	12-071-05093	17.6	17.0	66	JM-460/A-64-7A	19.5	18.4
22	12-071-10054	16.5	17.0	67	SL-972-A	15.7	17.0
23	P-30	15.8	17.0	68	WP 2654-A	15.8	17.0
24	P-134-1	20.1	18.2	69	NEC-562	17.2	17.6
25	P-200	17.6	17.2	70	NEC-2436	18.0	17.0
26	P-345	18.3	17.5	71	NEC-571	17.3	17.8
27	P-388	19.5	17.6	72	Annegiri	15.3	15.6
28	P-394-1	16.8	18.0	73	Baroda Dhakan Local	19.6	17.0
29	P-538	16.0	15.5	74	Bengal gram	21.5	17.1
30	P-619-1	17.4	16.5	75	BG 203	18.1	16.0
31	P-623	16.3	15.9	76	B-110	18.3	16.0
32	P-678	18.0	16.3	77	Caina	20.6	17.8
33	P-810	16.9	16.2	78	Chafa	18.9	17.0
34	P-947	16.4	16.1	79	C-104	21.8	16.4
35	P-993	20.7	17.6	80	C-214	18.4	15.6
36	P-1081	17.8	18.5	81	C-235	20.1	17.8
37	P-1081-1	17.7	17.4	82	F_3 Parner-4-14-1	22.1	19.6
38	P-1092	17.8	17.4	83	F-61	18.6	16.4
39	P-1181-A	18.0	17.5	84	F-404	19.1	16.6
40	P-1213-2	17.6	17.2	85	Giza	20.1	17.8
41	P-1231	17.7	16.1	86	GL-622	17.6	16.9
42	P-1265	19.9	17.2	87	GL-629	18.3	16.6
43	P-1363-1	17.2	18.1	88	GL-C30	19.1	17.3
44	P-1497	18.2	18.4	89	GL-651	20.2	17.7
45	P-1613	17.6	18.6				

90	G-130	19.4	16.2	109	NEC-108	19.3	16.9
91	G-543	18.7	16.9	110	NEC-123	18.8	16.5
92	H-208	18.3	16.8	111	NEC-143	19.3	18.0
93	H-355	17.5	16.4	112	NEC-175	22.4	18.6
94	Jam	17.9	16.6	113	NEC-197	18.7	16.3
95	JG-39	18.9	16.5	114	NEC-240	19.6	17.4
96	JG-62	18.9	16.0	115	NEC-249	19.4	19.7
97	JG-71	15.2	16.6	116	NEC-495	19.4	18.5
98	JG-221	17.7	17.2	117	NEC-721	21.3	17.6
99	JM466/DZ 10-4	20.5	18.3	118	NEC-802	17.3	16.5
100	Kaka	17.8	16.8	119	NEC-902	20.9	19.3
101	Kourosh	18.8	15.9	120	P-36	17.3	18.2
102	K-1189	20.3	17.4	121	NEC-30	19.4	17.9
103	K-56566	17.1	16.6	122	NEC-79	22.1	16.9
104	Lebabese Local	19.9	16.4	123	NEC-643	19.6	17.6
105	L-345	18.5	16.4	124	NEC-1639	19.8	17.9
106	L-534	20.0	16.1	125	NEC-1660	19.2	18.1
107	L-550	17.8	16.5	126	P-416	-	17.4
108	NEC-34	18.3	15.8				

[a] Analysed by Technicon Auto Analyser; [b] Average value of duplicate determination by Dye binding method.