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## Profiling of total nitrogen content and soluble proteins in popular varieties of *desi* chickpea, *Kabuli* chickpea and pigeon pea

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#### Abstract

In today's world of a growing population-with more than 800 million people suffering from acute or chronic malnutrition, India houses about 24% of world's malnourished and 30% of stunted children under the age of five years. Enhancing the diet with plant-based protein-rich food that are easily available as well as affordable to the larger mass is the need of the hour. Thus, a study was carried out on the popular varieties of two important Indian pulses which consisted of 12 genotypes of Desi chickpea, 12 of Kabuli chickpea and 10 genotypes of pigeon peas to determine the total nitrogen (N) content and the total soluble proteins present in the seed. Among chickpea varieties analysed, the nitrogen content ranged from 2.27 to 4.23% in which, N content in Desi genotypes ranged from 2.68 to 4.23% and the Kabuli genotypes showed 2.27 to 3.86% of N. In the case of pigeon pea genotypes, the nitrogen content varied from 2.32 to 3.91%. The Bradford assay for estimation of total soluble protein showed that among chickpea genotypes, Desi and Kabuli genotypes showed total soluble protein content of 15.46 g/100g to 25.57 g/100g and 13.14g/100g to 22.30g/100g respectively. Thus, our results suggest that, Kabuli genotypes possess lower amount of soluble protein compared to Desi genotypes. Similar results were found when the approximate content of crude protein was calculated from total nitrogen content. The findings will help in identifying suitable high-protein candidate lines for future breeding programmes which could help in combating the challenge of protein malnutrition.

Keywords: Pulses, chickpea, pigeon pea, total nitrogen content, total soluble protein

#### Introduction

The burgeoning population along with malnutrition are double burdens the world is facing right now and the situation is graver in the African and Asian countries. One of the facets of the multifactorial malnutrition is protein malnutrition which has been accounted for about 56% of child deaths in developing countries (Semba, 2016)<sup>[19]</sup>. A hopping number of nearly 144 million children under 5 were stunted, and over 47 million suffered from wasting in the world (Joint Child Malnutrition Estimates 2020)<sup>[26]</sup>. In Indian context, the huge population along with a large sector of people under the poverty level are major constraints in accessing nutritious food which leads to scarcity of proteins both in terms of quantity and quality in the diets. Since animal protein is not in the reach of every individual due to economic and/or cultural factors, supplementing the diet with plant-based proteins is the most accepted approach to meet this challenge. Pulses here have an undeniable role to play here since they are protein-rich; a storehouse of vitamins and minerals as well as pulses are environment friendly as they release nutrients to the soil and thus requiring minimum inputs for their cultivation (Venkidasamy et al, 2019)<sup>[24]</sup>. Including pulses in the diets on a regular basis lowers the risks of developing type 2 diabetes, coronary heart disease and some forms of cancer (Chibbar et al., 2010)<sup>[5]</sup>.

India ranks, 1<sup>st</sup> and 2<sup>nd</sup> in the production of chickpea and pigeon pea respectively. Chickpea is an important traditional staple food legume across Central Asia, the Indian sub-continent, the Middle East, East Africa and Mediterranean basin. World chickpea production is about 9.4 million metric tons out of which India produces approximately 74% (Thavarajah 2012)<sup>[22]</sup>.

Desi and Kabuli are the two main types chickpea: Kabuli type has large cream seeds with a skinny testa while Desi type usually has comparatively small, wrinkled, brown/dark brown seed with thick testa (Jambunathan and Singh 1981)<sup>[10]</sup>. Chickpea is a nutritionally enriched food legume having good quality proteins, carbohydrates, antioxidants, vitamins and minerals. The seeds contain moderately high protein ranging from 18% to 28% (S.A. Alajaji, 2006; el-Adawy, 2002; Ghavidel, 2007) <sup>[2, 6]</sup>, high available carbohydrate which includes starch, dietary fiber, and oligosaccharides (Chibbar et al., 2010)<sup>[5]</sup>. The chickpea protein is superior to many other legumes in terms of nutritional value (Khan et al., 1979)<sup>[12]</sup>. Chickpeas are also enriched with B-group of vitamins, essential minerals like K, Ca, Mg, P & Fe (Milan Carrillo et al., 2000)<sup>[13]</sup> as well as beneficial unsaturated fatty acids like oleic & linoleic acids (Wood & Grusak, 2007)<sup>[25]</sup>.

Pigeon pea is one of the oldest crops in India which accounts for 11.8% of the total area under pulse production (Venkidasamy *et al.*, 2019) <sup>[24]</sup>. Out of total production globally, India alone contributes about 92% of the share (Reddy et al., 2016)<sup>[16]</sup>. Pigeon pea, cultivated both for food and forage, is a sturdy crop which be grown under rainfed conditions with minimum inputs. It is unique among the food legumes as it has balanced nutritional profiles, provides highbiomass productivity, shows high tolerance to stresses and in addition, it contributes enrichment to the soil (Odeny, 2007) <sup>[14]</sup>. It is consumed either as decorticated splits (dhal) or green seeds as vegetables. It also occupies a very important place in human nutrition. The protein content generally ranges from 18-25% (Agarwal et al., 2015)<sup>[1]</sup>. In addition, it is also a valuable source of crude fibre; minerals such as K, Mn, Ca, Fe and S as well as provides water soluble B-vitamins (Saxena et al., 2010)<sup>[18]</sup>. Since pulses form the staple diet of most of the Indian population and is the cheapest source of proteins, it is now the need of the hour to identify cultivars having a good nutritional profile.

### Materials and Methods Materials

Seeds of 24 genotypes of Chickpea which consisted of 12 genotypes of *Desi* cultivar and 12 *Kabuli* cultivars and 10 genotypes of pigeon pea were procured from the Division of Genetics, IARI, New Delhi. The seeds were crushed to fine powder using grinder and the contents were passed through 80 mm sieve to have uniform powder which was stored for extraction and further assays.

# Determination of total Nitrogen content by Kjeldahl's method of $N_2$ estimation.

Total crude protein was estimated using Kjeldahl method (AOAC). Briefly, 0.5 g of finely ground sample flour was passed through 40 mm sieve. The sample was then transferred to a 500 mL Kjeldahl flask to which25 mL of conc. H<sub>2</sub>SO<sub>4</sub> was added and the mixture was allowed to stand for 30 minutes. Subsequently, 5 g of sodium thiosulphate was added and again allowed to stand for 30 minutes. Further, the catalyst mixture was added and Kjeldahl flask was first heated slowly till frothing continued and then heated briskly. The digestion was continued for 30 min after the digests got clean. The flask was then cooled and150 mL of water was added carefully and cooled again. 120 mL of 40% NaOH solution was then added along the sides of the flask along with a few glass beads and a drop of mineral oil and immediately connected to the distillation unit. Evolved ammonia was collected in 25mL boric acid solution containing mixed indicator. Distillation was continued till 150 mL of distillate was collected. Collected ammonia was titrated against 0.1 N sulphuric acid. A blank was run simultaneously with a piece of paper and other reagents excluding the sample.

## Estimation of total soluble protein

Total soluble protein of chickpea and pigeon pea genotypes was estimated by using Bradford (1976)<sup>[4]</sup> method with minor modifications.

## **Extract preparation**

1gm of finely ground flour was dissolved in 10 mL of 0.2 M phosphate buffer (pH 7.5) and was vortexed for 1 minute. The sample was then centrifuged at 10,000 rpm for 30 min. Supernatant was collected and stored at 4 °C till further analysis.

## **Protein estimation**

Briefly,  $10 \ \mu L$  crude extract was taken in a test tube and 2 mL Bradford reagent was added. The volume was made up to 3 mL by adding 990  $\mu L$  of distilled water. The mixture was incubated for 10 minutes at dark and at room temperature. Absorbance was then recorded at 595 nm. A same mixture minus crude extract served as blank and a known concentration of Bovine Serum Albumin (BSA) was used as standard.

## Statistical interpretation

The statistical analysis was done by SAS system where a twoway analysis of variance (ANOVA) and F-Test was done to asses any significant variation between the means (p < 0.05). Pairwise comparison was done by T test.

### **Results and Discussion**

## Determination of total Nitrogen content by Kjeldahl's method of $N_2$ estimation

Percentage of total nitrogen (N<sub>2</sub>) was determined using the Kjeldahl's method of nitrogen estimation (AOAC, 1990) and is presented in Table 1 and Table 2. In chickpea varieties, the nitrogen content ranged from 2.27 to 4.23% where, N content in Desi genotypes ranged from 2.68 to 4.23% and that of Kabuli genotypes showed 2.27 to 3.86% of N. Among Desi genotypes the significantly lowest and highest (p < 0.05) amount of total Nitrogen content was found in Pusa 547 (2.68%) and Pusa 362 (4.23%) respectively while the Kabuli genotypes showed the significantly (p < 0.05) lowest and highest amount of total Nitrogen content in Pusa 5023 (2.27%) and Pusa 1003(3.86%) respectively. In the case of pigeon pea genotypes, the nitrogen content varied from 2.32 to 3.91%. The variety Bahar showed the significantly lowest content of total crude protein of 2.32% while TJT 501 showed the significantly (p < 0.05) highest content of 3.91%.

Crude protein content of wild species, *Kabuli* and *Desi* was found to be 24.30%, 24.67% and 25.31% respectively (Kaur *et al.*, 2019) <sup>[11]</sup>. The protein content ranging from 15.7 and 31.5% was found in chickpea genotypes (Singh *et al.*, 2010) <sup>[20]</sup>. In the present study, we found higher total Nitrogen content and thus higher protein content in *Desi* genotypes as compared to *Kabuli* genotypes, in general. Similar results were reported in *Desi* chickpea and shown crude protein content of 29.2% and also further showed that, the protein content in *Desi* genotypes were found to be higher than *Kabuli* chickpea (Gaur *et al.*, 2008) <sup>[7]</sup>. Similarly, we found total protein content in pigeon pea ranged from 14.48g/100g to 24.46g/100g. Reddy *et al.* (1979) <sup>[16]</sup> have reported protein content in pigeon pea in the range of 18.4 to 28.8%. Srivastava and Vasishtha (2012)<sup>[21]</sup> reported crude protein in the range of 15.37 to 21.87%. Ghadge *et al.* (2008)<sup>[8]</sup> have reported 24.43% crude protein in raw seeds of pigeon pea.

Table 1: Total Nitrogen content (%) of Desi and Kabuli chickpeas

Desi Chickpea	Total N <sub>2</sub>	Kabuli Chickpea	Total N <sub>2</sub>
Varieties	Content (%)	Varieties	Content (%)
Pusa 256	$3.42\pm0.13^{\rm c}$	Pusa 5023	$2.27\pm0.15^{\rm f}$
Pusa 3043	3.30 ±0.10 <sup>cd</sup>	Pusa 3022	$3.40\pm0.16^{b}$
Pusa 372	$3.15 \pm 0.11^{d}$	Pusa 2085	$3.39\pm0.18^{b}$
Pusa 5028	3.40 ±0.18°	Pusa1105 k	$2.82\pm0.19^{d}$
Pusa 362	$4.40 \pm 0.16^{a}$	Pusa 1053	$3.30\pm0.15^{bc}$
Pusa 547	2.68 ±0.23 <sup>e</sup>	Pusa 3000	$3.28\pm0.09^{bc}$
Pusa 1103	2.74 ±0.04 <sup>e</sup>	Pusa 2024	$3.05\pm0.14^{\rm c}$
P 391	2.71 ±0.06 <sup>e</sup>	Pusa 1003	$3.86\pm0.04^{a}$
SDB 377	3.53 ±0.16 <sup>bc</sup>	BG 3028	$3.50\pm0.03^{b}$
BGD 72	4.23 ±0.17 <sup>a</sup>	BGD 128	$2.83\pm0.05^{\rm d}$
BGD 1005	3.31 ±0.13 <sup>cd</sup>	GNG 1969	$2.58\pm0.16^{e}$
BGD 112	3.75 ±0.14 <sup>b</sup>	KAK2	$2.59\pm0.09^{e}$

The values are mean of two replicates  $\pm$  SD. All the values are significant with p < 0.0001. Different letters within a column in each of the values represent significantly different value

 Table 2: Total Nitrogen content (%) in pigeon peas

Varieties	Total Nitrogen content (%)
Asha	$3.40\pm0.09^{b}$
Pusa 992	$2.55\pm0.08^{\rm d}$
PA 16	$3.25\pm0.21^{b}$
BSMR 736	2.97± 0.13°
MAL 6	$3.21\pm0.13^{b}$
TJT 501	$3.91\pm0.14^{\rm a}$
MAL 13	$3.38\pm0.10^{\mathrm{b}}$
MAL 3	$3.33\pm0.14^{b}$
Bahar	$2.32 \pm 0.17^{e}$
ICP 87	3.28 ±0.10 <sup>b</sup>

The values are mean of two replicates  $\pm$  SD. All the values are significant with p < 0.0001. Different letters within a column in each of the values represent significantly different value

## Determination of total soluble proteins

The total soluble protein content was analysed in all the genotypes of chickpea and piogenpea using Bradford assay and is presented in Table 3 and Table 4 respectively.

Among chickpea genotypes, *Desi* and *Kabuli* genotypes showed total soluble protein content ranging from 154.6 mg/100g to 255.7 mg/100g and 131.4 mg/100g to 223.0 mg/100g. Thus, our results suggest that, *Kabuli* genotypes possess lower amount of soluble protein as compared to *Desi* genotypes. Total soluble protein content was found to be lowest in Pusa 547 (154.6 mg/100g) and Pusa 5023(131.4 mg/100g), while significantly (p < 0.05) highest content was found in the varieties BGD 72 (255.7 mg/100g) and Pusa 1003 (223.0 mg/100g) of *Desi* and *Kabuli* chickpeas respectively. The pigeon pea genotypes showed a lower value for total soluble proteins as compared to chickpeas and it ranged from 13.91 g/100g to 207.9 mg/100g with the variety Bahar showing the lowest content and Asha showing the significantly (p < 0.05) highest content of soluble proteins.

Based on the soluble protein content the chickpea and pigeon pea genotypes were classified into low, medium and high groups by equal class distribution of T test. In case of *Desi* chickpea genotypes, six genotypes (BGD 72, Pusa 362, BGD 112, SDB 377, Pusa 256, Pusa 3043) had higher soluble protein content (> 200 mg/g), three genotypes (Pusa 1005, Pusa 5028, Pusa 372) had medium soluble protein content (170 to 200mg/g) and the remaining three genotypes (Pusa 1103, P 391, Pusa 547) had lower protein content ( $\leq$  170 mg/g). Among Kabuli chickpea genotypes, four genotypes (Pusa 1003, Pusa 3022, Pusa 2085, Pusa 1053) had higher protein content (190 to 220.30 mg/g), five genotypes (Pusa 3000, BG 3028, Pusa 2024, BGD 128, Pusa 1105) had medium protein content (170 to 190 mg/g) and the remaining three genotypes (KAK 2, GNG 1969, Pusa 5023) had lower protein content ( $\leq 170 \text{ mg/g}$ ). Among pigeon pea genotypes, six genotypes (Asha, ICP 87, MAL 6, MAL 3, MAL 13, PA 16) had higher soluble protein content (190-210 mg/g), two genotypes (BSMR 737, TJT 501) had medium soluble protein content (150-189 mg/g) and the remaining two (Pusa 992, Bahar) genotypes showed lowest content of protein ( $\leq 170$ mg/g). The content of protein was reported to be 18.8% in pigeon pea genotypes, 22% and 25.97% (Sabahelkhier et al., 2014) <sup>[17]</sup>. Protein content was ranged from 19.06 to 28.89% and 21.01 to 29.24% in kidney bean genotypes belonged to "hard to cook" and "easy-to-cook" category (Parmar et al., 2017) [15].

 Table 3: Soluble Protein content (mg/100g) of Desi and Kabuli chickpeas

<i>Desi</i> Chickpea Varieties	Total soluble Protein	<i>Kabuli</i> Chickpea	Total soluble Protein	
varieues	(mg/100g)	Varieties	(mg/100g)	
Pusa 256	$205.68 \pm 0.803^{cd}$	Pusa 5023	$131.35 \pm 0.917^{\rm f}$	
Pusa 3043	$201.89 \pm 1.032^{cde}$	Pusa 3022	$191.62 \pm 1.261^{b}$	
Pusa 372	189.73 ±0.153 <sup>e</sup>	Pusa 2085	$191.08 \pm 0.344^{b}$	
Pusa 5028	198.11 ±0.268 <sup>cde</sup>	Pusa1105 k	$170.54 \pm 0.573^{d}$	
Pusa 362	$255.14 \pm 1.223^{a}$	Pusa 1053	$190.00 \pm 0.115^{b}$	
Pusa 547	$154.59 \pm 0.153^{\rm f}$	Pusa 3000	$189.19 \pm 1.070^{b}$	
Pusa 1103	$167.57 \pm 0.229^{\rm f}$	Pusa 2024	$183.51 \pm 0.497^{bcd}$	
P 391	$157.30 \pm 0.535^{\rm f}$	Pusa 1003	$222.97 \pm 0.344^{a}$	
SDB 377	$211.89 \pm 0.229^{bc}$	BG 3028	$185.41 \pm 0.306^{bc}$	
BGD 72	$255.68 \pm 0.382^{a}$	BGD 128	$173.51 \pm 0.153^{cd}$	
BGD 1005	$193.78 \pm 0.344^{de}$	GNG 1969	$153.51 \pm 0.382^{e}$	
BGD 112	221.62 ±1.070 <sup>b</sup>	KAK2	$156.49 \pm 0.420^{e}$	
The values are mean of two replicates + SD. All the values are				

The values are mean of two replicates  $\pm$  SD. All the values are significant with p < 0.05. Different letters within a column in each of the values represent significantly different value

Table 4: Soluble Protein content (mg/100g) of pigeon peas

Varieties	Total soluble Protein(g/100g)
Asha	207.93± 0.250ª
Pusa 992	$144.68 \pm 0.174^{d}$
PA 16	$191.531 \pm 0.343^{bc}$
BSMR 736	186.67± 0.307°
MAL 6	201.98 ±0.967 <sup>a</sup>
TJT 501	182.70 ±0.780°
MAL 13	193.33 ±0.573 <sup>bc</sup>
MAL 3	$197.12 \pm 0.326^{ab}$
Bahar	$139.10 \pm 0.190^{d}$
ICP 87	203.60 ±0.624 <sup>a</sup>

The values are mean of two replicates  $\pm$  SD. All the values are significant with p < 0.05. Different letters within a column in each of the values represent significantly different value

### Conclusion

The present study will be useful in identification of genotypes and if judiciously explored, could be useful in breeding for high-protein lines. Further agronomic studies for total nitrogen requirement for quality seed harvest could be done and, when correlated with the hereby produced data of nitrogen content in seeds, will be useful to assess the amount of assimilates produced. Based on study it can be concluded that the chosen popular varieties of *Desi* chickpea, *Kabuli* chickpea and pigeon pea contain good quantity of protein which can be of use to plant breeders, researchers as well as the food industry to formulate nutrient-based food products.

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