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## Genetic characterization of biochemical contents of pigeon pea (*Cajanus cajan* (L.) Millsp)

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#### Abstract

**Purpose** – The purpose of this paper is to characterize 25 collections of pigeon pea from south-west Nigeria using nutritional and anti-nutritional contents of the seeds.

**Design/methodology/approach** – The nutritional and anti-nutritional constituents of 25 collections of pigeon pea were determined. Data collected were subjected to correlation, principal components and fastclus analyses.

**Findings** – Ariation among the collections for the nutrient contents was low. Trypsin inhibitor content had the highest variability ranging between 21.74 and 35.43 Tiu/mg. Protein and trypsin inhibitor contents were significant and negatively correlated. The first three principal components explained 74.0 per cent of the total variation. Fastclus procedures grouped the collections into three. Members of cluster 1 had the highest value for protein and the lowest concentrations of the anti-nutritional factors (ANFs). Cluster 2 possessed relatively low protein with high level of ANFs while cluster 3 was intermediate between clusters 1 and 2 for most characters.

**Originality/value** – The results indicate that members of cluster 1 with high protein and low levels of ANFs are good candidates in breeding/selecting pigeon pea cultivars with enhanced nutritional values.

Keywords Food crops, Nigeria, Nutrition Paper type Research paper

#### Introduction

Pigeon pea (*Cajanus cajan* is a tropical crop with high adaptability to semi-arid environments (Whiteman *et al.*, 1983; Nene and Sheila, 1990). In Nigeria, the dry seeds of the crop are used in the preparation of various meals and serves as a substitute for cowpea. The pods, seeds and leaves are excellent fodder for cattle, sheep and goats (Oyenuga, 1967). Pigeon pea like other grain legumes is an excellent food, low in fat and rich in protein, fiber, minerals and vitamins along with their traditional potential for enhancing efficient crop rotation and soil fertility. Despite the well known nutritional benefit of pigeon pea, the value of the crop is not well appreciated in Nigeria and other West African countries (Oyenuga 1967; Akande, 2006). This is unlike in the semi-arid tropics of Asia, where pigeon pea and soyabean based systems are rapidly replacing other systems because of higher monetary returns (Ali, 1996).

One of the major limiting factors to pigeon pea acceptability is the presence of some anti-nutritional factors (ANFs) (Fasoyiro *et al.*, 2005) which often reduce the digestibility of protein and other nutrients. Although these ANFs can be reduced or eliminated with appropriate heat treatment, an easier way is to select pigeon pea varieties which are genetically deficient in these ANFs at the beginning of a breeding project (Del Rosario *et al.*, 1980). The nutritional composition of pigeon pea has been studied in the past

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(Singh *et al.* 1990; Apata and Ologhobo 1994; Fasoyiro *et al.* 2005) but the variability of the nutritional and anti-nutritional factors in the existing germplasm particularly from Nigeria have not been studied. There is therefore, a need to study the existing variability for these seed constituents of pigeon pea with the aim of identifying genotypes with high nutritional values and low anti-nutritional contents. The results would guide in breeding for acceptable pigeon pea cultivars for the populace. Hence, the objective of this study was to characterize 25 pigeon pea collections from south-west Nigeria using nutritional and anti-nutritional contents of the seeds.

#### Materials and methods

The twenty five collections of pigeon pea used consisted of 13 from Ondo, 7 from Oyo, 4 from Ekiti States and one supplied by IITA (Table I). The seeds were sorted to remove extraneous materials, dried at 105 °C for 24 h, milled and stored in polythene bags at 4 °C.

Proximate analysis for crude fat, fiber and ash were carried out according to AOAC (1990), while nitrogen content was determined by the microKjedahl method at a conversion factor of 6.25. The tannin, trypsin inhibitor, phytate and oxalate contents were determined using the methods of Price *et al.* (1978), Kakade *et al.* (1969), Davies and Reid (1979) and Fasset (1966), respectively. All the analyses were in triplicates. Data were subjected to analysis of variance and correlation analysis. The resultant correlation matrix was used for principal component analysis SAS (1997) and then the Fastclus procedure also of SAS.

Accessions	Seed colour	Source of collection (State)		
1	Cross	Ondo		
1	Grey	Ondo Ondo		
2	Brown			
3	Grey	Оуо		
4	Brown	Oyo		
5	Brown	Ondo		
6	Brown	Ondo		
7	Brown	Ekiti		
8	Grey	Ondo		
9	Grey	Ondo		
10	Brown	Oyo		
11	Grey	Oyo		
12	Light brown	Oyo		
13	Brown	Ondo		
14	Brown	Ondo		
15	Light brown	Ekiti		
16	Brown	Оуо		
17	Brown	Ondo		
18	Grey with spots	Ondo		
19	Grey	Oyo		
20	Brown	Ondo		
20	Brown	IITA		
22	Grey	Ekiti		
23	Grey	Ondo		
23 24	Brown	Ekiti		
24 25	Cream	Ondo		

Biochemical contents of pigeon pea

 Table I.

 Seed colour and the source of collections evaluated

#### NFS Results

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The 25 collections of pigeon pea evaluated were significantly different with respect to the nutritional and anti-nutritional factors. As shown in Table II, protein content ranged from 21.65 to 24.47 per cent with a mean value of 23.14 per cent. Mean values for fat, fiber and ash contents were 2.05 per cent, 6.49 per cent and 4.11 per cent, respectively. Carbohydrate content however varied between 52.37 and 55.18 per cent. For the anti-nutrients, average values for tannin, phytate and oxalate contents were 3.81, 3.31 and 2.34 g/100 g, respectively. The greatest variability was observed in trypsin inhibitor content which ranged between 21.74 and 35.43 Tiu/mg.

Pearson correlation coefficients are shown in Table III. Protein content of the seeds of pigeon pea collections had significant but negative correlation with fat, fiber, carbohydrate and trypsin inhibitor contents. Fat content was significantly correlated with fiber and carbohydrate contents. Fiber content was significantly positively correlated with carbohydrate but negatively correlated with ash content. Ash content also had negative and significant correlation with carbohydrate and trypsin inhibitor contents. Carbohydrate content however had significant positive correlation with trypsin inhibitor content. Among the anti-nutrients, only trypsin inhibitor content had significant positive correlation with tannin content.

Results of the principal component (PC) analysis showed that the first three PCs explained 74 per cent of the total variation with the first PC1 alone being responsible for 40 per cent of the variation (Table IV). PC 1 attributed higher loadings to the

Seed constituents	Mean	Minimum	Maximum	Coefficient of variation
Protein (%)	23.14	21.65	24.47	3.55
Fat (%)	2.05	1.74	2.31	7.95
Fiber (%)	6.49	5.86	6.93	4.46
Ash (%)	4.11	3.59	4.74	5.85
Carbohydrate (%)	53.92	52.37	55.18	1.21
Tannin (g/100 g)	3.81	3.20	4.36	7.91
Phytate (g/100 g)	3.31	2.74	3.66	7.06
Oxalate (g/100 g)	2.34	2.07	2.76	6.44
Trypsin inhibitor (Tiu/mg)	29.60	21.74	35.43	13.25

**Table II.** Mean values, range and coefficient of variation of seed constituents of *Cajanus cajan* 

	$\mathcal{O}^{X}$	Protein	Fat	Fiber	Ash	Carbohydrate	Tannin	Phytate	Oxalate	Trypsin inhibitor
	Protein Fat		_							
	Fiber Ash	-0.71 -0.71** 0.22	0.82** -0.25	-0.40*	_					
	Carbohydrate	-0.91**	0.56*	0.51*						
	Tannin Phytate	0.04 0.06	-0.11 0.22	-0.25 0.21	0.28 0.02	$-0.02 \\ -0.21$	-0.12	_		
Table III.           Pearson correlation	Oxalate Trypsin	-0.18	0.22	0.21	-0.21	0.18	0.36	0.24	_	
coefficients of the seed	inhibitor	-0.39*	0.19	0.11	-0.40*	0.52**	0.42*	-0.03	0.30	_
constituents of pigeon pea	Notes: *Sign	ificant at	0.05 prob	ability l	evel; **s	ignificant at 0.	01 proba	ability lev	rel	

Seed constituents	PC 1	PC 2	PC 3	Biochemical
	0.40	0.01	0.1.4	contents of
Protein	-0.48	0.01	0.14	pigeon pea
Fat	0.45	-0.18	0.17	pi8con pou
Fiber	0.43	-0.28	0.16	
Ash	-0.28	0.03	0.10	
Carbohydrate	0.45	0.10	-0.31	0.00
Tannin	-0.04	0.66	0.13	263
Phytate	0.04	-0.18	0.75	
Oxalate	0.18	0.38	0.50	
Trypsin inhibitor	0.27	0.51	-0.08	Table IV.
Eigen value	3.61	1.71	1.34	Contribution of the seed
Proportion	0.40	0.19	0.15	constituents to PC 1,
Total variance (%)	40	59	74	PC 2 and PC 3

nutrient contents (Protein, fat, fiber and carbohydrate (Table IV). PC 2 which was responsible for 19 per cent of the variation was mainly associated with tannin and trypsin inhibitor contents. PC 3 explained 15 per cent of the variation and gave the highest weight to the phytate content (Table IV).

The results of the specific grouping of the 25 collections into three using fastclus procedure are shown in Table V. There were eight, seven and ten members in clusters 1, 2 and 3, respectively. Members of cluster 1 were characterized by high protein and ash contents but relatively low fat, fiber and carbohydrate contents and with the lowest concentrations of the ANFs. Cluster 2 had the highest values for the ANFs and the least for protein content. Most of the attributes of members of cluster 3 were intermediate between those of clusters 1 and 2. Mean values for protein and trypsin inhibitor contents of members of clusters 1 and 2 (clusters with extreme values for the two seed constituents) are shown in Table VI. Cluster 1 had relatively higher protein content ranging between 22.59 and 24.40 per cent with the lowest level of trypsin inhibitor content varying from 21.75 to 26.54 Tiu/mg. While in cluster 2, protein content was between 21.88 and 24.12 per cent with significantly high trypsin inhibitor content of between 32.80 and 35.40 Tiu/mg.

#### Discussion

The range of values for the nutrient contents of pigeon pea collections observed in this study were in agreement with the previous reports on the crop (Singh and Jamburatha,

Clusters	1	2	3	
Members	5, 8, 9, 10, 17, 18,	1,2, 6, 7, 13, 14,	3, 4, 11, 12, 15, 16, 22,	
	20, 21 = 8	19 = 7	23, 24, 25 = 10	
<i>Cluster means</i> Protein (%)	23.42	22.91	23.09	
Fat (%)	2.00	2.04	2.09	
Fiber (%)	6.46	6.47	6.53	
Ash $(\%)$	4.23	3.97	4.10	(D) 1 1 V
Carbohydrate (%)	53.61 3.66	54.31 3.95	53.91 3.84	Table V.
Tannin (g/100g) Phytate (g/100g)	3.29	3.95 3.34	3.30	Mean characteristics of each of the three
Oxalate (g/100)	2.28	2.40	2.35	clusters based on
Trypsin inhibitor (Tiu/mg)	24.65	33.98	30.49	fastclus grouping

NFS	Members of clusters 1 and 2	Protein (%)	Trypsin inhibitor Tiu/mg
39,3	Cluster 1		
	5	22.81e	26.54g
	8	24.24ab	24.54j
	9	22.59f	25.501
	10	22.29g	26.46h
264	17	24.40a	25.47i
	18	23.76c	24.41k
	20	22.97ed	22.511
	21	24.30ab	21.75m
	Mean for cluster 1	23.42	24.64
	Cluster 2		
	1	22.52f	33.45e
	2	21.88h	35.30b
	6	23.12d	32.81f
	7	23.61c	34.55c
	13	22.26g	33.55d
Table VI.	14	24.12b	32.80f
Mean values for protein	19	22.86e	34.40a
and trypsin inhibitor	Mean for cluster 2	22.91	33.98
contents of members of clusters 1 and 2	Notes: Means in the same column for		1.1.1.1.1.1.1.1.0.05

1981; Apata and Ologhobo, 1994; Fasoviro et al., 2005). Although the differences among the collections were statistically significant, the level of variability was low (Table II). Singh et al. (1984) also observed low variability for protein and other seed constituents of pigeon pea. The range of protein contents of the seeds of pigeon pea accessions observed however, compared well with those of cowpea (Fashakin and Ojo, 1988); African yam bean (Adeparusi, 2001; Ajibade *et al.*, 2005) and those of other grain legumes such as Vigna umbellata, V. radiata and V. mungo (Siddhuraju et al., 1992). The results indicate that pigeon pea could be an effective substitute for cowpea as a source of protein in both human and animal nutrition. The relatively low fat content (1.74-2.31 per cent) of pigeon pea recorded in this study, in comparison to soybean and groundnut (Fasoyiro et al., 2006) indicates their possible use in effective weight management. The range of tannin, phytate and oxalate contents observed were considerably higher than those in cowpea, soybean and groundnut but lower than in African yam bean, jack bean and lima bean (Ologhobo et al., 2003; Ajibade et al., 2005; Fasoyiro et al., 2006). The range of trypsin inhibitor content (21.74-35.43 Tiu/mg) was however higher than in the above-mentioned legumes. Faris and Singh (1990) also reported large variation for trypsin inhibitor content of pigeon pea. To enhance the utilization of the crop for human and animal nutrition, selection should be made from collections with low trypsin inhibitor content.

The significant negative correlation between protein content and fat, fiber and carbohydrate contents indicates that, simultaneous selection for high protein content and the later seed constituents may not be possible. While significant negative correlation between protein and trypsin inhibitor contents is a favorable phenomenon suggesting that in selecting for high protein content the trypsin inhibitor content is automatically being selected against. The positive correlations between trypsin inhibitor and tannin contents also indicate that the two ANFs could be simultaneously selected against in a breeding programme.

Higher loadings attributed to the nutrient contents by PC1 indicate their importance in differentiating among the collections, unlike in African yam bean, where the ANFs were more important in delineating the collections (Ajibade *et al.*, 2005). The clustering of the pigeon pea collections into three clusters was not associated with the colour of the seed or the source of collection (Tables I and V). One or more collections from each of the three states were found in each cluster. However, six out of the eight members of cluster 1 (5, 8, 9, 17, 18 and 20) and five of the seven members of cluster 2 (1, 2, 6, 13 and 14) were collected from Ondo State. While cluster 3, comprised of mixtures from the three states. Cluster 1 had high protein with low level of ANFs while cluster 2 possessed relatively low protein with high level of ANFs (Tables V and VI). Germplasm collection of pigeon pea could be intensified in Ondo state for possible identification of more genotypes with desirable nutritional quality. Members of cluster 1 are good candidates in breeding pigeon pea varieties with enhanced nutritional values. Seeds of three of the collections in cluster 1 (8, 9, 18) were grayish while five of them (5, 10, 17, 20, and 21) were brownish in colour (Tables I and V).

Since out of all the ANFs, only the trypsin inhibitor content had significant negative correlation with protein content of pigeon pea (Table III), and protein is of great importance in pigeon pea and other grain legumes used as human food, the protein and trypsin inhibitor contents of members of clusters 1 and 2 (clusters with extreme values for the two seed constituents) are shown in Table VI. The higher nutritional values of members of cluster 1 with low levels of ANFs are illustrated with collection 21 being the best candidate having protein content of 24.30 per cent with trypsin inhibitor content of 21.75 Tiu/mg. The high ANFs in members of cluster 2 may limit their selection for human and animal consumption. The ANFs are however, said to have some health benefits (Champ and Muzqiuz, 2001), if high level of ANFs are desired, then selection should be made from cluster 2. Collections 6, 7 and 14 combined relatively high protein content with high level of trypsin inhibitor content while collections 2 and 19 had low protein and the highest trypsin inhibitor contents.

This study has revealed a low level of genetic variability in the seed nutrient contents of the 25 collections of pigeon pea evaluated. Among the ANFs, trypsin inhibitor content exhibited the greatest degree of variability and it also had significant and negative correlation with protein content. Some collections with high protein and significantly low level of trypsin inhibitor contents were identified.

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