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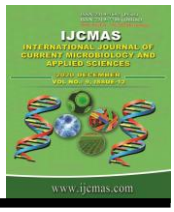

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Assessment of Nutritional Composition in Prominent *Cajanus cajan* Germplasm in India

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A B S T R A C T

Plant protein plays significant role in the world protein demand. It provides nearly 65% of the world human protein requirements. Pigeon pea, *Cajanus cajan*, [(L.) Millspaugh], a perennial legume, is a major grain legume crop ranked sixth in area and production globally. Pigeon pea is an adequate source of starch, protein, crude fiber, fat, trace elements, and minerals in human diets used in dhal and as a green vegetable. Nutritional composition is the most essential prerequisite for any successful crop improvement programme and this necessitates the analysis of pigeon pea germplasm from Assam Agricultural University (AAU) seed bank. In the present study, 10 pigeon pea germplasm were evaluated for their biochemical composition to check any genetic variation amongst them. Moisture content was in the range of 71.31- 132.53 (mg/g), crude fibre 53.28-103.25 (mg/g), ash 35.08 - 40.33 (mg/g) and crude fat 11.75 – 23.10 (mg/g), crude protein 23.37-26.82 % and nitrogen free extract (NEF) were found in the range of, 54.46 – 64.70% on dry weight basis respectively in the ten pigeon pea germplasm under study. Pigeon pea is a good protein source that poses a great potential in northeast parts of Indian foods products as well as for promotion the functional property in different food. Further, the two way cluster dendrogram and principal component analysis showed the influence of these biochemical parameters on nutritional composition in studied *Cajanus cajan* germplam.

Introduction

Plant Protein plays significant role in the world protein demand. It provides nearly 65% of the world human protein requirements (Akinhanmi *et al.*, 2008). Generally, dried seeds of edible legumes are often called as pulses. Out of the 13,000 species of legumes, only about 20 are commonly consumed by humans (Witold *et al.*, 1998). Pulses or legumes are popularly known as "Poor man's meat" and "rich men vegetable" (Singh, 2015). In comparison to other, legumes are second only to cereals as a source of proteins, carbohydrates, including fiber, certain minerals, and B-complex vitamins. Nutritionally, they are 2 to 3 times richer in protein than cereal grains (Salunkhe *et al.*,

1986). In addition, pulses are highly water efficient, can be grown in drought prone areas, can be used as fodder, fuel, soil conservator and also help to improve soil fertility by fixing atmospheric nitrogen to the soil (Tiwari, 2017). Pigeon pea (*Cajanus cajan* L.), a diploid legume crop species (2n = 2x = 22), is a member of the tribe Phaseoleae. Genus *Cajanus* comprises of 32 species, and of these, only *Cajanus cajan* is cultivated (Mudaraddi, 2013). In general, pigeonpea seeds consist of 85% cotyledons, 14% seed coat, and about 1% embryo within its seed, and contain a variety of dietary nutrients. The cotyledons are rich in carbohydrates (66.7%) while a major proportion (about 50%) of seed protein is located in embryo (Saxena, 2010).

The proteins present in legume seeds can be broadly classified into metabolic proteins, which are involved in normal cellular activities, and storage proteins, which are synthesized during seed development (Singh, 1984). The protein content of the pigeon pea varies from 15.5 to 28.8% and depends on genetic and environmental factors Similar to other legumes, Pigeon pea protein is deficient in sulfur containing amino acids (methionine and cysteine) and contains a surplus of lysine (limiting amino acid in cereals). About one-third of seed coat is made up of fiber (Saxena, 2010). Pegionpea seeds are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, pharmaceutical intermediates and lead compounds in synthetic drugs. The leaves and seeds are known to possess antihelmenthic, antiparasitic, antibacterial, antifungal, antitumor, antioxidant and antidiabetic activities (Gnanaraja, 2014). The leaf and seed are applied as poultice over the breast to induce lactation (Aja, 2015). High trypsin inhibitor activity in legumes prevent protein metabolism while phytate phosphorus compromises mineral absorption (Pele, 2016). Despite its popularity, the cultivation of pigeon pea in NE region of India is not very encouraging. There is a scattered cultivation of this crop in this region. The main reason might be the low productivity of the crop.

The relatively low crop yields may be attributed to non availability of improved cultivars, poor crop husbandry practices and exposure to number of biotic and abiotic stresses in the environment. Therefore, there is an urgent necessity to develop high yielding varieties of pigeon pea. However, no systematic efforts were made so far to document the nutritional composition among the pigeon pea germplasm collection.

Materials and Methods

The present investigation was designed to estimate the compositional quality of the *Cajanus cajan* seeds grown in Assam.

Experimental site

The present research experiment was conducted at experimental site, Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat.

Germplasm

Ten pigeon pea (*Cajanus cajan*) germplasm, *viz*. ICPH- 2671, ICPH-2740, ICPH- 11330, ICPL-11305, ICPL-88039, LOCAL-1, LOCAL-2, LOCAL-3, TS-3R and BAHAR were collected from the Assam Agricultural University seed bank located at Biswanath College of Agriculture, Biswanath Chariali, Assam. The seeds were cleaned after harvesting and were dried in hot air oven at 70℃ ± 5 for 16 hours to a constant weight in order to reduce its moisture content. Dried seeds were converted to powder using stainless steel grinder and stored in desiccators until used.

Methods

Determination of moisture content

Moisture content was determined by the AOAC (1970) method. 5gm of powdered sample was weighed in aluminum moisture boxes and dried in an oven at 70°C (± 2°C) for 16 hours, cooled in desiccators and weighed again.

Calculation

$$\text{Moisture content (g/100g sample)} = \frac{\text{Initial weight (g)} - \text{final weight(g)}}{\text{Weight of the sample taken}} \times 100$$

Estimation of crude fibre

Crude fibre was determined as per the procedure of AOAC (2000).Powdered seed sample (2g) was extracted with petroleum ether to remove the fat. After fat extraction, the material was boiled with 200ml of H_2SO_4 for 30min with bumping chips, filtered through muslin cloth and washed with the boiling water until washings were no longer acidic.

Then again boiled with 200ml of sodium hydroxide solution for 30 min, filter through muslin cloth and washed with 25ml of boiling 1.25% H_2SO_4, then 50ml portions of water and 25ml alcohol. Removed the residue and transferred to ashing dish (pre-weighed dish W_1). The residue was dried for 2h at 130±2°c. Cooled the dish in desiccator and weighed (W_2), and finally ignite at 600±15°C for 30 min. Cooled in desiccators and weighed again.

Calculation

$$\text{Percentage of crude fiber} = \frac{(W2-W1)\ (W3-W1)}{\text{Weight of the sample}} \times 100$$

Determination of Ash content

Ash (total mineral) content was determined as per the procedure of AOAC (2000). For this, 5 g moisture free powdered sample was taken in a silica crucible , charred with low Bunsen flame, and finally ignited at 600℃ for 6 hours in the muffle furnace. The ash percentage is calculated by dividing the weight of the ash by the weight of the sample taken and multiplied by 100

Calculation

$$\text{Ash content (g/100g sample)} = \frac{\text{weight of the ash (g)}}{\text{weight of the sample taken (g)}} \times 100$$

The ash was moistened with a small amount of distilled water and added 5 ml of distilled HCl. The mixture was evaporated to dryness on a boiling water bath. Another 5 ml of HCl was added again and the solution evaporated to dryness as before. Four ml of HCl and few ml of water were then added and the solution warmed over a boiling water bath and filtered into a 100ml volumetric flask using Whatman No. 40 filter paper. After cooling, the volume was made up to 100 ml and suitable aliquots were used for mineral estimation.

Estimation of crude fat

Crude fat or ether extract were determined from oven dried sample using a Soxhlet extraction apparatus (AOAC, 1970). In a thimble, 5g dried and powdered sample was taken. The top of the thimble was plugged with fat free cotton. The thimble was placed in the fat extraction tube of Soxhlet extraction apparatus. The bottom of the extraction tube was attached to the empty, dried extraction flask which is previously weighed (W_1). Approximately 75 ml or more anhydrous petroleum ether (boiling point 60 to 80℃**)** was poured through the sample into the extraction tube, which came down to the

flask. The top of the fat extraction tube was connected with the condenser. Extraction was started by heating the petroleum ether by a regulated heater. The temperature should be regulated in such a way that volatilized ether could condense and then dropped continuously upon the sample without any appreciable loss. The extraction was continued for about 16 hr when petroleum ether in the Soxhlet became totally colorless. The thimble was removed from the apparatus and most of the ether was distilled off and was collected in the extraction tube to be used for next sample. When a small portion of ether is present in the extraction flask, the thimble was removed from the apparatus, dried over a water bath at low temperature, cooled and weighed. This was repeated until a constant weight (W_2) was obtained. The difference in weight (W_2-W_1) gives the amount of fat soluble material present in the sample.

Calculation

Percentage of crude fat (on dry weight basis) $= \frac{\text{Weight (g) of fat soluble material}}{\text{weight of dried sample (g)}} \times 10$

The estimation was done in triplicate and their mean was recorded as percentage of crude fat content in moisture free sample.

Estimation of crude protein content

Crude protein content of the sample was determined by micro Kjeldahl method (AOAC, 2000). In determining the total nitrogen, the organic form of nitrogen in the sample was first converted into inorganic form (ammonium sulfate) by wet digestion with conc. sulphuric acid in the presence of catalysts, copper sulphate and potassium sulphate and subsequent decomposition of ammonium sulphate by excess alkali (40% NaOH). The librated ammonia was collected in 4 % boric acid solution and was then titrated with standard hydrochloric acid (0.1 N). 500mg of powdered sample, 1g catalyst mixture and 10 ml of concentrated sulphuric acid were taken in a digestion tube and kept overnight for pre-digestion. Next day the pre digested sample was digested in automatic digestion unit (KEL-PLUS, model KES O4L, Pelican, India) till a clear solution was obtained.

The digested sample were distillated using an automatic distillation unit (KEL PLUS, model ELITE EX, Pelican, India) in presence of 40% sodium hydroxide solution and liberated ammonia was trapped in 4% boric acid solution. Distilled sample in the conical flask mixed with indicator was titrated against 0.1N HCl till the colour changes from bluish green to pale pink.

Calculation

Nitrogen content (g/100g sample) $= \frac{(a-b)\times \text{Normality of HCl} \times 14 \times 100}{\text{g of sample} \times 100}$

Where,

a = ml standard acid of sample
b = ml standard acid of blank
If total nitrogen value is X, protein contain in 100 g = X × 6.25

The estimate was done in triplicate for each sample and the mean of the estimation was recorded for interpretation of the result. The protein content was expressed as percentage on moisture free basis.

Estimation of Nitrogen free Extract/ Total carbohydrate content

Total carbohydrate content was determined as described by Jeon (1995). Total carbohydrate was calculated by subtraction of the sum of weight of crude protein (CP), crude fat (CF),

moisture (M) and ash (A) from the total weight of the sample used.

Total carbohydrate (%) = 100 – (% crude protein + % crude fat + % moisture + % ash)

Statistical analysis

The experiment was conducted as randomized block design with three replications and the data was analyzed using JMP 2009 (JMP, Version 9.0.0. SAS Institute Inc., Cary, NC) and SPSS (Statistical Package for Social Science, SPSS Inc., Chicago, IL). The principal component analysis (PCA) and two way cluster analysis were used to evaluate the variations in ten different *Cajanus cajan* germplasm.

Results and Discussion

Pigeon pea (*Cajanus cajan*) is one of the commonly grown pulses in India and one of the major pulses of NE India. There are 32 species in the genus *Cajanus*, but *Cajanus cajan* is the only cultivated species. Pigeon pea is a rich source of protein, carbohydrate and minerals. It is also a good source of water soluble vitamins *viz.*, thiamine, riboflavin and niacin. Pigeon pea is used for food, feed and fuel. It is most widely eaten in the form of split seeds. A wide variability exists in the chemical composition of pigeon pea seeds due to genotype, growth conditions and duration/condition of storage. The protein content of commonly grown pigeon pea cultivars ranges between 17.9 and 24.3 g/100g for whole grain sample (Salunkhe *et al.,* 1986). Wild species of pigeon pea have been found to be very promising sources of high protein and several high protein genotypes have been developed with a protein content as high as 32.5% (Singh *et al.,* 1990). However, cultivation of pigeon pea in NE region of India is not very encouraging and only a scattered cultivation of this crop can be seen in this region. Low productivity of the crop because of non availability of improved cultivars, poor crop husbandry and exposure to number of biotic and abiotic stresses may be some of the reasons. Assam Agricultural University (AAU), Jorhat has maintained a collection of pigeon pea germplasm over the years. However, no systematic efforts were made so far to document the nutritional composition and to assess the range of genetic variability in pigeon pea germplasm collection. The present study aimed to gather information on the nutritional composition of the pigeon pea germplasm of AAU and the results and discussion of the present study are discussed here.

Moisture content

The moisture content is a measure of yield and quantity of solid matters and its distribution is an important factor for storage and preservation of germplasm. The moisture content of ten *Cajanus cajan* germplasm were found to be in the range of 71.31- 132.53 (mg/g), highest moisture content in ICPH-2671 and lowest moisture content in Local 3 *Cajanus cajan* germplasm (Fig. 1). Different values for moisture content in different germplasm had been reported by other workers. Hassen *et al.,* (2011) reported the moisture content of the whole seeds of pigeon pea as 9.53% where as Mwanjala *et al.,* (1999) reported average moisture content to be 13.12 %. Pele *et al.,* (2016) reported the moisture content range from 7.91 % to 13.65 % in pigeon pea. Thus the finding of the present study was found similar to the values reported earlier. The varietal effect on moisture content was found significant and such variation in the moisture contain in ten different *Cajanus cajan* germplasm might be due to their stage of harvest, climate from where the sample was collected, genetic makeup, as well as method used for moisture determination.

Table.1 The principal component analysis of biochemical components in *Cajanus cajan* germplasm

Germplasm	Principal Component 1 (PC1)	Principal Component 2 (PC2)
ICPH-2671	2.558	-0.506
ICPH-2740	1.710	1.508
ICPH-11330	-1.640	-0.666
ICPL-11305	-0.181	-0.126
ICPL-88039	0.907	-0.801
LOCAL-1	-1.553	-0.692
LOCAL-2	-0.105	1.006
LOCAL-3	-3.813	0.251
TS-3R	1.576	-1.645
BAHAR	0.542	1.672

Fig.1 Moisture and crude fibre content on dry-weight basis in seeds of ten different *Cajanus cajan* germplasm

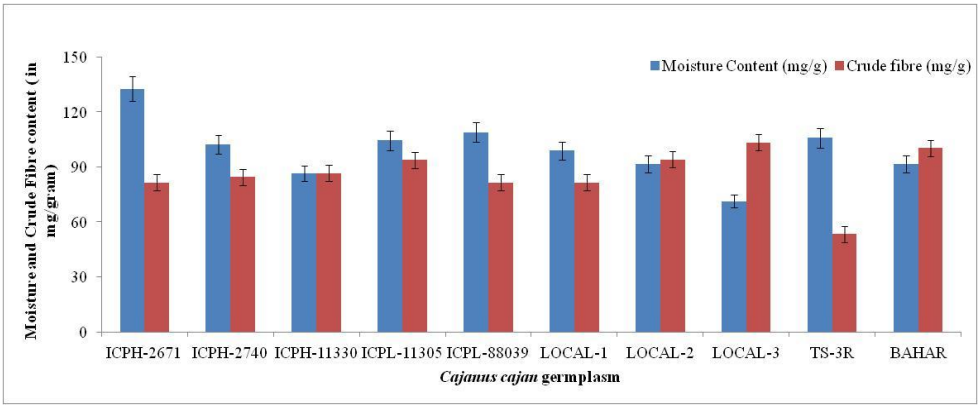


Fig.2 Ash and crude fat content on dry-weight basis in seeds of ten different *Cajanus cajan* germplasm

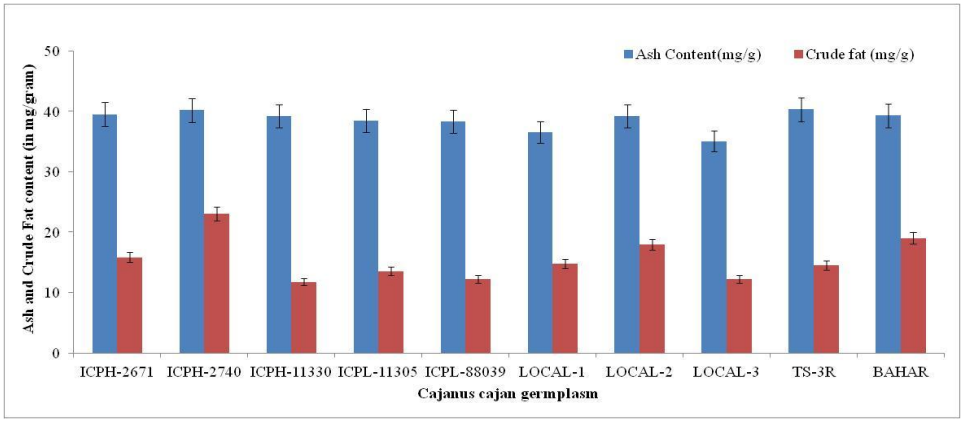


Fig.3 Crude protein and nitrogen free extract on dry-weight basis in seeds of ten different *Cajanus cajan* germplasm

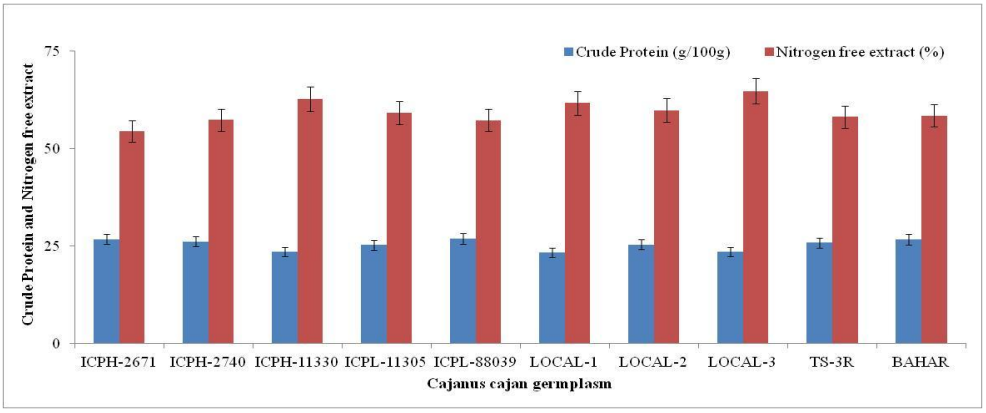


Fig.4 Two way cluster dendrogram representing the variations biochemical parameters in ten different *Cajanus cajan* germplasm

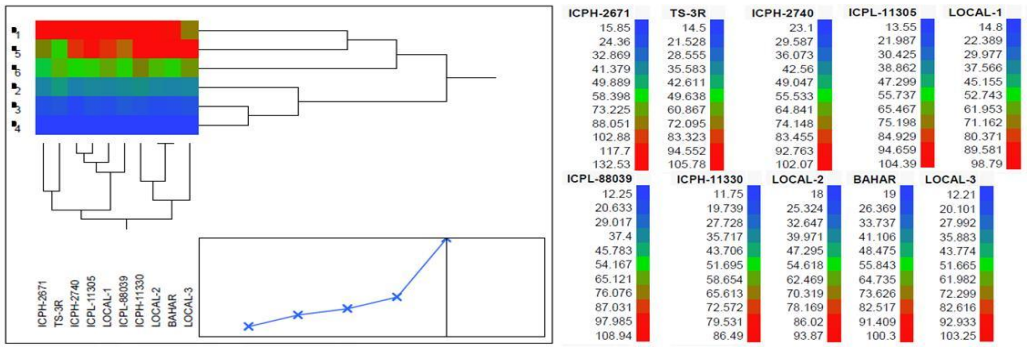
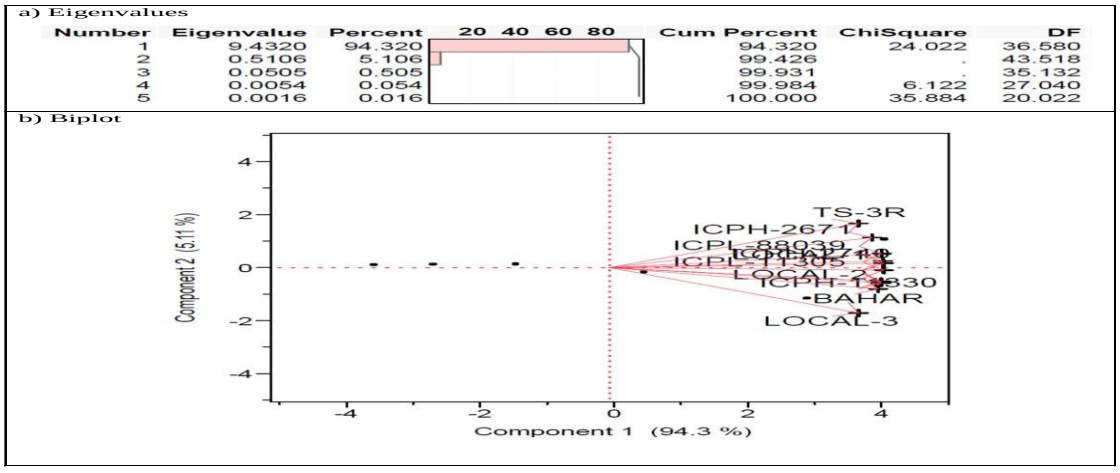


Fig.5 Principal component analysis with a) Eigenvalues, and b) Biplot based on biochemical parameters in ten different *Cajanus cajan* germplasm



Ash content

Ash content represents the total amount of minerals matters in a sample. The minerals constituents of plants and their products vary in amounts according to the stage of maturity, condition of growth, fertilization and the nature of soil. In the present study the ash contents in *Cajanus cajan* germplasm were found to be in range of 35.08 - 40.33 (mg/g) on dry weight basis (Fig. 2). The highest ash content was recorded in TS-3R and lowest ash content was recorded in Local 3 *Canajus cajan* germplasm. Mwanjala *et al.,* (1999) reported average ash content of *C. cajan* seeds to be 4.10 % which was almost similar to the present finding. Akande *et al.,* (2010) reported ash content in raw pigeon pea seeds as 3.76 %. Ash content in whole pigeon pea flour was found to be 3.96 % (Hassan *et al.*, 2011). Pratima and Mathad (2017) reported higher values for the total ash in seed coat and cotyledon of *C. cajan* as 14.5% and 11.5% respectively. These results were in agreement with the results obtained in the present study.

Crude protein

Protein quality or nutritional value of a protein means the usefulness of the proteins for specific vital purposes such as growth, replacement of metabolic losses of damage tissues, reproduction, location and general well being. Pigeon pea is a valuable source of low cost vegetable protein. In the present study crude protein content was found to vary from 23.37 to 26.82 g/100g (Fig. 3). Results obtained were in good agreement with the earlier reports by Mathew *et al.,* (2015). Hulse (1977) found that the protein content of pigeon pea seed samples ranged between 18.5 and 26.3% with a mean value of 21.5%. Pele *et al.,* (2016) investigated the effect of processing methods on the nutritional and anti-nutritional properties of pigeon pea seeds and reported crude protein content ranged from 16.74 to 28.43%. However lower values for crude protein contents (19.0 - 21.7%) in pigeon pea grown at Sebele, Botswana was also reported (Amarteifio *et al.,* 2002). Such variations in crude protein content might be due to genetically difference, location, stage of maturity and also by level of fertilization and nature of soil. Dahiya *et al.,* (1977) reported a high environmental influence on protein content, and a negative correlation between yield and percentage-seed protein.

Crude fat

Crude fat or lipids are the group of heterogeneous compounds which are classified together because of their solubility in organic solvents such as chloroform, ether or benzene. This solubility differentiates them from constituents such as protein, carbohydrates, and nucleic acids of cells and tissues. They include free fatty acids, mono, di or triacylglycerols, phospholipids and lipoproteins. They are important as a source of essential fatty acids and a concentrated source of energy. In the present investigation the crude fat contents in pigeon pea germplasm were found to be in range of 11.75 – 23.10 (mg/g) on dry weight basis (Fig. 2). Crude fat was recorded highest in ICPH-2740 and lowest in ICPH-11330 *Cajaus cajan* germplasm. The crude fat content was found to be within the range previously reported. Sinha (1977) reported much wider range (0.6-3.8%) of lipids in pigeon pea. Mwanjala *et al.,* (1999) studied physiochemical properties of pigeon pea collected from Nairobi, Kenya and reported average crude fat content to be 1.84%. The whole seeds of pigeon pea were found to have 2.13% fat (Hassan *et al.,* 2011). However, Akande *et al.,* (2010) reported much higher values for crude fat content in pigeon pea seeds (4.43%). Oloyo (2002) also reported the fat content in pigeon pea seeds as 2.7%. The total fat and triglycerides values were evaluated and results showed that total

fat ranged from (1.94–1.75 g) and triglycerides (1.46–1.07 g). Such a variation in the fat content might be due to the genetic composition of cultivar/variety used for the analysis as well as maturity and harvesting stage and the agroclimatic condition.

Crude fibre

Crude fibre represents the ash less material that remains after vigorous digestion with hot sulphuric acid and hot sodium hydroxide whereas, the dietary fibre include all plant constituents that are not digested by human digestive system, mainly cellulose, hemicelluloses, pectin and gums and non carbohydrate like lignin. The crude fibre contents obtained in *Cajanus cajan* germplasm were shown in Fig. 1. Among selected germplasm, the crude fibre content ranged from 53.28 to 103.25 (mg/g) on dry weight basis with highest crude fibre in Local 3 and lowest in TS-3R germplasm. Oloyo (2002) found crude fibre content in seeds of *Cajanus cajan* L. cv IITA 8860, collected from the International Institute of Tropical Agriculture, Ibadan, Nigeria to be 8.3%. Akande *et al.,* (2010) reported a lower value for crude fibre content in pigeon pea (7.16%). Mwanjala *et al.,* (1999) reported average crude fibre content in commercial varieties of pigeonpea as 6.27%. The variation in crude fibre content in pigeon pea may be attributed to the differences in genetic makeup as well as maturity stage.

Nitrogen free extract

Nitrogen free extracts is the total soluble carbohydrate content (by difference). It represents soluble non fibrous carbohydrates like sugar and starch and other digestible and easily utilized non- nitrogenous substances in feed stuff. When moisture content, ash content, crude protein, crude fat and crude fibre content are added and sum is subtracted from 100, the difference is called as nitrogen free extracts (NFE). The range of NFE was found to be 54.46 – 64.70 % (Fig. 3). Slightly higher values of NFE in pigeon pea were reported in Local 3 and lowest NFE content in ICPH- 2671 germplasm. Oloyo (2002) found NFE in pigeon pea seeds to be 62.55% whereas Akande *et al.,* (2010) reported it to be 59.51%. Such variation in NFE content in pigeon pea germplasm might be attributed to the genetic makeup of the materials used as well as the seed maturity at harvest.

Principal component analysis

The numerous biochemical components such as moisture content, ash content, crude protein, crude fat, crude fibre and nitrogen free extract play an important role in improving nutritional aspects in seeds of *Cajanus cajan* germplasm. Therefore, a two-way cluster dendrogram was performed to identify the grouping impact of these biochemical components in ten *Cajanus cajan* germplasm (Fig. 4).

Further, the first principal component (PC1) was the most imperative and explained 94.3 per cent of the total variations with 9.43 of eigenvalue (Fig. 5a and 5b). The PC1 was attributed to ICPH-2671, ICPH-2740, TS-3R, ICPL-88039 and BAHAR for positive loadings, whereas rest of the *Cajanus cajan* germplasm had negative loadings for the traced biochemical components (Table 1).

The second principal component (PC2) explained further 5.11 per cent of the total variations with eigenvalue to 0.51 and was attributed to positive loadings of BAHAR, ICPH-2740, LOCAL-2 and LOCAL-3, rest all had negative loadings. Hence, results of the present study showed the significant impact of above said biochemical components among the studied *Cajanus cajan* germplasm.

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