

Phenotypic and Seed Protein Analysis in 31 Lima Bean (*Phaseolus lunatus*) Accessions in Ghana

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Abstract

Phenotypic and seed protein analyses were performed on 31 accessions of Lima bean assembled in Ghana. Data on 16 phenotypic characters consisting of eight quantitative and eight qualitative were analysed. There were significant differences among the accessions based on the eight quantitative characters. Seed protein analysis showed 17 bands with relative mobility of bands, which ranged from 0.01 to 0.86. An ordinal logistic regression analysis showed significant evidence for seed coat, pod beak shape and seed size association. Cluster analysis based on both phenotypic and protein data provided evidence for differences among the accessions. Quantitative characters were associated with some specific clusters.

Introduction

Lima bean (*Phaseolus lunatus* L.) is the most important food legume within the neotropical genus *Phaseolus* (Baudoin *et al.*, 1991). In Ghana it is fourth in importance after cowpea, groundnut and bambara groundnut (Doku, 1977) while in Nigeria, it is placed second in importance to cowpea (FAO/CCTA, 1958). In tropical Africa the crop is interplanted with crops such as maize, sorghum, sweet potatoe, coffee, cotton and yam. The crop is hardy and may be advantageous in adverse conditions where other leguminous vegetables do not grow well (FAO, 1984).

The banding patterns produced by seed protein electrophoresis have been used to effectively characterize cultivars of pasture grasses and legumes (Sheidai *et al.*, 2000), *Capsicum annuum* and *C. frutescens* (Odeigah *et al.*, 1993). Multiple domestication centres have been suggested through the seed protein electrophoresis analysis from different wild and cultivated accessions of common bean (Gepts *et al.*, 1986). Seed storage protein electrophoresis has been used to estimate diversity among accessions in genetic resources collection and species inter-relationships (Gardiner & Forbe, 1992; Badr, 1995). Generally, seed storage proteins have been used as markers in the following four main areas: analysis of genetic diversity within and among populations, plant domestication in relation to genetic resources conservation and breeding, genome relationships especially in polyploid series, and as a tool in plant breeding (Gepts, 1990).

Storage seed protein satisfies the requirements of genetic markers for the following reasons: they are highly polymorphic, their polymorphism is genetically determined and the molecular sources of their polymorphism are known; their control of qualitative genetic variations is simple and involves limited number of loci of nuclear genome; and they show that most protein variants are unique and can, therefore, be used as evolutionary markers. Finally, homologies have been established between seed storage proteins of different taxa (Gepts, 1990). The objective of the study was to use seed protein and phenotypic analyses to study variety inter-relationships and provide data on differences among accessions of *P. lunatus* in Ghana.

Materials and methods

Morphometry

The 31 accessions of Lima bean used for the experiment were collections from local farmers and market places. They form the total number of Lima bean collections at the Plant Genetic Resources Centre of Ghana at Bunso in the Eastern Region. The accessions were as follows:

99/001, 99/002, 99/003, 99/004, 99/005, 99/006, 99/007, 99/008, 82/293, 82/292, 99/009, 99/010, 99/011, 99/012, 82/414, 82/125, 87/160, 87/134, 82/166, 87/118, 99/013, 82/482, 82/493, 82/469, 82/489, 99/014, 99/015, 99/017, 99/018, 82/496 and 82/505.

The experiment was conducted in 2003 in the University of Ghana Research Farms of the Department of Crop Science. The accessions were planted in a completely randomized design (CRD) with three replications. Each entry had two rows per replication and each row was 5.0 m long. The rows were 50 cm apart and plants within a row were 30 cm apart. Two seeds were planted per hill and thinned out to one plant per hill at 10 days after seedling emergence. There was no fertilizer application. The morphological characters studied were grain yield, number of seeds per pod, 100-seed weight, seed width, seed length, cyanide content (mg/100 g), pod beak shape, flower wing colour, keel colour, pod hairiness, wing opening position, pod dehiscence, raceme position, leaf persistence, terminal leaflet length and seed coat colour. These characters were selected on the basis that they are among the standard characters in the Lima Descriptor by the Plant Genetic Resources Institute.

Protein extraction and electrophoresis

Protein electrophoresis by SDS-PAGE was performed according to modified method of Sanchez-Yelamo *et al.* (1995), using 0.39 M tris phosphate buffer (pH 6.8) for extraction. The crude extracts were boiled for 2 min. in tris 0.0625 M tris/HCl (pH 6.8), 2% sodium dodecyl sulphate (SDS), 2% 2-mercaptoethanol and 10% glycerol. The gels were loaded with 15 µl of the seed protein extracts and were electrophoresed at a constant 30 mA for 8 h. Coomassie Brilliant Blue G-250 was used for staining the gel and stored in 20% glycerol solution.

Data analysis

Gowers similarity coefficient was used to estimate similarity of accessions based on the phenotypic traits morphological characters. The Jaccard's index was used to estimate similarity among the accessions as indicated by protein electrophoresis patterns. All similarity coefficients were based on the UPGMA by using the SPSS package. The ordinal logistic regression analysis was performed by using Minitab Software package to test for association between quantitative and qualitative traits.

Results and discussion

Phenotypic characters

A summary of variability of the eight quantitative characters is presented in Table 1. There were significant differences among the accessions for all the eight quantitative characters as indicated by the standard error of difference. Number of pods per plant ranged from 40.34 to 87.00 with a mean of 56.86. The 100-seed weight ranged from 23.84 to 71.81 g with a mean of 50.84 g. Grain yield per plant ranged from 31.73 to 144.40 g with a mean of 83.86 g. Cyanide content ranged from 5.20 to 7.18 mg/100 g with a mean of 6.15 mg/100 g. Seed width ranged from 0.62 to 1.25 cm with a mean of 0.99 cm. Seed length ranged from 0.86 to 1.77 cm with a mean of 1.45 cm. Terminal leaflet width and length ranged from 2.44 to 7.59 cm and 6.47 to 10.58 cm, with means of 5.97 cm and 7.79 cm, respectively. Table 2 shows a summary of the qualitative characters studied. Four seed coat colour patterns were observed among the 31 accessions. Three different classes were observed for each of pod beak shape, flower wing colour and pod raceme position. Two different classes were observed for each of flower keel colour, hairiness of standard petal, nature of wing opening and pod dehiscence.

TABLE 1
Quantitative characters of 31 Lima bean accessions

<i>Accessions</i>	<i>Number of pods per plant</i>	<i>100-seed weight (g)</i>	<i>Grain yield per plant (g)</i>	<i>Seed width (cm)</i>	<i>Seed length (cm)</i>	<i>HCN (mg/100 g)</i>	<i>Terminal leaflet width (cm)</i>	<i>Terminal leaflet length (cm)</i>
99/001	65.03	63.50	124.00	1.16	1.75	5.80	6.01	7.85
99/002	62.47	41.12	73.69	0.96	1.40	7.80	7.59	8.51
99/003	44.07	23.84	31.73	0.62	0.86	7.10	4.94	6.78
99/004	64.33	38.96	69.61	0.95	1.30	6.10	6.90	10.09
99/005	53.14	61.25	96.69	1.12	1.69	6.25	6.20	7.95
99/006	59.27	65.53	108.40	1.25	1.88	5.50	7.19	9.18
99/007	54.00	27.39	44.58	0.70	0.98	5.35	4.59	7.69
99/008	69.60	32.37	63.51	0.89	1.08	5.20	5.23	7.67
82/293	60.73	70.36	122.80	1.21	1.77	5.60	7.19	8.81
82/292	87.80	25.06	64.06	0.74	0.90	5.50	5.53	7.85
99/009	57.27	29.45	49.57	0.74	0.97	7.85	2.44	10.58
99/010	44.80	67.72	92.33	0.95	1.53	6.25	6.69	7.80
99/011	46.53	62.94	85.11	1.08	1.67	5.80	7.09	7.55
99/012	54.80	60.47	100.90	1.15	1.70	5.50	5.42	7.09
82/414	48.80	69.66	102.80	1.26	1.72	6.45	5.55	7.53
82/125	43.87	66.97	88.96	0.76	1.64	5.98	6.19	7.70
87/160	58.08	59.33	103.30	1.17	1.74	6.16	6.62	7.98
87/134	73.20	32.10	60.76	0.81	1.00	5.35	4.11	6.97
82/166	59.73	70.19	126.10	1.15	1.77	5.80	6.87	7.89
87/118	42.87	67.53	85.23	1.17	1.74	5.80	6.13	7.29
99/013	87.00	31.53	80.50	0.74	1.13	5.65	6.17	7.78
82/482	40.34	59.77	71.90	1.07	1.64	5.80	5.85	7.48
82/493	50.87	63.40	94.50	1.11	1.68	5.35	6.01	7.22
82/469	67.73	23.90	87.48	0.73	0.90	5.65	5.25	6.47
82/489	56.60	54.84	88.70	1.07	1.57	5.35	6.73	8.78
99/014	46.33	55.83	76.60	1.08	1.62	5.50	5.79	6.59
99/015	51.33	71.81	109.30	1.16	1.70	5.95	6.89	9.21
99/017	45.87	57.81	81.00	1.15	1.73	5.65	6.21	7.40
99/018	46.08	61.83	86.40	1.09	1.70	7.18	6.31	7.31
82/496	51.47	54.01	80.46	1.15	1.65	5.35	5.49	6.87
82/505	63.27	57.51	144.40	1.07	1.66	5.20	4.89	6.63
CV (%)	20.94	31.12	28.40	18.60	21.92	11.76	17.74	12.05
LSD (5%)	4.91	0.79	7.08	0.05	0.02	0.17	0.12	0.10

Loi (1994) and Esquivel *et al.* (1990) identified five different morphotypes of Lima based on seed weight, seed length and seed width as follows: Big Lima (100–110 g, 25 mm long, 14 mm wide), Sieva (30–45.3 g, 12 mm long), Potato (35.5 g, 9 mm long, 8 mm wide), Potato-Sieva (36.3 g, 11 mm long, 8 mm wide) and Sieva-Big (77.5 g, 17 mm long, 11 mm wide). Based on these criteria, the 31 accessions of the present work could be representatives of the Potato, Potato-Sieva and Sieva-Big morphotypes of Loi (1994) and Esquivel *et al.* (1990). To confirm the absence of the Big Lima type in Ghana large number of accessions must be collected and subjected to further analysis. The use of seed morphotype for classification must be used with caution because seed weight, seed length and seed width can be influenced by the environment and also the level of heritability of these characters must be determined.

TABLE 2
Summary description of band pattern in 31 Lima bean accessions

<i>Band</i>	<i>Relative distance</i>	<i>Total number of bands</i>	<i>Band frequency</i>
1	0.01	1	0.003

2	0.07		3	0.008
3	0.10		11	0.030
4	0.14		23	0.062
5	0.17		28	0.075
6	0.21		30	0.081
7	0.29		31	0.083
8	0.36		31	0.083
9	0.47		31	0.083
10	0.53		30	0.081
11	0.57		1	0.003
12	0.59		30	0.081
13	0.61		31	0.083
14	0.64		22	0.059
15	0.68		23	0.062
16	0.76		25	0.067
17	0.86	31	0.083	

Association between seed coat colour and seed characteristics, pod beak length and seed characteristics

An ordinal logistic regression analysis was performed to test for association between the quantitative and qualitative characters. The following characters were found to have effect on seed coat colour: seed weight (constant = -0.091, $z = -3.34$, $P = 0.001$, odd ratio = 0.91), seed width (constant = -6.145, $z = -2.83$, $P = 0.005$, odd ratio = 0.00) and seed length (constant = -4.161, $z = -3.21$, $P = 0.001$, odd ratio = 0.02) in the same manner seed weight (constant = 0.999, $z = 3.24$, $P = 0.001$, odd ratio = 1.10), seed length (constant = 4.588, $z = 3.11$, $P = 0.002$, odd ratio = 98.28), and seed width (constant = 5.604, $z = 2.54$, $P = 0.011$, odd ratio = 271.49) showed effect on pod beak shape.

Association among characters could be attributed to either genetic linkage or pleiotropy. Sax (1923) found linkage between seed colour and seed size in segregating population of *Phaseolus vulgaris*. Brittingham (1950) reported association between genes controlling buff seed-coat and pod length, the genes controlling general colour factor and seed size in the *Vigna unguiculata*, Saunders (1960) also suggested that seed-coat colour and date of maturity are associated in a quantitative-qualitative linkage in the same species.

In the present study there was evidence between a quantitative-qualitative association for seed coat, pod beak shape (qualitative), and seed weight, seed length and seed width (quantitative). This association could be attributed to either genetic linkage or pleiotropy. Further study is underway to develop mapping populations to confirm this finding. It, therefore, remains to comment that in addition to seed morphotypes as the main basis of Lima classification, polymorphisms in seed coat colour and pod beak shape observed in the present work can be used as criteria for further classification in Lima bean. This association can also give an evidence of coevolution of seed coat colour, pod beak shape and seed size in the Lima bean.

Seed protein

Seed protein analysis of the Lima bean accessions studied revealed the presence of 17 bands. Table 3 shows a summary description of band pattern. Relative mobility of bands ranged from 0.01 to 0.86. Bands 7, 8, 9, 13 and 17 were present in all 31 accessions, while bands 1 and 11 were present in only accessions 87/166 and 82/144, respectively. Band 2 was present in accessions 82/134, 99/013 and 82/414. Bands 1 and 11 recorded the lowest frequency of 0.003 each (Table 3).

TABLE 3

Mean of relevant characteristics for each of the six clusters

<i>Characteristic</i>	<i>Cluster</i>					
	<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>
No. of seeds per pod	52.10	48.80	63.40	44.07	57.27	73.22
100-seed weight (g)	68.92	69.66	40.04	23.84	29.45	28.72
Grain yield (g)	98.36	102.8	71.65	31.78	49.57	66.82
Seed width (cm)	1.11	1.26	0.96	0.62	0.74	0.76
Seed length (cm)	1.69	1.72	1.35	0.86	0.97	1.00
HCN (mg/100g)	5.79	6.45	7.00	7.10	7.85	5.54
Terminal leaflet length (cm)	6.29	5.55	7.24	4.94	2.44	5.04
Terminal leaflet width (cm)	7.74	7.53	9.30	6.78	10.58	7.36

Phenetic analysis

UPGMA cluster analysis of phenotypic (quantitative and qualitative) data based on Gower General Similarity Coefficient grouped the accessions into six major clusters at 0.76 similarity level (Fig. 1). The first cluster comprised 20 accessions. The second cluster contained only one accession (Accession 82/414) and the third cluster contained two accessions (Accessions 99/002 and 99/004). The fourth and fifth clusters contained one accession each. Means of the eight quantitative characters associated with each cluster are presented in Table 4. The sixth cluster was associated with the highest mean number of seeds per pod (73.22), while the fourth cluster was associated with the lowest number of seeds per pod. The fourth cluster had the lowest mean 100-seed weight (23.84 g). The second cluster gave the highest mean grain yield (102.8 g), while the fourth cluster was associated with the lowest mean grain yield (31.73 g). The highest mean seed width (1.26 mm) was associated with the second cluster, while the lowest mean seed width (0.62 mm) was associated with the fourth cluster. The highest mean terminal leaflet length (7.24 cm) was associated with the third cluster, while the lowest mean terminal leaflet length (2.44 cm) was associated with the fifth cluster.

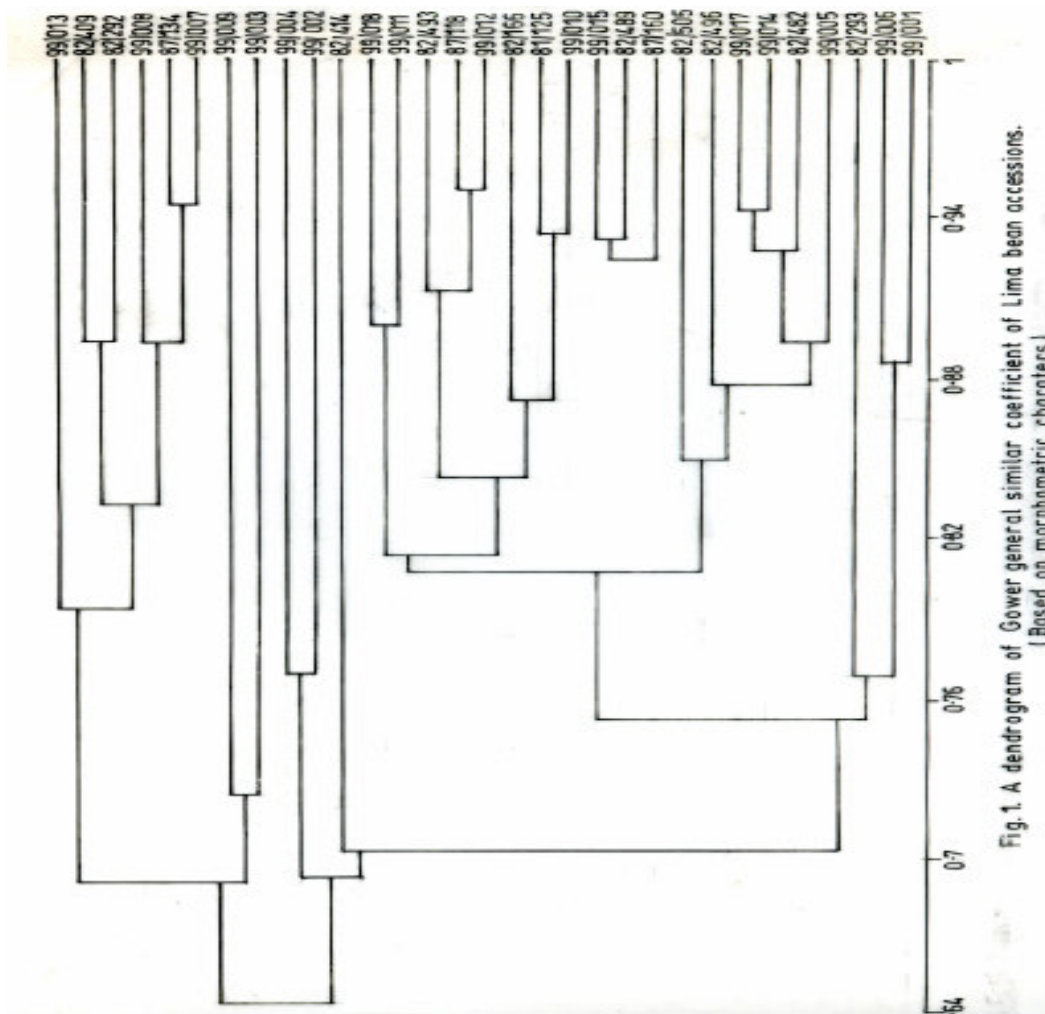


Fig. 1. A dendrogram of Gower general similar coefficient of Lima bean accessions. (Based on morphometric characters)

TABLE 4
Qualitative characteristics of 31 accessions of lima accessions

Accession	Wing opening	Pod raceme position	Pod dehiscence (initial)	Pod raceme position at 90% full pod	Persistence of leaf
99/001	Parallel	Within foliage	Non shattering	Within foliage	Intermediate
99/002	Parallel	Intermediate	Non shattering	Intermediate	Intermediate
99/003	Parallel	Within foliage	Non shattering	Intermediate	Intermediate
99/004	Parallel	Intermediate	Non shattering	Within foliage	Few leaves remaining
99/005	Parallel	Within foliage	Non shattering	Emerging from leaf canopy	Intermediate
99/006	Parallel	Intermediate	Non shattering	Within foliage	Intermediate
99/007	Parallel	Emerging from leaf canopy	Dehisce no shattering	Emerging from leaf canopy	Few leaves remaining
99/008	Parallel	Emerging from leaf canopy	Non shattering	Emerging from leaf canopy	Few leaves remaining
82/293	Intermediate	Intermediate	Non shattering	Within foliage	Few leaves remaining
82/292	Parallel	Intermediate	Non shattering	Intermediate	Intermediate
99/009	Parallel	Within foliage	Non shattering	Emerging from	Intermediate

				leaf canopy	
99/010	Parallel	Intermediate	Non shattering	Intermediate	Intermediate
99/011	Intermediate	Intermediate	Non shattering	Intermediate	Intermediate
99/012	Parallel	Intermediate	Non shattering	Intermediate	Few leaves remaining
82/414	Parallel	Within foliage	Non shattering	Within foliage	Few leaves remaining
82/125					
87/160	Parallel	Within foliage	Non shattering	Within foliage	Few leaves remaining
87/134	Parallel	Emerging from leaf canopy	Non shattering	Emerging from leaf canopy	Few leaves remaining
82/166	Parallel	Intermediate	Non shattering	Within foliage	Intermediate
87/118	Parallel	Intermediate	Non shattering	Intermediate	Few leaves Remaining
99/013	Parallel	Emerging from leaf canopy	Dehisce non shattering	Emerging from leaf canopy	Intermediate
82/482	Parallel	Within foliage	Non shattering	Intermediate	Intermediate
82/493	Parallel	Intermediate	Non shattering	Intermediate	Intermediate
82/469	Parallel	Intermediate	Non shattering	Intermediate	Few leaves remaining
82/489	Intermediate	Within foliage	Non shattering	Within foliage	Few leaves remaining
99/014	Parallel	Within foliage	Non shattering	Within foliage	Intermediate
99/015	Parallel	Within foliage	Non shattering	Within foliage	Few leaves remaining
99/017	Parallel	Within foliage	Non shattering	Within foliage	Intermediate
99/018	Intermediate	Within foliage	Non shattering	Within foliage	Intermediate
82/496	Parallel	Within foliage	Non shattering	Intermediate	Intermediate
82/505	Parallel	Within foliage	Non shattering	Intermediate	Intermediate

Table 4 cont'd
Qualitative characteristics of 31 accessions of Lima accessions

<i>Accession</i>	<i>Seed testa colour</i>	<i>Beak shape</i>	<i>Flower wing colour</i>	<i>Colour of keel</i>	<i>Hairiness of standard</i>
99/001	brown	Long	Light pink	Pink	Sparsely on tip
99/002	cream	Short	Light pink	Pink	Moderate
99/003	cream	Short	Violet	Pink	Sparsely on tip
99/004	purple brown	Medium	Light pink	Pink	Sparsely on tip
99/005	white	Medium	White	Green	Sparsely on tip
99/006	brown	Medium	Light pink	Pink	Sparsely on tip
99/007	brown	Long	White	Green	Sparsely on tip
99/008	purple red	Short	White	Green	Sparsely on tip
82/293	white	Long	White	Green	Sparsely on tip
82/292	cream	Short	White	Green	Sparsely on tip
99/009	brown	Short	White	Green	Moderate
99/010	white	Medium	White	Green	Sparsely on tip
99/011	white	Medium	White	Green	Sparsely on tip
99/012	white	Medium	White	Green	Sparsely on tip
82/414	purple brown	Long	Light pink	Pink	Moderate
82/125	white	Long	White	Green	Sparsely on tip
87/160	white	Short	White	Green	Sparsely on tip
87/134	purple red	Short	White	Green	Sparsely on tip
82/166	white	Medium	White	Green	Sparsely on tip
87/118	white	Medium	White	Green	Sparsely on tip
99/013	cream	Medium	White	Green	Sparsely on tip

82/482	white	Medium	White	Green	Sparsely on tip
82/493	white	Medium	White	Green	Moderate
82/469	cream	Short	White	Green	Sparsely on tip
82/489	white	Medium	White	Green	Sparsely on tip
99/014	white	Long	White	Green	Sparsely on tip
99/015	white	Medium	White	Green	Sparsely on tip
99/017	white	Medium	White	Green	Sparsely on tip
99/018	white	Medium	White	Green	Sparsely on tip
82/496	white	Medium	White	Green	Moderate
82/505	white	Medium	White	Green	Sparsely on tip

UPGMA cluster analysis of the protein profile based on Jaccard's index grouped the accessions into three major clusters at 0.76 level of similarity (Fig. 2). The first cluster contained 29 accessions. Within the first cluster there were four sub-clusters of which the first, second, third and fourth sub-clusters contained seven, 10, six and six accessions, respectively. The second and third major clusters comprised one accession each, namely accession 82/293 and 99/007, respectively. Eighty-five per cent of the accessions in the first cluster of the morphological data formed about 62% of the accessions in the first cluster of the protein data.

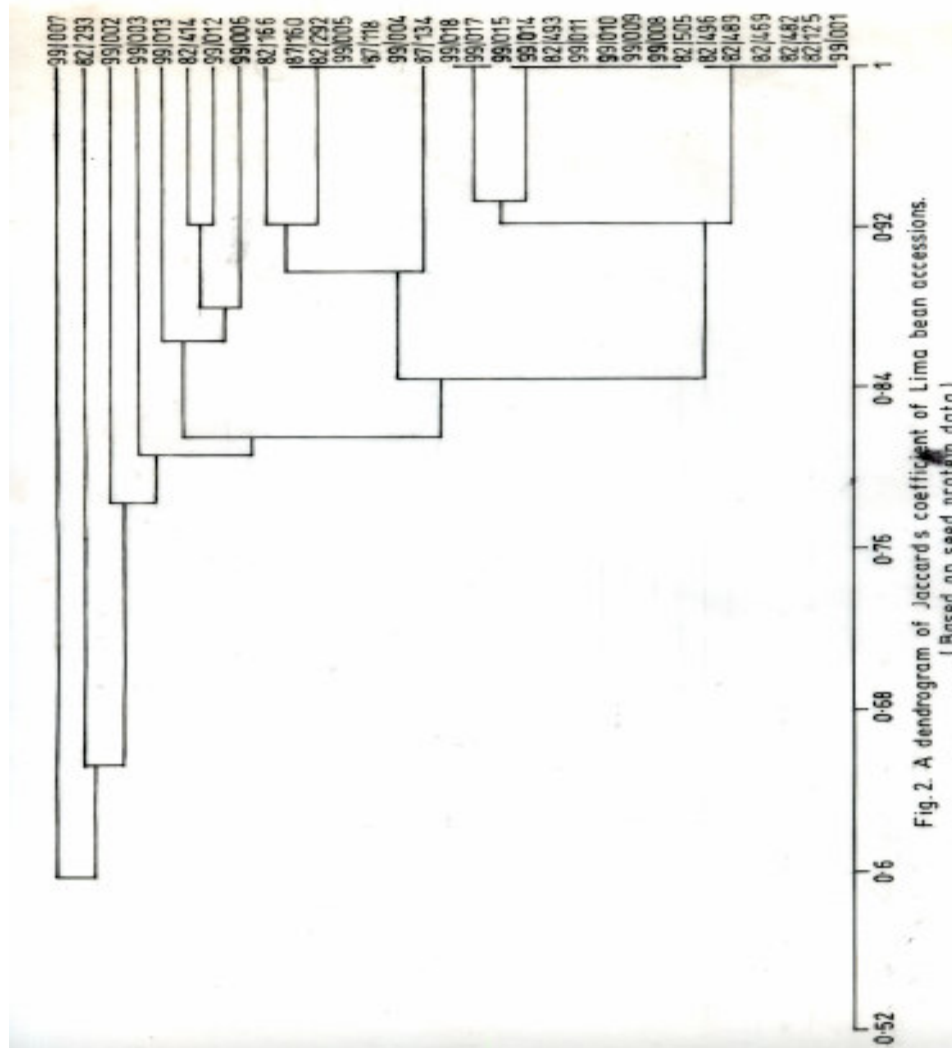


Fig. 2. A dendrogram of Jaccard's coefficient of Lima bean accessions. (Based on seed protein data)

Accessions 82/293 and 99/007 in clusters 1 and 2 of the morphological data clustered into two separate clusters based on the protein data. The present study gives evidence of morphological and protein variation among the Lima bean accessions studied. The study also showed intervarietal variations within the accessions by the use of total seed protein data. The electrophoretic descriptors of the seed proteins can be used as effective technique in genebanks involved in Lima bean identification and differentiation. The differences observed among the accessions available in Ghana would be of immediate importance for broadening the Lima bean gene pool and may be used in hybridization and breeding programmes.

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References

- Badr A.** (1995). Electrophoretic studies of seed proteins relation to chromosomal criteria and relationships of some taxa of *Trifolium*. *Taxon* **44**: 183–191.
- Baudoin J. P., Barthelemy J. P and Ndungo V.** (1991). Variability of cyanide contents in the primary and secondary gene pools of lima bean (*Phaseolus lunatus*). *Pl. Genet. Resour. Newsl.* **85**: 5–9.

- Brittingham W. H.** (1950). The inheritance of date of pod maturity, pod length, seed shape and seed size in the southern pea, *Vigna sinensis*. *Proc. Am. Soc. Hort. Sci.* **51**: 281–288.
- Doku E. V.** (1977). Grain legume production in Ghana. In *Proceedings of the Joint University of Ghana Council for Scientific and Industrial Research Symposium on Grain Legumes in Ghana*. (E. V. Doku, ed.), pp. 1–7. The Institute of Adult Education, University of Ghana.
- Esquivel M, Castineiras L. and Hammer K.** (1990). Origin, classification, variation and distribution of lima bean (*Phaseolus lunatus* L.) in the light of Cuban material. *Euphytica* **49**: 89–97.
- FAO** (1984). *Legumes inoculates and their use*. Food and Agriculture Organization of the United Nations, Rome.
- FAO/CCTA** (1958). *Report of the FAO/CCTA technical meeting on legumes in agriculture and human nutrition in Africa*. FAO, Rome.
- Gardiner S. E. and Forbe M. B.** (1992). Identification of cultivars of grasses and forage legumes by SDS-PAGE of seed protein. In *Seed analysis*. (H. F. Linskens and J. F. Jackson, ed.), pp. 43–61. Springer-Verlag, Berlin, New York.
- Gepts P.** (1990). Genetic diversity of seed storage proteins in plants. In *Plant populations genetics, breeding and genetic resources*. (A. H. D. Brown, M.T. Clegg, A. L. Khaler and B. S. Weir, ed.), pp. 64–82. Sinauer Associates Inc., Sunderland, Massachusetts.
- Gepts P., Osborn T. C., Rashka K. and Bliss F. A.** (1986). Phaseolin-protein variability in wild form and landraces of the common bean (*Phaseolus vulgaris*); evidence for multiple centres of domestication. *Econ. Bot.* **40**: 451–468.
- Loi L.** (1994). Morphotype relationships in Lima bean (*Phaseolus lunatus* L.) deduced from variation of the evolutionary marker phaseolin. *Genet. Resour. Crop Evol.* **41**: 81–85.
- Odeigah P. G. C., Oboh B. and Aghalope I. O.** (1999). The characterization of Nigerian varieties of pepper, *C. annum* and *C. frutescens* by SDS polyacrylamide gel electrophoresis of seed protein. *Genet. Resour. Crop Evol.* **46**: 127–131.
- Sanchez-Yelamo M. D, Espenjo-Ibanez M. C., Francisco-Ortega J. and Santose-Guerra A.**(1995). Electrophoretical evidence of variation in populations of the fodder legume *Chmaecytisus proliferus* from the Canary Islands. *Biochem. Syst. Eco.* **23**: 53–63.
- Saunders A. R** (1960). Inheritance in the cowpea (*Vigna sinensis* Endb.) 3. Mutations and linkages. *S. Afr. J. agric. Sci.* **3**: 327–348.
- Sax K** (1923). The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetica* **8**: 552–560.
- Sheidai M., Hamta A., Jaffari A. and Noori-Daloli M. R.** (2000). Morphometric and seed protein studies of *Trifolium* species and cultivars in Iran. *Pl. Genet. Resour. Newsl.* **120**: 52–54.