

# Morphological Characterisation and Cyanide Content Determination in *Phaseolus lunatus* Linn. (Lima Beans) Accessions

## ABSTRACT

Lima bean (*Phaseolus lunatus* Linn) is one of the important legumes in developing countries. The study was carried out to evaluate the morphological characters and determine the amount of cyanide in lima beans. A total of 15 Lima bean accessions were obtained from the genebank of Genetic Resource Centre, International Institute of Tropical Agriculture (IITA) Ibadan, Oyo state, Nigeria and laid in Complete Randomised Design with three replicates. Morphological characters were evaluated based on the descriptors proposed by the International Plant Genetic Resources Institute, while hydrocyanide (HCN) content was determined using alkaline picrate colorimetric method. Six of the accessions (2005-014, 2006-003A, 2006-003B, 2006-005A, PS-16 and TPI-2429) having best yield, moderate yield and lowest yield were selected for cyanide determination. Result showed that the accessions varied morphologically. The highest yielding accessions were TPI-2429, 2005-014, 2006-003A and 2005-011, while 2006-003B and TPI-178 had moderate yields. Accession 2005-014 had the highest growth characters, followed by 2006-015 and 2006-003A which showed good quality. Lower amounts of cyanide were detected in 2005-014 and PS-16 accessions, while 2006-003A, 2006-003B and 2006-005A accessions did not show cyanide contents. Therefore, TPI-2429, 2006-003A and 2005-011 accessions which showed higher growth and yield characters with no cyanide content could be further improved in breeding of lima beans.

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Keywords: Lima beans, Cyanide, Morphological traits and Breeding

## 1.0 INTRODUCTION

Lima bean (*Phaseolus lunatus* L.), also known as 'Butter bean' is one of the under-utilized legumes of the fabaceae family, second most important economical species of the genus *Phaseolus* which comprises other legumes such as common bean (*P. vulgaris*), scarlet runner bean (*P. coccineus*) and tepary bean (*P. acutifolius*) (Fofana *et al.*, 1999; Adeniran *et al.*, 2013; Soetan and Atanda, 2018; Adebo, 2023). Its origin in tropical America, specifically from Peru, extended to West and Central Africa and well adapted to humid rainforest in the Southern Nigeria (Baudet, 1977; Gutiérrez-Salgado *et al.*, 1995; Akande and Balogun, 2007; Adebo, 2023). Lima bean seeds are good sources of proteins, carbohydrates, fibers and minerals which can overcome the malnutrition problems for people in developing countries (Kathirvel and Kumudha, 2011; Bonita, *et al.*, 2020). The composition of amino acid containing phenol in Lima bean stimulate human gastrointestinal digestion (Arora, 2014; Kyeremateng, 2015; Salawu, *et al.*, 2019). In

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some parts of West Africa, Lima bean is cooked with maize, rice or yam and used for making special soup and stews. Young pods and leaves are mostly taken as vegetables in Ghana and Malawi (Broughton *et al.*, 2002). The Yoruba tribe in Nigeria processed the seed into cakes, puddings and porridge (Akande and Balogun, 2007; Soetan and Atanda, 2018).

The potential uses of lima bean cultivars can be determined on the basis of morphological characterisation which involve measuring of phenotypic traits using standard plant descriptors (CIP/AVDRC/IBPGR, 1982; 2015; Edmund, 2014). Cyanide is an effective cytochrome oxidase inhibitor which interferes with aerobic respiratory system. Acute hydrogen cyanide poisoning could cause difficulties in breathing and result into death (Osuntokun, 1973; Ologhobo, *et al.*, 1984; Montero-Rojas, *et al.*, 2013; Adebo, 2023). Cyanogenic potential varies with respect to maturity, stage of development, genotype and cultivation conditions. Young leaves and seeds of lima bean had high nutritional contents and varied cyanide compounds such as cyanogenic glucosides and linamarase which have the potential to cause rapid respiration, drop in blood pressure, headache, vomiting and convulsions (Akande and Balogun, 2007; Ballhorn *et al.*, 2008b; Kathirvel and Kumudha, 2011; Heuzé *et al.*, 2013; Andueza-Noh, *et al.*, 2015; Bolarinwa, *et al.*, 2016).

Lima bean (*Phaseolus lunatus* L.) is scarcely available in most Nigerian markets compared to other common legume species such as cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogea*) and bambara groundnut (*Vigna subterranea*). It is underutilized because it has been cultivated and maintained by few traditional farmers over many years, despite its diversity and yield potential. Moreso, young leaves and seeds of some accessions of lima bean with high level of cyanide content can pose great threat to our health. This necessitate the characterisation of promising lima bean accessions of high yielding and low cyanide traits. Therefore, this study was carried out to evaluate the morphological characters and determine the amount of cyanide in lima beans.

## 2.0 MATERIALS AND METHODS

### 2.1 Seed Collections and Study Location

The Seed of Fifteen accessions of *Phaseolus lunatus* (Lima beans) were obtained from the genebank of Genetics Resource Centre, International Institute of Tropical Agriculture (IITA) Ibadan, Oyo State, Nigeria as shown in Table 1.

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**Table 1. Sources and collection of *Phaseolus lunatus* accessions**

Lima Beans accessions	Sources
2006-003A	IITA
2006-015	IITA
TPI-2429	IITA
TPI-189	IITA
TPI-198	IITA

118-2	IITA
198-3	IITA
2006-007	IITA
2005-014	IITA
2005-011	IITA
TPI-178	IITA
2006-005A	IITA
Ps-16	IITA
2005-015	IITA
2006-003B	IITA

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## 2.2 Experimental Design, Seed Processing and Planting Procedure

The field experiment was carried out from June to October, 2021 at the research farm of the Department of Botany, University of Ibadan, Nigeria. The experiment was laid out in a complete randomised design with three replicates. Mechanical scarification and planting of the seeds were done using standard procedures. Each accession was planted in bags properly labeled with the accession number for easy scoring and evaluation. The plants were staked 3 weeks after planting. Agronomic practices such as watering and weeding of the plants were duly carried out.

## 2.3 Determination of growth and yield characters in Limabeans

Morphological Data were taken at weekly intervals. Growth characters; plant height, petiole length, number of leaves, leaf length and leaf widths were taken with a meter rule and manual counting. The plant height measurement was taken from the soil level to the apex of the plant. Leaf with the best appearance is used for the measurement of leaf length and leaf width. The length of the leaf was taken from the base of the leaf to the apex, while the width was taken from the horizontal mid-point of the leaf margin. The number of leaves was done by counting each leaf on the plant stand. The evaluation of the yield parameters; length of pod, number of pods per rep, number of locules per pod, fresh pod weight, number of seeds per rep, seed length, seed width, seed thickness, number of seeds in each pod, pod width, 100 seed weight were done using the descriptors described by IBPGR, (1982; 2015).

## 2.4 Determination of Hydrogen Cyanide (HCN)

The seeds of Six accessions (2005-014, 2006-003A, 2006-003B, 2006-005A, PS-16 and TPI-2429) were tested for cyanide. Hydrogen cyanide (HCN) content was determined using alkaline picrate colorimetric method of Balagopal *et al.* (1998). Two grams each, of the sample was dispersed in 50 ml of distilled water in a 25 ml conical flask. An alkaline picrate paper was hung over the sample mixture and the blank in their respective flasks. These setups were incubated overnight and each

picrate paper was eluted or dipped into 60ml of distilled water. A standard cyanide solution is prepared and diluted to a required solution. The absorbance of the eluted solutions was measured with colorimeter at 540nm wavelength with the reagent blank at zero.

The cyanide content is determined by the formula shown below.

$$\text{HCN mg/kg} = \frac{1000 \cdot au \cdot C \cdot D}{w \cdot as}$$

Where W=weight of sample analyzed; au=absorbance of test sample

as=absorbance of standard HCN solution; C=concentration of the standard in

mg/dD=dilution factor where applicable

### 2.5 Data analysis of Morphological characters

Morphological data were analysed by Analysis of variance, while differences in means were estimated by Duncan Multiple Range Test. Cluster analysis was conducted by Unweighted Pair Group Methods using Arithmetic averages using Multivariate Statistical Program v.3.1 software. Principal component analysis was also performed to define the role of each character in grouping of the accessions.

### 3.0 Results

The result of growth characters in 15 accessions of Lima beans is shown in Table 2. The plant height, petiole length, number of leaves, leaf length and leaf width varied for all the accessions. All the accessions were significantly different in Plant height and Number of leaves. Accessions 193-3, 015, 003b were significantly different, while other accessions were not for leaf length. The PS-16, 2006-015, 2006-003B and 198-3 accessions were significantly higher for leaf width as well as 2005-011 and TPA-198 accessions are also significantly different. PS-16, 198-3, 2006-003A was highly significant, while 2005-011, TPI-178 and TPI-198 are significantly different at  $p < 0.05$  for Number of leaves.

The result of variations of characters of yield stage of 15 accessions of Lima beans is shown in Table 3. The accessions were varied for no of pods per stand, no of seeds per pod, no of locules per pod, no of seeds per stand, pod length, pod width, weight of fresh pod, seed length, seed width and seed thickness.

The 2006-005A, PS-16, 2006-007 and 2006-015 accessions were significantly different, while accessions 2005-015 and TPI-198 are highly significant for number of pod per rep. Accessions TPI-2429, TPI-178, 198-3, TPI-189, 2005-015 and 2006-003B are significantly higher and different. For the Number of locules per pod, TPI-2429, TPI-178, 118-2, 198-3, TPI-189, 2005-015 and 2006-003B accessions are highly significant but different at  $p < 0.01$ , while 2006-005A, PS-16, 2006-007, 2006-015 accessions are significantly different at  $p < 0.05$ . Accessions 2005-011, 2006-003A as well as 118-2, TPI-198, 198-3 and 2005-015 are highly significant. The pod length in TPI-2429, TPI-178, 2005-014, 118-2, TPI-189, 2005-015 and 2006-003B are highly significant, while TPI-2429, TPI-178, 118-2, TPI-198, 198-3, TPI-189, 2006-003A, 2005-015 and 2006-003A accessions are highly significant for pod width. The accessions; 2005-011, TPI-178, 118-2 and 2006-003B are highly significant for weight of fresh pod, while TPI-2429, 198-3, TPI-189, 2006-003A

accessions are highly significant for seed length. The 2005-011 and 2005-014 accessions are highly significant, while 2005-011, TPI-178, 2006-005A, PS-16, 2006-007 and 2006-015 are significant at  $p < 0.05$ . The result of the mean square variance of lima beans growth characters is shown in Table 4. The plant height and number of leaves had no significant effect, but had significant effect on leaf length across the accession number ( $r = 0.05$ ). Leaf width and petiole length was highly significant in accessions but not significant in replicates. Plant height, leaf width, petiole length and number of leaves had no significant effect across replicate, but was significant on leaf length.

The result of mean square variance of genotypic effect on the yield characters in table 5 shows that the number of pods per rep, number of seeds per pod, number of locules per pod, number of seeds per rep, pod length, pod width, weight of fresh pod, seed length, seed width, seed thickness and 100 seed weight were significant. The result of growth character associations in Lima beans accessions in Table 6 shows that strong positive correlation exists between the leaf length and leaf width ( $r = 0.82$ ) and also positively associated with petiole length ( $r = 0.71$ ). More so, leaf width had strong positive relationship with petiole length that  $r = 0.74$ .

The associations of yield traits in Lima beans accessions are shown in a correlation matrix (Table 7). Seed length had strong significant positive correlation with seed width ( $r = 0.87$ ) and 100 seed weight ( $r = 0.78$ ). Seed width is positive and strongly correlated with 100 seed weight ( $r = 0.76$ ). Number of pods per rep had positive and strong association with number of seeds per pod ( $r = 0.82$ ), number of seeds per rep ( $r = 0.95$ ), number of locules per pod ( $r = 0.82$ ), fresh pod weight ( $r = 0.89$ ), pod length ( $r = 0.81$ ), and pod width ( $r = 0.73$ ) while, number of seeds per pod had strong positive correlation with number of seeds per rep ( $r = 0.74$ ), number of locules per pod ( $r = 1.00$ ), fresh pod weight ( $r = 0.71$ ), pod length ( $r = 0.07$ ), and pod weight ( $r = 0.94$ ). Number of seeds per rep had strong positive correlation with number of locules per pod ( $r = 0.74$ ), fresh pod weight ( $r = 0.82$ ), pod length ( $r = 0.72$ ) and pod width ( $r = 0.64$ ). Number of locules per pod had strong positive correlation with fresh pod weight ( $r = 0.71$ ), pod length ( $r = 0.97$ ) and pod width ( $r = 0.94$ ). More so, fresh pod weight is positive and strongly related with pod length ( $r = 0.73$ ) and pod width ( $r = 0.64$ ). Also, pod length had positive and strong correlation with pod width ( $r = 0.93$ ).

The data of qualitative character are represented in Tables 8. The qualitative traits scored from 15 accessions of lima beans were plant type, leaf type, seed shape, seed colour, flower wings, pod colour, pod tip, pattern colour, and pod curvature. Accessions 2005-014, 2005-011, TPI-178 and 2006-005A were bush plant type, also called bush lima, while 2006-003A, 2006-015, TPI-2429, TPI-189, TPI-198, 118-2, 198-3, PS-16, 2005-015, 2006-003B and 2006-007 were vined. The leaf shape was Ovate-lanceolate in 2006-003A, 2006-015, 198-3, PS-16, 2005-015 and 2006-003B, lanceolate in TPI-2429 and TPI-189, round in TPI-198, 118-2 and 2006-007. Ovate in 2005-014, 2005-011, TPI-178 and 2006-005A.

The flower wings for all accessions appeared white yellow wings except for TPI-178 that is white. Similarly, pod colour (young), pod colour, pod tip in all accessions were green, short and brown, while the pod curvature was slightly curved in 13 accessions, but straight in 2006-015 and 2006-007 accessions. The seed shape was wide flat kidney for accessions TPI-198 and 118-2, but round in 2006-007, 2005-

014, 2005-011, 2006-005A, PS-16 and 2005-015. The 193-3, medium flat kidney in TPI-178, Oval cuboid in 2006-003B, kidney in 2006-003A and very wide flat kidney in TPI-2429. The seed colour of accessions 2006-003A, 2006-015, 2005-014, 2006-005A and 2005-015 were buff, while 2005-11, TPI-178, PS-16 and 2006-003B accessions were maroon and accession TPI-2429 was dark brown, TPI-189 and 2006-007 were white, 198-3 was brown and TPI-198 and 118-2 were light brown. The pattern colour trait was dark brown in 2006-015, TPI-198, 118-2 and 198-3 accessions, black in accessions 2006-007, 2005-011 and 205-015, while no pattern was observed in 2006-003A, TPI-2429, TPI-189, 2005-014, TPI-178, 2006-005A, PS-16 and 2006-003B accessions.

Three accessions (2006-003A, 2006-003B, and 2006-005A) came out not decided. Accession PS-16 had the highest level of cyanide with 0.03mg/kg followed by TPI-2429 (0.02mg/kg) and then 2005-014 (0.01mg/kg) than other accessions as shown in Table 9. The result in figure 1 showing PC1 of growth characters revealed that leaf width, leaf length and petiole length are closely related to each other but not closely related to plant height and number of leaves which are closely associated to leaf width, leaf length and petiole length are not related to one another.

The result of PC2 showing yield characters in figure 2 revealed that seed length, seed width, and 100 seed weight are closely related but not related to pod width, pod length, number of locules per pod, number of seeds per pod, seed thickness, fresh pod weight, number of pods per rep and number of seeds per rep. The pod width, pod length, number of locules per pod and number of seeds per pod are closely related to one another, but not related to seed length, seed width, 100 seed weight, seed thickness, fresh pod weight, number of pods per rep and number of seeds per rep. Seed thickness, fresh pod weight, number of pods per rep and number of seeds per rep are closely related to one another but not closely related to pod width, pod length, number of locules per pod, number of seeds per pod, seed length, seed width and 100 seed weight.

**Comment [mm5]:** (0.02mg/kg)

**Table 2: Growth Performance of Lima Beans Accessions.**

ACCESSION NO	PH(cm)	LL(cm)	LW(cm)	PL(cm)	NOL
TPI-2429	6.80 <sup>a</sup>	6.38 <sup>h</sup>	4.95 <sup>e</sup>	5.17 <sup>f</sup>	8.90 <sup>a</sup>
2005-011	8.17 <sup>a</sup>	7.02 <sup>fgh</sup>	7.84 <sup>bcd</sup>	7.25 <sup>bcdef</sup>	12.43 <sup>a</sup>
TPI-178	8.26 <sup>a</sup>	6.76 <sup>gh</sup>	9.23 <sup>ab</sup>	6.89 <sup>bcdef</sup>	10.57 <sup>a</sup>
2005-014	8.64 <sup>a</sup>	7.60 <sup>efgh</sup>	7.59 <sup>cd</sup>	5.17 <sup>a</sup>	10.76 <sup>a</sup>
118-2	10.01 <sup>a</sup>	8.73 <sup>bcde</sup>	7.59 <sup>cd</sup>	6.61 <sup>cdef</sup>	7.63 <sup>a</sup>
TPI-198	11.86 <sup>a</sup>	8.73 <sup>bcde</sup>	8.03 <sup>bcd</sup>	7.42 <sup>bcdef</sup>	7.48 <sup>a</sup>
2006-005A	12.20 <sup>a</sup>	8.10 <sup>defg</sup>	6.96 <sup>d</sup>	7.67 <sup>abcde</sup>	9.76 <sup>a</sup>
2006-007	14.80 <sup>a</sup>	8.57 <sup>cde</sup>	8.33 <sup>abcd</sup>	8.50 <sup>abcd</sup>	10.76 <sup>a</sup>
PS-16	12.42 <sup>a</sup>	9.48 <sup>abcd</sup>	9.07 <sup>abc</sup>	9.06 <sup>abc</sup>	8.57 <sup>a</sup>
198-3	15.56 <sup>a</sup>	9.83 <sup>abc</sup>	8.51 <sup>abc</sup>	8.91 <sup>abc</sup>	10.95 <sup>a</sup>
TPI-189	15.80 <sup>a</sup>	9.548 <sup>a</sup>	9.548 <sup>a</sup>	6.44 <sup>def</sup>	10.38 <sup>a</sup>

2006-015	16.02 <sup>a</sup>	7.87 <sup>bcd</sup>	7.87 <sup>bcd</sup>	5.85 <sup>ef</sup>	7.71 <sup>a</sup>
2006-003A	16.32 <sup>a</sup>	7.02 <sup>d</sup>	7.02 <sup>d</sup>	8.91 <sup>abc</sup>	9.95 <sup>a</sup>
2005-015	16.94 <sup>a</sup>	8.98 <sup>abc</sup>	8.98 <sup>abc</sup>	9.89 <sup>a</sup>	10.05 <sup>a</sup>
2006-003B	18.20 <sup>a</sup>	8.83 <sup>abc</sup>	8.83 <sup>abc</sup>	9.31 <sup>ab</sup>	7.71 <sup>a</sup>

Mean with different letters in the same column are significantly different from each other at  $p < 0.05$  according to Duncan Multiple Range Test (DMRT).

PH-Plant height, LL-leaf length, LW-leaf width, PL-petiole length, NOL-number of leaves.

**Table3:YieldPerformanceofLimaBeansAccessions**

ACCESSION O	NOPPR	NOSPP	NOLP P	NOSPR	PL(cm)	PW(cm)	WOFP(g)	SL(cm)	SW(cm)	ST(mm)
TPI-2429	36.33 <sup>ab</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	109.00 <sup>abc</sup>	7.66 <sup>ab</sup>	1.90 <sup>ab</sup>	129.33 <sup>a</sup>	11.26 <sup>h</sup>	8.25 <sup>gh</sup>	4.08 <sup>h</sup>
2005-011	26.33 <sup>bcd</sup>	2.67 <sup>b</sup>	2.67 <sup>b</sup>	132.00 <sup>ab</sup>	5.50 <sup>b</sup>	1.40 <sup>b</sup>	52.33 <sup>bc</sup>	15.23 <sup>c</sup>	10.77 <sup>b</sup>	3.50 <sup>i</sup>
TPI-178	19.67 <sup>cde</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	59.00 <sup>bcd</sup>	6.66 <sup>ab</sup>	1.70 <sup>ab</sup>	52.67 <sup>bc</sup>	16.67 <sup>b</sup>	9.49 <sup>de</sup>	3.64 <sup>i</sup>
2005-014	47.00 <sup>a</sup>	4.00 <sup>a</sup>	4.00 <sup>a</sup>	175.33 <sup>a</sup>	8.10 <sup>ab</sup>	2.23 <sup>a</sup>	109.00 <sup>a</sup>	14.11 <sup>e</sup>	10.91 <sup>b</sup>	4.23 <sup>gh</sup>
118-2	18.00 <sup>de</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	54.00 <sup>cd</sup>	6.66 <sup>ab</sup>	2.07 <sup>ab</sup>	50.33 <sup>bc</sup>	13.87 <sup>e</sup>	9.11 <sup>f</sup>	6.15 <sup>ab</sup>
TPI-198	13.33 <sup>def</sup>	2.67 <sup>b</sup>	2.67 <sup>b</sup>	38.33 <sup>cd</sup>	5.80 <sup>a</sup>	1.83 <sup>ab</sup>	33.00 <sup>c</sup>	14.67 <sup>d</sup>	9.69 <sup>c</sup>	4.39 <sup>g</sup>
2006-005A	0.00 <sup>f</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>j</sup>	0.00 <sup>i</sup>	0.00 <sup>j</sup>
PS-16	0.00 <sup>f</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>j</sup>	0.00 <sup>i</sup>	0.00 <sup>j</sup>
2006-007	0.00 <sup>f</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>j</sup>	0.00 <sup>i</sup>	0.00 <sup>j</sup>

198-3	10.67 <sup>ef</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	32.00 <sup>cd</sup>	5.66 <sup>b</sup>	1.83 <sup>ab</sup>	40.33 <sup>c</sup>	11.38 <sup>h</sup>	8.11 <sup>h</sup>	5.97 <sup>b</sup>
TPI-189	11.00 <sup>ef</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	33.00 <sup>cd</sup>	6.46 <sup>ab</sup>	2.17 <sup>ab</sup>	8.33 <sup>cd</sup>	12.15 <sup>h</sup>	8.45 <sup>g</sup>	4.70 <sup>f</sup>
2006-015	0.00 <sup>f</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>c</sup>	0.00 <sup>c</sup>	0.00 <sup>d</sup>	0.00 <sup>e</sup>	0.00 <sup>i</sup>	0.00 <sup>j</sup>
2006-003A	32.67 <sup>bc</sup>	4.00 <sup>a</sup>	4.00 <sup>a</sup>	130.67 <sup>ab</sup>	8.46 <sup>a</sup>	1.90 <sup>ab</sup>	96.00 <sup>ab</sup>	11.54 <sup>h</sup>	8.23 <sup>gh</sup>	5.67 <sup>c</sup>
2005-015	14.33 <sup>def</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	43.00 <sup>cd</sup>	7.33 <sup>ab</sup>	2.03 <sup>ab</sup>	37.00 <sup>c</sup>	13.79 <sup>e</sup>	9.22 <sup>ef</sup>	5.43 <sup>d</sup>
2006-003B	23.00 <sup>bcde</sup>	3.00 <sup>ab</sup>	3.00 <sup>ab</sup>	69.00 <sup>bcd</sup>	6.33 <sup>ab</sup>	1.97 <sup>ab</sup>	48.33 <sup>bc</sup>	10.05 <sup>i</sup>	8.06 <sup>h</sup>	5.16 <sup>e</sup>

Means with different letters in the same column are significant at  $p < 0.05$  according to Duncan Multiple Range Test (DMRT)

NOPPR-Number of Pods per rep, NOSPP-Number of Seeds per pod, NOLPP-Number of floccles per pod, NOSPR-Number of Seeds per rep, PL-Pod length, PW-Pod Width, WOFP-Weight of fresh Pod, SL-Seed Length, SW-Seed Width, ST-Seed thickness

**Table4:MeanSquareVarianceof GrowthCharactersin LimaBeans accessions**

Source of Variation	Df	PH	LL	LW	PL	No of Leaves
<b>Corrected</b>	44	144.01	28.06	19.04	23.36	35.94
<b>Model</b>						
<b>Intercept</b>	1	48485.79	21062.34	18505.67	17027.62	26829.36
<b>Accession Number</b>	14	285.16 <sup>ns</sup>	43.52 <sup>**</sup>	33.32 <sup>**</sup>	45.01 <sup>**</sup>	48.41 <sup>ns</sup>
<b>Replicate</b>	2	39.55 <sup>ns</sup>	21.46 <sup>**</sup>	16.30 <sup>ns</sup>	12.25 <sup>ns</sup>	8.71 <sup>ns</sup>
<b>Error</b>	264	445.46	2.63	2.97	10.86	171.92
<b>Corrected Total</b>	308					

\*\*ishighlysignificant atP<0.01, ns=notsignificant

Df-degreeoffreedom,PH-Planheight, LL- leaf length,LW-leafwidth,PL-petiolelength,NOL- numberofleaves.

**Table 5: Mean Square Variance of Yield Characters in Lima Beans accessions**

Source of variation	Df	SL	SW	ST	NOPPR	NOSPR	NOSPP	NOLPP	WOFPP	PL	PW	100 SEED WEIGHT
<b>Corrected Model</b>	14	22.21	6.41	2.76	621.99	6.56	9134.87	6.56	4883.29	310.7	2.41	405.72
<b>intercept</b>	1	223917.1	145034.37	78656.01	203.14	624.06	92.45	624.06	107.52	598.04	568.80	0.00
<b>Accession Number</b>	44	22.21**	6.41**	2.76**	621.99**	6.56**	9134.87**	6.56**	4883.29**	310.7**	2.41**	405.72**
<b>Error</b>	30	0.04	0.03	0.02	62.68	0.37	1657.53	0.37	837.48	1.86	0.15	0.00
<b>Corrected total</b>	44											

\*\*is highly significant at  $P < 0.01$

Df- degree of freedom, NOPPR- Number of Pods per rep , NOSPP- Number of Seeds per pod, NOLPP- Number of locules per pod, NOSPR-Number of Seeds per rep, PL-Pod length, PW-Pod Width, WOFPP-Weight of fresh Pod, SL-Seed Length, SW-Seed Width, ST-Seed thickness

Table6:CorrelationonGrowthCharactersofLimaBean

	<b>LL</b>	<b>LW</b>	<b>PL</b>	<b>NOL</b>
<b>PH</b>	-0.16	-0.26	-0.27	0.33
<b>LL</b>		0.82**	0.71**	0.09
<b>LW</b>			0.74**	0.09
<b>PL</b>				-0.05
<b>NOL</b>				

\*\*is highly significant at  $P > 0.5$

PH-Plant height, LL-leaf length, LW-leaf width, PL-petiole length, NOL-number of leaves.

**Table 7** Correlation on yield characters of Limbean

	SW	ST	NOPPR	NOSPP	NOSPR	NOLPP	WOFPP	PL	PW	100 SEED WEIGHT
<b>SL</b>	0.87**	-0.8	-0.27	-0.36	-0.19	-0.36	-0.29	-0.36	-0.37	0.78**
<b>SW</b>		-0.24	-0.12	-0.33	-0.23	-0.33	-0.19	-0.34	-0.36	0.76**
<b>ST</b>			-0.29	-0.13	-0.29	-0.13	-0.25	-0.14	-0.09	-0.01
<b>NOP</b>				0.82**	0.95**	0.82**	0.89**	0.81**	0.73**	-0.16
<b>NOSP</b>					0.74**	1.00**	0.71**	0.97**	0.94**	-0.23
<b>NOS</b>						0.74**	0.82**	0.72**	0.64**	-0.25
<b>NOLP</b>							0.71**	0.97**	0.94**	-0.23
<b>WOFPP</b>								0.73**	0.64**	-0.21
<b>PL</b>									0.93**	-0.26
<b>PW</b>										0.29

\*\*is highly significant at  $P > 0.5$

NOPPR-Number of Pods per rep, NOSPP-Number of Seeds per pod, NOLPP-Number of locules per pod, NOSPR-Number of Seeds per rep, PL-Pod length, PW-Pod Width, WOFPP-Weight of fresh Pod, SL-Seed Length, SW-Seed Width, ST-Seed thickness.

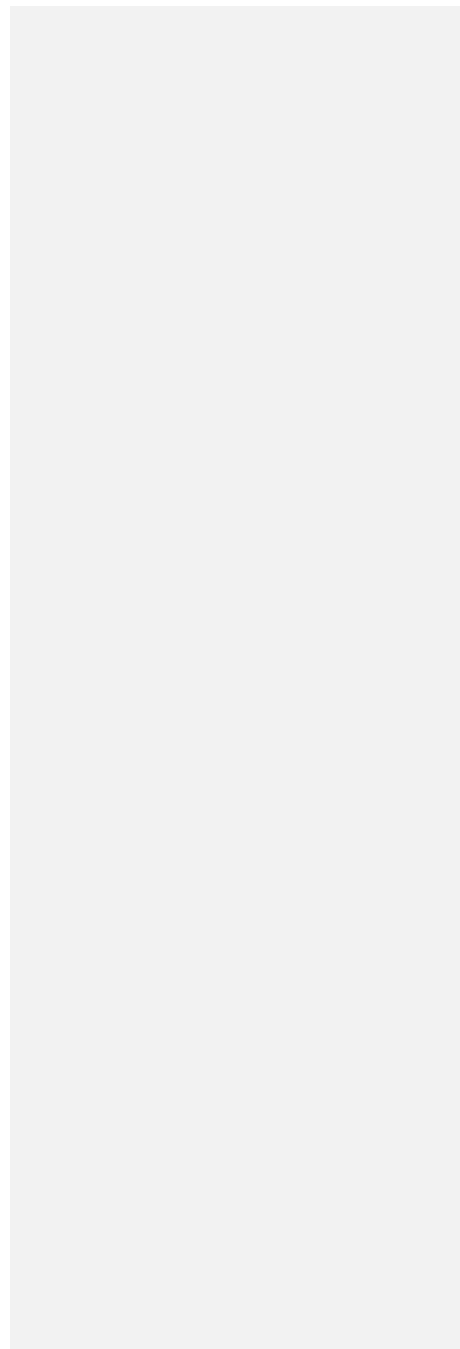
**Table 8a: Quantitative Characters of Lima Beans Accessions**

Accession No	Plant Type	Leaf Shape	Flower Wing	Pod colour (young)	Pod Tip	Pod curvature	Seed Shape	Seed Colour	Pod Colour	Pattern Colour
2006-003A	Vine	Ovate lanceolate	white-yellow	Green	short	slightly curved	kidney	buff	brown	no pattern
2006-015	Vine	Ovate lanceolate	white-yellow	Green	short	Straight	wide kidney	buff flat	brown	dark brown
TPI-2429	Vine	lanceolate	white-yellow	Green	short	slightly curved	very wide kidney	dark brown	brown	no pattern
TPI-189	Vine	lanceolate	white-yellow	Green	short	Slightly curved	flat kidney	white	brown	no pattern
TPI-198	Vine	Round	white-yellow	Green	short	Slightly curved	wide kidney	flat light brown	brown	dark brown
118-2	Vine	Round	white-yellow	Green	short	slightly curved	wide kidney	flat light brown	brown	dark brown
198-3	Vine	Ovate lanceolate	white-yellow	Green	short	slightly curved	wide kidney	brown flat	brown	dark brown

2006-007 Vine Round white-  
yellow Green short Straight round white brown black

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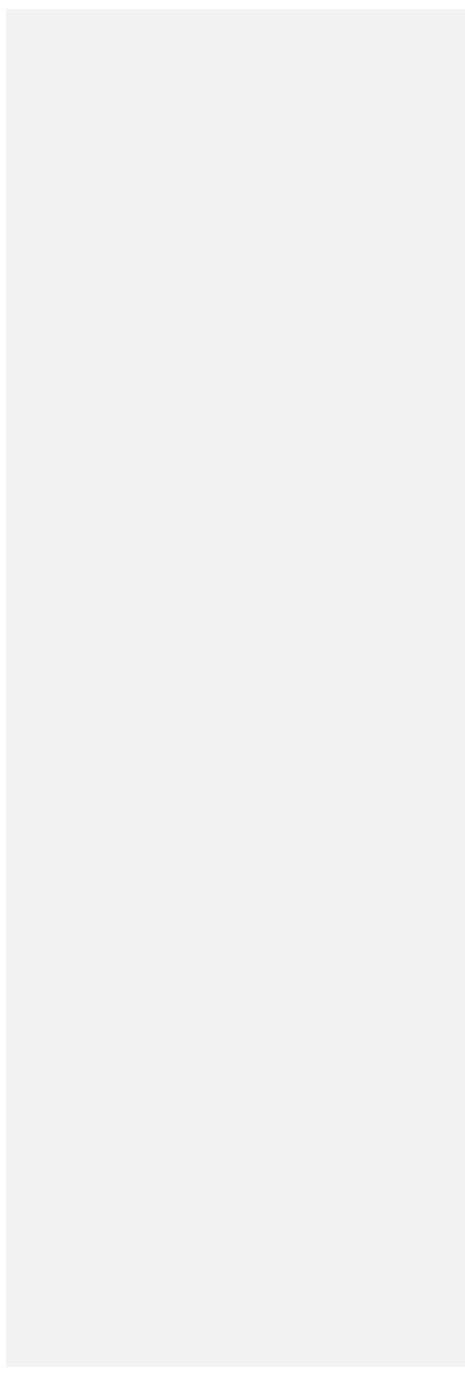
**Table 8b: Quantitative Characters of Lima Beans Accessions**

Accession No	Plant Type	Leaf Shape	Flower Wings	Pod colour(young)	Pod Tip	Pod curvature	Seed Shape	Seed Colour	Pod Colour	Pattern Colour
2005-014	Bush	Ovate	white-yellow	Green	short	Slightly curved	round	buff	brown	no pattern
2005-011	Bush	Ovate	white-yellow	Green	short	slightly curved	round	maroon	brown	black
TPI-178	Bush	Ovate	white	Green	Short	Slightly curved	medium flat kidney	maroon	brown	no pattern
2006-005A	Bush	Ovate	white-yellow	Green	Short	slightly curved	round	buff	brown	no pattern
PS-16	Vine	Ovate lanceolate	white-yellow	Green	Short	slightly curved	round	maroon	brown	no pattern
2005-015	Vine	Ovate lanceolate	white-yellow	Green	Short	slightly curved	round	buff	brown	black
2006-003B	Vine	Ovate lanceolate	white-yellow	Green	short	slightly curved	oval cuboid	maroon	brown	no pattern

**Table9: Cyanidetest**

<b>SAMPLE</b>	<b>REPLICATES</b>	<b>SAMPLECYANIDE(mg/kg)</b>
2005-014	1	0.01
	2	0.01
	3	0.01
2006-003A	1	ND
	2	ND
	3	ND
2006-003B	1	ND
	2	ND
	3	ND
2006-005A	1	ND
	2	ND
	3	ND
PS-16	1	0.03
	2	0.02
	3	0.03
		39

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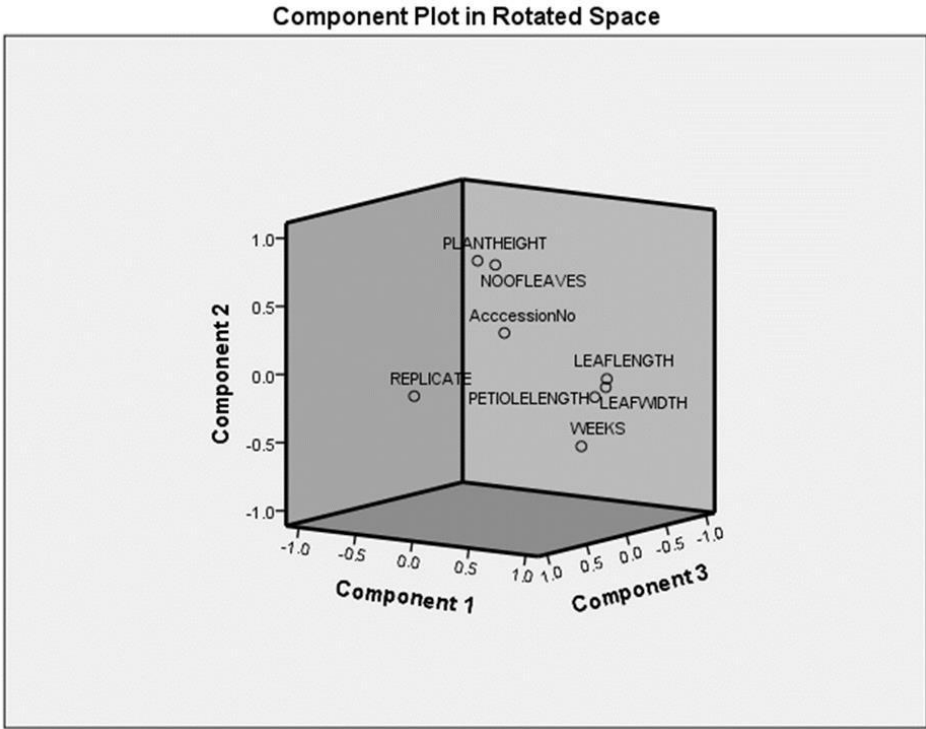


TPI-2429	1	0.02
	2	0.02
	3	0.01

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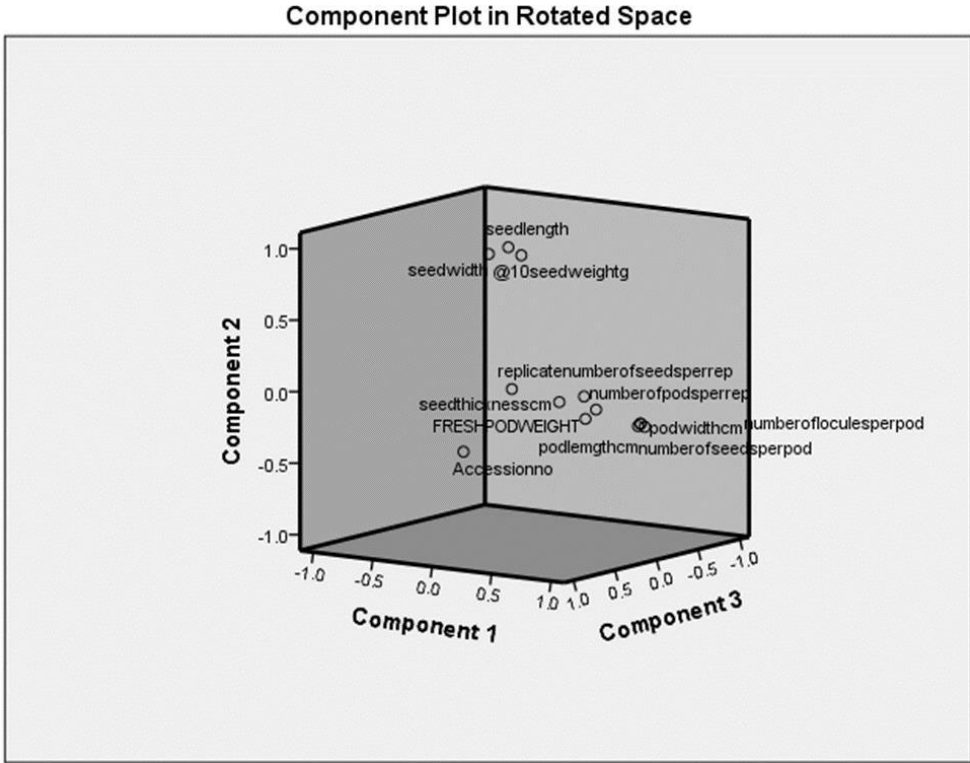
ND=NotDecided

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**Figure1:Principal Component of showing the Growth Characters of Lima Beans Accessions.**

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**Figure2:Principal Component of Yield Characters in Lima BeansAccessions**

## 4.0 DISCUSSION

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This study revealed variation in morphological characteristics of fifteen accessions of Lima beans. 2005-014 accessions performed best for most of the character scored. Accessions TPI-2429 and 2006-003A performed best in yield related characters. The variation shown by the characters could be as a result of high genetic diversity, differences of growing type and difference in the type of adaptation as similarly reported by Kulakow (1987), Mujica and Jacobsen (2003) and Montero-Rojas *et al.* (2013). It was observed that 2005-011, 2005-014 and TPI-178 accessions germinated earlier, flowered with white-yellow, podded earlier and had good yield than other accessions are in agreement with the findings made by Yaguiet *al.* (2003) and Gepts (2012) who reported yield and pattern of color on the skin of the seeds as a factor of biochemical pigmentation process and a characteristics of wild type character of the cultivated species.

Findings from correlation of growth characters revealed that the leaf length and leaf width of Lima bean significantly influenced petiole length positively, but did not affect the height of the plant. Again, the yield characters such as number of pods per rep, number of seeds per pod, number of locules per pod, number of seeds per rep, pod length, pod width, weight of fresh pod, seed length, seed width, seed thickness and 100 seed weight significantly associated with one another. This implies that these characters can be improved based on this selection criterion to enhance growth and performance of various legumes in plant population as similarly reported by Porbeniet *al.* (2018).

Levels of cyanide is significantly far lower than oral toxicity standard of 50 to 90 mg HCN equivalent/Kg body weight (WHO, 2004), less than the lethal dose of cyanide intoxication of human which has been reported as 200 to 300 mg/kg by Akiyama *et al.* (2006) in mushroom and also, less than permissive dosage reported by Orjiekweet *al.* (2013)) as well as lower than 100- 120 ppm/100-120mg/kg stated by Baudoin (2006) who observed that domesticated Lima bean species contained plant rich nutrients, and had shown that cooking and different other modifications were used to reduce cyanide content present in the beans.

## CONCLUSION AND RECOMMENDATION

Accession 2005-014 had the highest growth and yield performance. The Leaf length, Leaf width and Petiole length are foliar characters as well as seed length, seed width, number of pods per rep, number of seeds per pod, number of seeds per rep, number of locules per pod, fresh pod weight, and pod length which are yield characters should be considered in breeding of lima beans. The three accessions (2006-003A, 2006-003B, 2006-005A) which

hadnocyanidecontentscannot be harmful to the body system. Therefore, TPI-2429, 2006-003A and 2005-011 accessions which showed higher growth and yield characters with no cyanide content could be further improved in breeding of lima beans.

## REFERENCES

- Adebo, J.A. A Review on the Potential Food Application of Lima Beans (*Phaseolus lunatus* L.), an Underutilized Crop. Appl. Sci. 2023; 13. doi.org/10.3390/app13031996
- Adeniran, H.A., Farinde, E.O. and Obatolu, V.A. Effect of heat treatment and fermentation on the anti-nutrients content of Lima bean (*Phaseolus lunatus*) during production of Daddawa analogue. Annual Review and Research in Biology 2013;3 (30):256-266.
- Akande, S.R. and Balogun, M.O. Evaluation and heritability studies of local Lima bean (*Phaseolus lunatus* L.) cultivars from south-west Nigeria. Revista UDO Agrícola 2007;7(1):22-28.
- Akiyama, H., Toida, T., Sakai, S., Amakura, Y., Kondo, K., Sugita-Konishi, Y. and Maitani, T.. Determination of cyanide and thiocyanate in sugihiratake mushroom using HPLC method with fluorometric detection. J. Health Sci. 2006; 52:73-77.
- Andueza-Noh, R.H., Martínez-Castillo, J. and Chacón-Sánchez, M.I. Domestication of small-seeded Lima bean (*Phaseolus lunatus* L.) and traces in Mesoamerica: evidence from microsatellite markers, Genetica 2015;143(6): 657-669.
- Arora, R.K. Diversity in Underutilized Plant Species – An Asia-Pacific Perspective. Bioversity International, New Delhi, India. 2014
- Balagopalan C, Rajalekshmy L. Cyanogen accumulation in environment during processing of cassava (*Manihot esculenta* Crantz) for starch and sago. *Water Air Soil Pollut.* 1998;102:407–413.
- Ballhorn, D.J., Schiwy, S., Jensen, M. and Heil, M. (2008b). Quantitative variability of direct chemical defense in primary and secondary leaves of Lima bean (*Phaseolus lunatus*) and consequence for an natural herbivore. *Journal of Chemical Ecology* 2008b. 34:1298–1301.
- Baudet, J.C. The taxonomical status of cultivated types of Lima bean (*Phaseolus lunatus* L.). *Tropical Grain Legume Bull.* IITA, Ibadan, Nigeria 1977;7: 29–30.
- Baudoin, J.P. *Phaseolus lunatus* L. PROTA 1: Cereals and pulses/Céréales et légumes secs [ed. by Brink, M., Belay, G.]. Wageningen, the Netherlands: PROTA. 2006.
- Bolarinwa, I.F., Oke, M.O., Olaniyan, S.A. and Ajala, A.S. A review of cyanogenic glycosides in edible plants, in: M.L. Larramendy (Ed.), *Toxicology-New Aspects to This Scientific Conundrum*. In Tech Open publishers, London, UK, 2016; 179-19.

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- Bonita, L.C.; Shantibala Devi, G.A.; Singh, B.C.H. Lima bean (*Phaseolus lunatus* L.) a health perspective. *Int. J. Sci. Technol. Res.* 2020; 9: 5638–5649.
- Broughton, W.J., Jabbouri, S. and Perret, X. Keys to symbiotic harmony. *Journal Bacteriology* 2002; 182 (20): 5641-5652.
- Edmund Teye, M. K. Morphological and Genetic Characterization of some Lima bean (*Phaseolus lunatus* L.) Cultivars and their nodulating rhizobia. A thesis is submitted to the University of Ghana. 2014
- Fofana, B., Vekemans, X., du Jardin, P. and Baudoin, J.P. Genetic diversity in Lima bean (*Phaseolus lunatus* L.) as revealed by RAPD markers. *Euphytica* 1999; 95: 157–165.
- Gepts, P. Domestications of Phaseolus Beans and Their Importance for Conservation and Genetic Improvement, viewed in April 2017, from [http://www.ias.csic.es/grainlegumesmagazine/legum\\_perspect\\_2.pdf](http://www.ias.csic.es/grainlegumesmagazine/legum_perspect_2.pdf). 2014
- Gutiérrez-Salgad, A., Gepts, P. and Debouck, D. Evidence for two gene pools of the Lima bean, *Phaseolus lunatus* L., in the Americas. *Genetic Resource Crop Evolution* 1995; 42: 15–28.
- Heuzé, V., Sauvant, D., Tran, G., Bastianelli, D. and Lebas, F. 2013. Lima bean (*Phaseolus lunatus*). *Feedipedia.org*. 2013
- International Board for Plant Genetic Resources (IBPGR). Lima bean descriptors. 1982.
- International Board for Plant Genetic Resources (IBPGR). Genetic resources, diversity and breeding. 2015 Retrieved from: [www.cgiar.org](http://www.cgiar.org) and [www.fao.org](http://www.fao.org). Accessed on 5, December, 2022 at 4:10 pm.
- Kathirvel, P. and Kumudha P. A Comparative Study on the Chemical Composition of Wild and Cultivated Germplasm of *Phaseolus Lunatus* L. *International Journal of Applied Biology and Pharmaceutical Technology*, 2011; 2(4):296–305.
- Kulakow, P.A. Genetics of grain amaranths. *Journal of Hereditary* 1987; 78: 293-297
- Kyeremateng, D.O. Determination of Compositional Characteristics, Functional Properties and Cluster Analysis of Lima Bean Accessions (*Phaseolus Lunatus*). *Tesis*. Kwame Nkrumah University of Science and Technology Kumasi, Ghana. Department of Food Science and Technology College of Science. 2015
- Montero-Rojas, M., Ortiz, M., Beaver, J.S. and Sirtunga, D. Genetic, morphological and cyanogen content evaluation of a new collection of Caribbean Lima bean (*Phaseolus lunatus* L.) landraces. *Genetic Resource Crop Evolution* 2013; 60: 2241–2252.
- Mujica, A. and Jacobsen, S.E. 2003. The genetic resources of Andean grain amaranths (*Amaranthus caudatus* L., *Amaranthus cruentus* L. and *Amaranthus hypochondriacus*) in America. *Plant Genetic Resources Newsletter* 2003; 133: 41-44.
- Ologhobo, A.D., Fetuga, B.L. and Tewe, O.O. The cyanogenic glycoside contents of raw and processed Lima bean varieties. *Food Chemistry*. 1984; 13: 117–128.
- Orjiekwe C.L., A. Solola, E. Iyen and S. Imade, 2013. 18. Determination of cyanogenic glucosides in cassava products sold in Okada, Edo State, Nigeria, *African Journal of Food Science* 2013;

7(12): 468-472

Osuntokun, B.O. Chronic cassava toxicity: An interdisciplinary workshop. B. Nestel and R. Macintyre, London, England, 1973:127-138.

Porbeni, J.B.O. Ayo-Vaughan, M.A. and Osunniyi, O. Genetic variability among some miscellaneous legumes for yield-related traits. *Nigeria Journal of Biotechnology*. 2018; 35(2): 1-7. DOI: <https://dx.doi.org/10.4314/njb.v35i2.1>

Salawu, S.O., Folorunso, O.M., Akindahunsi, A.A. and Boligon, A.A. Antioxidant indices and amino acid composition of phenolic containing Lima beans (*Phaseolus lunatus*) after simulated human gastrointestinal digestion. *International Journal of Food Studies* 2019; 8: 97–110.

Soetan, K. O. and Atanda, T. N. Proximate composition, phytochemical screening and some antinutritional factors of three accessions of lima beans an underutilized legume. *Nigerian Journal of Animal Production* 2018; 45(3): 71 – 78.

WHO. Hydrogen cyanide and cyanides: Human Health Aspect. WHO, 2004. Geneva.

Yagui, A., Machado-Neto, N.B., and Cardoso, V.J. M. Grouping of Brazilian Accesses of Lima Beans (*Phaseolus lunatus* L.) According To SDS-PAGE Patterns and Morphological Characters. *Maringa*. 2013; 25(1):7-12.

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