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# SDS-Page characterization of some elite cowpea (*VIGNA UNGUICULATA* L.WALP) varieties

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**Abstract**
**The shortcomings of genotype x environment interaction necessitated the use of molecular methods in characterizing many plant species and in determining their phylogenetic relationships. In this study, some selected cowpea lines (27 varieties) from Obafemi Awolowo University, Ile – Ife, the Institute of Agricultural Research (IAR), Samaru, Kaduna and Genetic Resource Centre, IITA, Ibadan were characterized using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) profiling. The protein banding profiles of the 27 cowpea varieties were scored and subjected to cluster analysis using Ward's minimum-variance method (WMVM) for dendrogram grouping. The dendrogram generated from the SDS-PAGE profiles grouped the varieties into seven clusters at 52% similarity coefficient. Hence, the biochemical characterization revealed more precise discrimination among the 27 cowpea varieties studied.**

**Keywords:** Cowpea, electrophoretic banding profiles, dendrogram grouping, total proteins

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## Introduction

Cowpea (*Vigna unguiculata*), belongs to the family Fabaceae. It belongs to the section catiang, which comprises four subspecies one of which is cultivated species (*unguiculata*), while the other three (*dekindtiana, stenophylla* and *tenuis*) are wild relatives (Ng and Marechal, 1985). *Vigna unguiculata* has been subdivided into four cultivar groups, namely, *unguiculata*, *biflora*, *sesquipedalis* and *textilis*. The cultivar group *unguiculata* is the most diverse and is widely grown in Africa, Asia and Latin America.

Cowpea is a single crop species but the varietal requirements in terms of plant types, seed types, maturity and use pattern are extremely varied from region to region making breeding programmes for cowpea more complex than other crops. Wide genetic variability in plant morphology has also been observed in cowpea. Oladejo *et al.* (2017) found diverse genetic variability in number of pods per plant, number of peduncles per plant and response to biotic stress like flower – bud thrips among the cowpea lines studied in Nigeria. Agbogidi and Egho (2012) also reported appreciable diversity among the cowpea varieties used in the tropical humid agroecology of Southeastern Nigeria.

Most morphological and phenological traits are multigenic, quantitative or continuous characters and their expression is mainly influenced by environmental conditions. Reports by various researchers have shown that the action of biochemical markers on total protein and isozymes proved to have better diagnostic tendencies in detecting genetic variability and their actions are usually free from genotype x environment interactions (Lombard *et al.*, 2001; Torkpo *et al.*, 2006) unlike the traditional approach of characterization and evaluation based on morphological features.

Gel electrophoretic studies have shown that many isoenzymes and polymorphic proteins are widely

distributed in plants (Cherry, 1975, Agbahoungba *et al*., 2018). Considerable variation in protein (Agbahoungba *et al*., 2018) and molecular marker electrophoretic banding profiles (Vaillancourt and Weeden, 1992; Vaillancourt *et al*., 1993; Panella *et al*., 1993) have been reported in cowpea. Electrophoresis has an advantage that it can directly equate variation in protein banding profiles to genes encoding these proteins (Gottileb, 1971). Several studies have been carried out in distinguishing *Vigna* species (Rao *et al*., 1992; Omitogun *et al*., 1999), *Sida* species (Illoh *et al.,* 1993) and *Okra* species (Torkpo, *et al*., 2006) where sodium docecylsulphate polyacrylamide gel electropheresis (SDS-PAGE), native PAGE and isozymes have been used. In line with the proven and innate utility of biochemical markers as genetic markers for distinguishing gene expressions and genetic variability in species, this study was carried out to evaluate and characterize 27 selected cowpea lines using SDS-PAGE profiling.

## Materials and Methods

The twenty seven varieties of cowpea used in this study were obtained from the Genetic Resource Centre, International Institute of Tropical Agriculture Ibadan, the Department of Crop Production and Protection, Obafemi Awolowo University (OAU), Ile – Ife, Nigeria and the Institute of Agricultural Research (IAR), Samaru, Kaduna (Table 1).

*Total Protein Extraction and Electrophoresis*

The extraction of total protein and electrophoresis of the cowpea varieties studied was carried out at the Biotechnology Laboratory of the Faculty of Agriculture, OAU, Ile-Ife. Four seeds each from 27 cowpea varieties (Table 1) were planted in the Seed Laboratory for 2-3 weeks after which the young immature leaves of each of the plant variety were harvested for protein extraction following the methods of Agbahoungba *et al*., (2018).

Young leaves (0.8g) of each plant variety were washed with distilled water and macerated with sterile mortar and pestle in 0.8% phosphate buffered saline (PBS) containing 0.4 M NaCl at pH 8.0. The extract was centrifuged at 5,000 rpm for 10 min and the supernatant of each sample was collected. 15 µl of each of the extract was subjected to electrophoresis in 12% polyacrylamide gel. Gels were stained with 0.3 % Coomassie brilliant blue. De-staining in methanol, acetic acid and distilled water (1:3:5 v/v) was done overnight to reveal the protein bands for scoring.

| Serial no | Varieties | Code | Source |
|---|---|---|---|
| 1. | IT 99K – 216 – 24 | IT99K | Ibadan |
| 2. | IT 98D – 1399 | IT98 | Ibadan |
| 3. | IFOB WELL 101 | IFOBW | Ile-Ife |
| 4. | IT 93K – 573 – 5 | IT 93K1 | Ibadan |
| 5. | LDP 08 OBLW | LDP 08 | Ile-Ife |
| 6. | IT 97 568 – 18 | IT97K1 | Ibadan |
| 7. | IT 89 KD – 391 | IT89 | Ibadan |
| 8. | IT 90K – 277 – 2 | IT90K | Ibadan |
| 9. | IFOB /01/9/IB | IFOB1 | Ile-Ife |
| 10. | Ife – 98 -12 | IFE | Ile-Ife |
| 11. | IAR – 06-1035 | IAR | Samaru |
| 12. | IAR 00 – 1006 | IAR0 | Samaru |
| 13. | IT – 98 – 131 – 1 | IT980 | Ibadan |
| 14. | IT – 98K -131 – 2 | IT98K3 | Ibadan |
| 15. | LDP10 – OBR1 | LDP10 | Ile-Ife |
| 16. | IT 95 – 222-3 | IT95 | Ibadan |
| 17. | IT 99K – 1066 | IT99 | Ibadan |

| | | | |
|---|---|---|---|
| 18. | IT 93K – 8- 21-6 | IT93 | Ibadan |
| 19. | IFOB/99/94/ow | IFOB9 | Ile-Ife |
| 20. | IT95k – 2011 – 11 | IT95k | Ibadan |
| 21. | IT 96D – 610 | IT900 | Ibadan |
| 22. | IT 98K – 356 – 1 | IT98K1 | Ibadan |
| 23. | IT 98K – 506 – 1 | IT98K2 | Ibadan |
| 24. | IT 95K - 193 – 1 | IT95K | Ibadan |
| 25. | MDIT98 K -132 – 3 | MDIT | Ibadan |
| 26. | IT 97K – 1072 – 1 | IT97K3 | Ibadan |
| 27. | IT97K – 499 – 35 | IT97 | Ibadan |
| 28. | Oloyin | OLYN | Ife Local Market |
| 29. | Ife BPC | IFEBP | Ile-Ife |
| 30. | Ife Brown | IFEBR | Ile-Ife |

*Sample Preparation for SDS-PAGE*

Seven percent (7%) of B-mercaptoethanol (Sigma) in sample buffer (Table 2) was used for the preparation of each cowpea sample under a fume hood. A discontinuous system of employing 12% separation gel overlaid with a 4% stacking gel was adopted. Sample buffer and B-mercaptoethanol was added in addition to 30µl of high molecular and low molecular weight protein markers following the method of Omitogun *et al*., 1999.

The samples were heated at $95^0$ C for 5 min in a water bath. 25µl of the heated sample was added to the prepared SDS PAGE gels and sample buffer with 40% sucrose solution being loaded in each well. The separation of protein was carried out with the use of Bio-Rad Electrophoresis Power Supply Model 200/2.0 in the Bio-Rad Mini Protean 11 Cell at 150 Volts for 55 min.

**Coomassie-blue Gel Staining for SDS-PAGE**

Gels were carefully removed and placed in a fixing or staining solution (Table 3). The staining was done for about 18 hours. The staining solution was removed and the de-staining solution was then added and left to de-stain for 3 hours with coomassie until the colour of the bands are clearly seen. The gels were scanned with a table scanner (HP 3320).

*Scoring of Protein Bands*

Data were collected on the scanned gels by scoring the presence (1) or absence (0) of protein bands directly from the computer screen. The protein bands on each gel were compared with the known molecular weights (kDa**)** of the following protein markers**:** 94kDa-phosphorylase B, 67-kDa-bovine serum albumin, 43 kDa ovaalbumin, 30 kDa-carbonic anhydrase, 20.1-kDa trypsin inhibitor, 14.4 kDa - α-amylase inhibitors.

Jaccard's coefficient of similarity (Jaccard, 1901) was used to calculate the similarities and contrast in them. The Jaccard's coefficient of similarity (j) was calculated as: $S_{ij} = a/(a+B+C)$; where $S_{ij}$ =

**Table 2:** Solutions for 4% stacking gel, 12% resolving gel for SDS-PAGE

| **Substance** | **Resolving gel** | **Stacking gel** |
|---|---|---|
| Acrylamide/Bis (Sigma) | 4.0ml | 1.3ml |
| Lower Tris buffer (1.5 M Tris-HCl, pH 8.8) | 2.5ml | - |
| Upper Tris buffer (0.5 M Tris-HCl, pH 6.8) | - | 2.5ml |
| Distilled water | 3.5ml | 6.1ml |
| 10% (w/v) SDS | 100 µl | 100 µl |
| 10% APS (Ammonium persulphate) (Sigma) | 50ul | 25 µl |
| TEMED (Tetramethylethylenediamine) (ROTH) | 5 µl | 10 µl |

**Table 3:** Composition of solutions for staining and de-staining of gels

| Staining Solution | *De-staining Solution |
|---|---|
| 45 ml Ethanol | 405 ml Ethanol |
| 90 ml Glacial acetic acid | 90 ml Glacial acid |
| 55 ml Distilled water | 505 ml Distilled water |
| 3g Coomassie blue R (Sigma R250) | |

*De-staining solution was diluted 1:2 with distilled water for de-staining gels.

similarity between two individual i and j; a = number of bands present in both i and j; B= the number present in i but not in j; C = the number present in j but not in i.

The electrophoretic banding profiles scored were subjected to cluster analysis using the Ward's minimum-variance method (WMVM) for phenogram (dendrogram) grouping (Sneath and Sokal, 1973).

**Results and Discussion**

The SDS-PAGE electrophoretic profiles of the cowpea varieties studied revealed 17 bands (Table 4) of molecular weights ranging from 96 kDa to 10 kDa. The visible bands were mostly between the ranges of 10 - 43 kDa which were in the range of molecular weight of vicilins (Omitogun *et al.*, 1999). On the top of every well in all the gels, there was presence of condensed band unit which were situated above the 90 kDa band. The frequencies of protein bands in the gels represented by the letters A to Q ranged between 0.41 and 1.0. Their molecular weights that ranged from 122 kDa– 65 kDa, 64 kDa – 45 kDa, 44 kDa– 15 kDa and 14 kDa – 10 kDa represent their corresponding proteins: albumins, globulins, vicilins and, α-amylase inhibitors respectively.

The letters in the first column (Table 4) represent different bands in descending order of migration starting from letter A which stands for the heaviest protein (globulins) with its sub-units, to letter P which represents the lightest and purest protein (α-amylase inhibitors) sub-units in the referenced cowpea varieties. Column 2 represents the varieties displaying each band while the column 3 represents the allelic frequencies.

The Jaccard's coefficient of similarity values ranged from 0.14 - 0.93. The phenogram produced by the WMVM (Figure 1) grouped the genotypes into six distinct clusters at 78% level of similarity coefficient, and each cluster has peculiar characteristics linked with some agronomic traits. The first main cluster consisted of five genotypes namely IAR, IT95K1, IFEBR, IT97K1 and IT99 which were relatively genetically distant from other genotypes. The five genotypes have identical phenological

**Table 4:** Distribution of identified bands across the cowpea varieties studied and their frequencies

| Allele (gene) | Number of occurrence | Frequencies |
|---|---|---|
| A | 27 | 1.0 |
| B | 13 | 0.48 |
| C | 18 | 0.67 |
| D | 17 | 0.62 |
| E | 12 | 0.44 |
| F | 16 | 0.59 |
| G | 11 | 0.41 |
| H | 17 | 0.62 |
| I | 20 | 0.74 |
| J | 20 | 0.74 |
| K | 17 | 0.62 |
| L | 20 | 0.74 |
| M | 20 | 0.74 |
| N | 23 | 0.85 |
| O | 17 | 0.62 |
| P | 24 | 0.88 |
| Q | 19 | 0.70 |

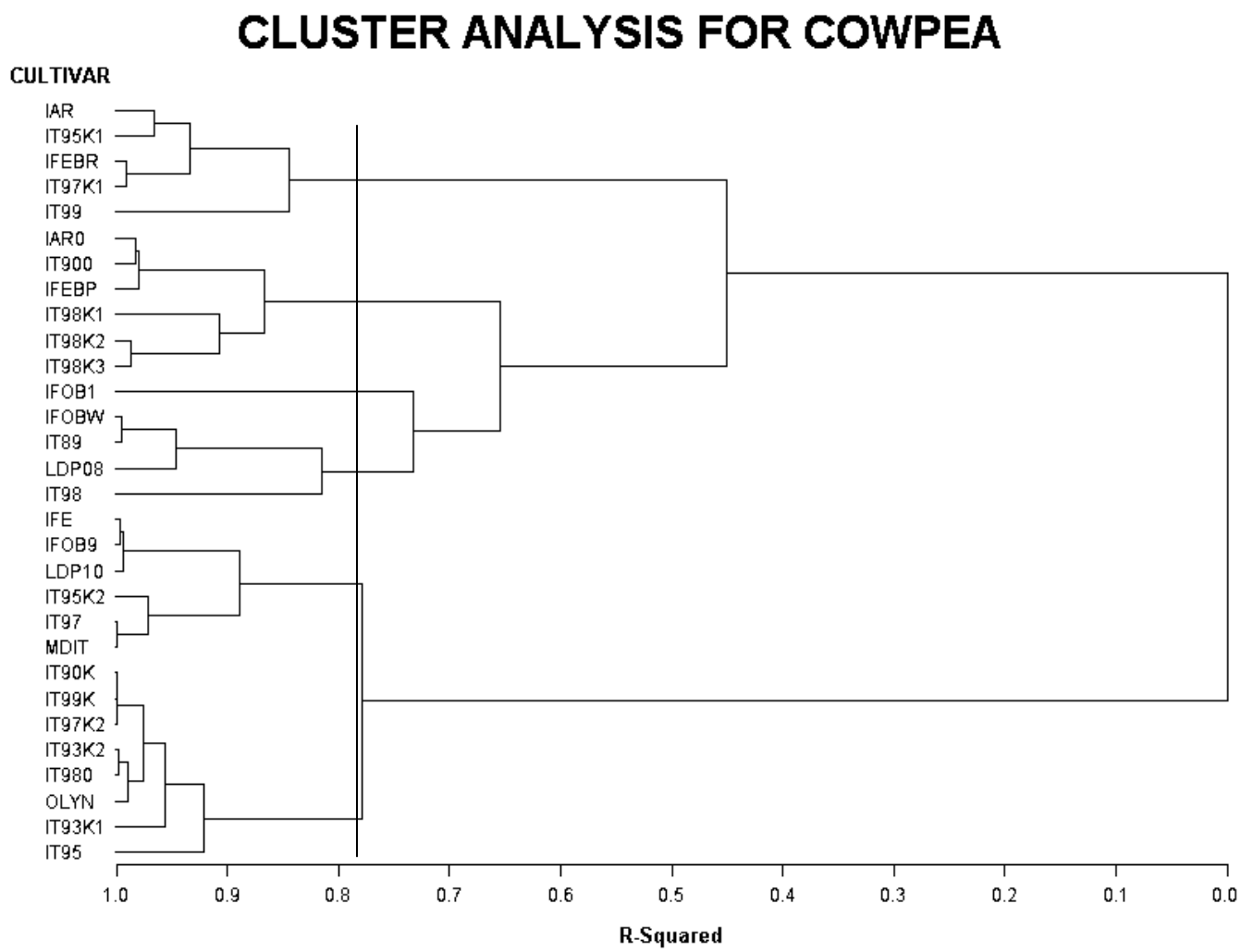


**Figure 1:** Phenogram of the cowpea varieties studied using Ward's minimum variance cluster analysis. (See Table 1 for codes of the cultivars).

traits like days to 50% flowering, 50% pod formation, and 50% harvest maturity (Oladejo *et al.*, 2017). It separated from other major clusters at 50% level of similarity.

The second cluster comprised 17 genotypes which include IA01, IT11, IT02, IT01, IT07, IF09, IF01, IA02, IF06, IT12, IT07, IT17, IT20, IT03, IF02, IF04 and IF08. In this second main cluster there were two subdivisions namely; IA01, IT10, IT02, IT01, IF07, IT09, IF01 IA02, IF06, IT12,

IT07, IT17, IT20, IT03, IF02 and IF08 comprising one sub cluster, while the other sub cluster comprised a single variety (IF08). The third main cluster comprised two varieties namely, IT15 and IT18. Three varieties (IT04, IT10 and IT09) belong to the fourth clusters and they were separated out at 45% level of similarity. The fifth, six, and seventh cluster consisted of single variety each namely IF05, IT08 and IF05 respectively. They were separated out from the preceding clusters at 40%, 38% and 35% levels respectively.

The critical explanation of this study is best done by reconciling the molecular cluster with both the physiological and morphological attributes of the 27 cowpea varieties studied. The critical assessment of each group revealed that the varieties that clustered shared some specific attributes in common. The first group consisted of two varieties that shared common agronomic

traits such as equal number of seed per pod, vigour index, days to fifty percent flowering, and days to harvest maturity (70 days). The second group constituted the largest number of varieties (17) and they shared similar physiological and morphological traits such as days to physiological maturity of mean value of 63 days after planting, days to harvest maturity (72 days), number of pods per plant (13), and yield growth rate (3.34). Only two varieties constituted the third cluster and they had common physiological traits of vigour index of mean value of 36.1, yield growth rate of 1.62, Fifty percent days to physiological maturity of 63 days and days to harvest maturity of 69 days.The fourth group comprised three varieties with average days to 50% flowering (47 days), and 50% days to harvest maturity (68) days. The fifth, sixth and seventh clusters consisted of one variety each possesses peculiar agronomic characters.

Oladejo *et al*., (2016) identified seven clusters in the study carried out on some cowpea lines using morphological descriptors. In this study, it was found that the cowpea varieties that clustered together into distinct groups possessed peculiar agronomic traits in common such as vigours index, days to 50% flowering, days to 50% to physiological maturity, days to 50% harvest maturity and number of pods per plant which are all polygenically inherited. It was evident that there were genes controlling each character and these genes could be isolated by molecular biologists to complement and hasten the work of breeders in cowpea improvement programme.

## Conclusion

The UPGMA dendogram generated from the SDS characterization of the 27 cowpea varieties studied identified 7 distinct clusters each cluster sharing some vital traits of agronomic importance such as vigours index, days to 50% flowering, days to 50% to physiological maturity, days to 50% harvest maturity and number of pods per plant in common. Further investigations could be carried out on these groups to harness the inherent genetic variability within them thus enhancing the improvement of cowpea by breeders.

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