COLLECTION AND CHARACTERIZATION OF SOME UNDERUTILIZED COWPEA (*Vigna unguiculata* L.) CULTIVARS OF SOUTHWESTERN NIGERIA

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Abstract
Genetic erosion of most tropical crops, especially legumes, has become a matter of global concern. The primary objectives of this study were to collect the underutilized cowpea cultivars indigenous to southwestern Nigeria and characterize their accessions.
Four States were purposively selected for collection exercise. Seed samples of the legumes were collected from farmers' fields, seed stores, orchard gardens and market places. The accessions were planted out in three-row plots using a spacing of 0.75 cm x 0.25 cm at the Teaching and Research Farm, Obafemi Awolowo University, Ile-Ife in 2002. Morphological and phenological traits were recorded and the data generated were summarized using means and standard error of mean. Proximate analysis was carried out on the accessions in two replicates and analysis of variance was used to ascertain variability among accessions for their nutrient composition. A total of eight accessions belonging to two distinct types (*solojo* and *ereguru*) were collected. Results showed wider variability among the *ereguru* accessions for most agronomic traits. Flowering traits were of primary importance in characterizing the accessions and cluster analysis grouped the accession according to their origin. Within each type, statistical analysis of the proximate composition revealed that seed coat colour was a good indicator of the crude protein content. Accessions with lighter coat colour had significantly higher crude protein than the darker accessions. In conclusion, there exist wide diversity among neglected cowpea variants in this region which is an indication of their great potential for preserving variability for sustainable cultivation of cowpea in the region.
Keywords: legumes, accessions, cowpea, characterization, diversity

INTRODUCTION

Conservation has been described as the rational use of earth's resources to achieve highest quality of living for mankind (Dasmann et al., 1973). The milestone international Convention on Biological Diversity (CBD) signed at the United Nations Conference on the Environment and Development in 1992 emphasized in Article 8 that conservation of agricultural biodiversity is important in farmers' field as well as in protected areas and genebanks (UNEP, 1994).

Cowpea (*Vigna unguiculata* L. Walp) is a very important, widely adapted, and versatile grain legume. It is mainly produced and consumed in Africa where it provides a major low-cost dietary protein for millions of smallholder farmers and consumers who cannot afford high protein foods, such as fish and meat. On the average, cowpea contains 24% protein, 62% soluble carbohydrates, and small amounts of other nutrients (IITA, 2010). It is a very low-input crop, traditionally grown in intercropping systems. Cowpea contributes to soil fertility through nitrogen fixation and is also cultivated to prevent soil erosion. Cowpea can be found approximately between 15°N and 20°S, and over a large range of climates but most likely found in subtropical to tropical conditions characterized by warm temperatures (annual average >20°C) and relatively high annual precipitation (>250 mm) (IITA, 2010). The worldwide cultivation of cowpea in 2008 was estimated to be 11.8 million ha of land with an annual production of 5.4 million tons of dried grains (FAOSTAT 2010).

The Food and Agriculture Organization of the United Nations (FAO) statistics reported that cowpea is grown on an estimated worldwide area of 14 million ha of which about 8 million ha of cowpea are grown in West and Central Africa, especially in Nigeria, Niger, Burkina Faso, Mali, Senegal, and Cameroon.

In Nigeria, the production trend shows a significant improvement with an increase of about 440% in area planted to cowpea (Ortiz, 1998). According to Singh (2000), Nigeria produces about 2 million tons of cowpea on 5 million ha of land annually. However, the intensity of cowpea cultivation in the southwestern region of Nigeria is ranked low and insignificant (Agboola, 1979). This sub-region has a great potential for high genetic biodiversity of cowpea because it falls within the humid tropics where the world's widest biodiversity of most crops is available (National Academy of Science, 1979). However, field observation, cultivation of cowpea in this region has dwindled considerably and farmers go for the improved cowpea cultivars which has very narrow genetic base as the expense of the local indigenous cultivars which over decades evolved as a result of cultivation and selection by farmers in this region. Only cowpea transported from the northern regions are found in most market places in this region.

Despite the potential for further yield increases, cowpea production faces several production challenges including low yield, insect and disease attacks, *Striga gesneroides* (Wild.) and *Alectra vogelii* (Benth.) parasitism, and heat and drought stresses. Most of the elite cultivars are susceptible to these constraints. Therefore, there is the need to explore the genetic potential among indigenous germplasm of cowpea in this region and characterize them in order to determine the extent of genetic diversity for grain yield and yield components as well as their nutritional values. Thus, the objectives of this study were to collect indigenous neglected cowpea cultivars in this region and characterize them with a view to assess their genetic diversity for agronomic traits and nutritional composition. This results of this study is expected to provide information on the appropriate breeding scheme to employ in order to develop cowpea cultivars that will attract farmers acceptance which will in turn lead to sustainable cultivation of cowpea in this region and in general increase productivity in Nigeria.

MATERIALS AND METHODS

Collection exercise and characterization

Four States namely; Oyo, Osun, Ondo and Ekiti, were purposively selected in the southwestern Nigeria for the collection exercise due to high level of cultivation of cowpea, and the exercise spanned between August and December, 2002. The region lies within. The region is characterized by evergreen rainforest bounded in the northern part by Lat. 6^0N and 10^0N of the equator and Long. 2^0E and 6^0E of the Greenwich meridian derived savanna vegetation and in the south by mangrove forest vegetation. The mean annual rainfall is between 1016 mm and 2032 mm with relatively high ambient temperature of 27^0C and relative humidity of about 60%. Collection was done in the farmers' fields, market places, seed stores, wild habitats, and backyard orchards from the four selected States). The quantity of seeds collected depended on the site of collection and the available quantity at the time of collection. On the farmers' fields, 10-50 seeds were collected from a plant and 50 plants were sampled at random. In market places, 0.5 kg of seeds was collected. For some that were near extinction, available quantities were collected. The accessions were given names that indicated their sources, first letters of their scientific names, and serial numbers (Table 1). Seeds from each accession were planted out in three-row plots using a spacing of 0.75 cm x 0.25 cm at the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife, Nigeria in September, 2003. Morphological and phenological traits were recorded according to the procedure of International Plant Genetic Resources Institute (now Bioversity) (IPGRI, 1984). These traits include days to 50 % emergence, days to flower, days to pod maturity, vigour index obtained by the formula (Height + Width)/2, which was calculated 6 weeks after planting, number of seeds per pod, number of pods per plant seed crowding index, and 100 seed weight. Parameters analyzed for proximate composition included crude protein, ether extract, crude fibre and ash content according to the procedure described by Association of Official Analytical Chemistry (AOAC, 1995). The analysis was done n three replicates. N-content was determined by Kjeldahl method and the value obtained was multiplied by 6.25 to obtain the crude protein content. Ether extract content was determined by Soxhlet extraction method using petroleum ether and ash content was determined by digesting 5g of ground samples in a muffle furnace at 550^0C for 1 hr. The crude fibre was determined by passing ground samples through 1 mm sieve and 1g of sample was weighed into crucible dish. Then, about 50 mls of boiling tetra-oxosulphate (VI) acid was added. The mixture was boiled for 1 hr, filtered and the residue washed with hot distilled water until it was free from acid. Hot 1.25% sodium hydroxide (50 ml) was added and the mixture was boiled for 1 hr. It was thereafter filtered and residue washed out with distilled water and with 10 ml of cold 98% alcohol and then rinsed with acetone. The residue was dried at 110^0C in a forced air oven for about 2 hrs and the weight was recorded. The dry residue was burned to ashes and weighed. The fibre content was calculated by subtracting the residue weight from the weight of the blank crucible. The data obtained were subjected to analysis of variance to assess variability in the nutrient composition of the accessions.

Statistical analysis

Means and standard errors of the traits of each accession were recorded. Principal component analysis was used to determine the effectiveness of the traits measured in characterizing the germplasm and Ward's minimum variance cluster analysis was used to assess genetic variability among the accessions. These analyses were carried out using SAS software, version 9.2. (SAS Institute, 2002).

RESULTS AND DISCUSSION

It is not surprising that most of the world cowpea production comes from Africa (about 91%) FAOSTAT, 2008). Most interestingly, West Africa, with 10.7 million ha, accounts for most of Africa's production, with Nigeria and Niger being the leading cowpea growing countries (FAOSTAT, 2008). This region is noted as the center of origin of this crop (Okigbo, 1994) and thus, the widest genetic diversity is expected in this region.

In this study, a total of eight accessions belonging to two discrete cowpea cultivar types were collected; two accessions of *solojo* and six accessions of *ereguru*. *Solojo* accessions were collected in relatively larger quantities from farmers' field and market places in the northern part of Oyo State while small quantities of *ereguru* accessions were available during the collection exercise. This indicates that *solojo* may not be under serious threat of extinction even though its cultivation is only limited to the northern part of Oyo State, which shares more of savanna climate than the rest of the State in the rainforest zone. *Ereguru*'s accessions were collect from Osun, Ondo, and Ekiti State, signifying that it has a wide adaptability in this region. However, it is evident that the relatively small quantity of these accessions available for collection showed that *ereguru* cultivar is under serious threat of going into extinction. Therefore, efforts towards collection and conservation of *ereguru* germplasm should be intensified.

Results from characterizing the germplasm revealed wide genetic variation among the eight accessions. Assessment of the qualitative traits showed wide variability in seed coat colour, pod and seed colour and shape, type of peduncle, nature of flower (determinate or indeterminate), life cycle, growth habit etc (Tables 1 and 2; Plates 1-3). Characterization based on morphological and phenological traits also revealed wide genetic variability. Subjecting the traits into principal component analysis revealed the level of importance of these traits in characterizing the germplasm (Table 3). From the analysis, the first six principal component (PC) axis accounted for 99% of the total variations among the accessions. Following a statistical restriction that only eigenvectors equal to or greater than 0.3 made a significant contribution to variation at a particular PC axis (Badu-Apraku and Lum, 2007), five traits namely; emergence, days to flower, days to ripe pod, days to harvest maturity, and seed crowing index had high loadings on PC1, indicating that they are of primary importance in characterizing the germplasm (Table 3). The high eigenvectors of traits at a PC axis implies high correlation among the traits with similar sign. Thus, the days to flower, days to ripe pod, and days to harvest maturity are expected to show high correlations while emergence and seed crowding index with negative signs are also significantly correlated. Similarly, vigour index, number of seeds per pod, plant height and seed crowding index are highly important at the secondary principal axis while pod development period, pods per plant, peduncle length and 100 seed weight had high loadings on the PC3 implying that they are tertiary importance in characterizing these accessions (Table 3). These results were similar to the findings of Oladejo (2010), when he evaluated 30 cowpea cultivars for their physiological, phenological, and morphological traits.

A major problem of cowpea production is low yield and productivity. Nkonye et al. (2010) reported that there is a wide gap between the current grain yield of cowpea (0.5 mt ha^{-1}) and its potential yield (2.3 mt ha^{-1}). A major objective of this study is to estimate the extend of genetic diversity for yield and yield components so as to plan breeding crosses to generate populations, varieties and hybrids that will be high yielding and possess better nutritional qualities. Constructing a dendrogram based on the 12 traits which were identified by principal component analysis as important at either primary, secondary, or tertiary levels revealed an interesting pattern in the genetic diversity of the accessions (Fig 1). The *solojo* accessions were clustered separately, in the first cluster, from the *ereguru* accessions. This indicate that even though, they belong to the same family, they might not be of the same sub-species and/or cultivar. The second cluster consists of the two *ereguru* accessions collected from Osun State.

The third cluster was the *ereguru* accession collected from Akure in Ondo State. The fourth cluster includes the white- and black-seeded *ereguru* accessions while the light-brown accession of *ereguru* falls into a different group. From this diversity assessment, it is interesting to note that the light-brown seeded *ereguru* accessions, despite the same seed coat colour were clustered according to their origin. This implies that they might not have co-evolved from the same ancestry or might have undergone genetic reconstitution or recombination through continuous cultivation by farmers. From these results, it evident that *ereguru* germplasm had inherent genetic variability and breeding efforts at improving its performance will be a worthwhile venture in this region. Diversity among *Solojo* accessions was not adequately assessed owing to their few numbers. Seeds harvested from the dark grey seeded type segregated to give a white-seeded type which indicates that there may be wider genetic variability than this study could explain.

Proximate analysis revealed that the *solojo* accessions were not significantly different from one another but *ereguru* accessions exhibited wider genetic variability especially for crude protein content (Table 4). It was also observed from the results that crude protein content is greatly determined by the seed coat colour. White-seeded accessions of cowpea had highest protein content, followed by light-brown accessions and lastly, the black-seeded accession. However, the crude protein percentage recorded in this study is not significantly different from the elite cowpea reported in literatures (IITA, 2010)

One major weakness of this paper is lack of well defined experimental laid-out (in a replicated trial) in the assessment of the performance of these accessions. However, information sufficient for characterizing the accession was obtained. Therefore, the accessions should be planted for about two more seasons to allow for possible segregation within accession and subsequently, a well-conducted field evaluation of the resulting entries is necessary to confirm the results reported in this study.

In conclusion, there is wide variability among the cowpea cultivars accessions characterized. By implication, although the number of the cowpea accessions is seemly small, the genetic diversity within the germplasm is sufficient for breeders to develop a breeding program to improve these cultivars for sustainable cultivation of cowpea in the south western region of Nigeria. In addition, the results of this study recommend more intensive and concerted efforts at collecting and conserving more accessions of these cultivars to prevent from genetic erosion.

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Table 1. Passport data on 8 accessions of underutilized cowpea cultivars collected in the southwestern region of Nigeria, in August, 2002.

	Accession code	Local Name	Scientific Name	Source	Pod Colour / Shape	Seed Colour	Dates of Collection
1	OY/Vu01	Solojo dudu	*Vigna unguiculata*	Saki, Oyo State	Light brown / straight	Dark Ash	25/ 8/ 2002
2	OY/Vu02	Solojo pupa	*Vigna unguiculata*	Saki, Oyo State	Light brown / straight	Light brown	10/ 8/ 2002
3	OS/Vu01	Awuje wewe (*Ereguru*)	*Vigna unguiculata*	Ikire, Osun State	Dark brown / curve	Reddish brown	15/ 8/ 2002
4	OS/Vu02	Awuje wewe (*Ereguru*)	*Vigna unguiculata*	Ilesha, Osun State	Light brown / straight	Reddish brown	10/ 8/ 2002
5	EK/Vu01	*Ereguru* dudu	*Vigna unguiculata*	Ido-Ekiti, Ekiti State	Light brown / straight	Black	10/ 8/ 2002

6	EK/Vu02	*Ereguru* pupa	*Vigna unguiculata*	Ado-Ekiti, Ekiti State	Light brown / straight	Reddish brown	10/ 8/ 2002
7	EK/Vu03	*Ereguru* funfun	*Vigna unguiculata*	Ado-Ekiti, Ekiti State	Light brown / straight	Cream	10/ 8/ 2002
8	OD/Vu01	*Ereguru* pupa	*Vigna unguiculata*	Akure, Ondo State	Light brown / straight	Reddish brown	10/ 8/ 2002

Table 2. Qualitative characteristics of the 8 accessions of underutilized cowpea cultivars collected in southwestern Nigeria, 2002.

	Accessions	Crop	Life Cycle	Growth Habit	Determinacy	Peduncle Type
1	OY/Vu01	*Vigna unguiculata*	Annual	Herbaceous erect	Determinate	Unbranched
2	OY/Vu02	*Vigna unguiculata*	Annual	Prostrate	Determinate	Branched
3	OS/Vu01	*Vigna unguiculata*	Annual	Climbing	Indeterminate	Branched
4	OS/Vu02	*Vigna unguiculata*	Annual	Climbing	Indeterminate	Unbranched
5	EK/Vu01	*Vigna unguiculata*	Annual	Prostrate	Indeterminate	Unbranched
6	EK/Vu02	*Vigna unguiculata*	Annual	Climbing	Indeterminate	Unbranched
7	EK/Vu03	*Vigna unguiculata*	Annual	Prostrate	Determinate	Branched
8	OD/Vu01	*Vigna unguiculata*	Annual	Climbing	Indeterminate	Unbranched

Table 3. Eigenvectors and cumulative proportions of the first six principal component axes extracted from the principal component analysis of the phenological and morphological traits of the eight accessions of the underutilized cowpea cultivars.

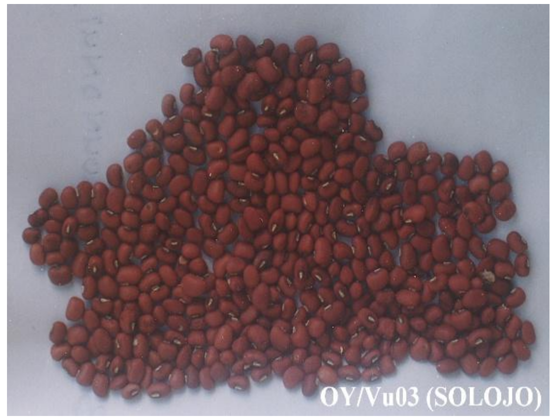
†PC =Principal component.

¶ Values in bold lettering indicate strong loadings (>0.30) on the corresponding PC axis.

Traits	PC†1	PC2	PC3	PC4	PC5	PC6
Emergence	**-0.40**¶	-0.04	0.28	0.06	-0.11	0.46
Days to flower	**0.46**	0.14	0.07	-0.13	0.15	0.08
Days to ripe pod	**0.39**	0.26	0.14	-0.15	0.36	0.09
Days to harvest maturity	**0.48**	0.11	0.06	-0.04	-0.04	0.00
Pod development period	0.07	-0.05	**-0.40**	0.48	0.49	0.40
Vigour index	0.18	**-0.51**	0.11	0.24	0.14	-0.27
Seeds per pod	0.19	**0.41**	-0.06	0.31	-0.47	0.40
Plant height (cm)	0.25	**-0.44**	-0.04	0.26	-0.33	0.23
pods per plant	0.01	-0.16	**0.55**	0.33	0.27	0.11
Peduncle length	0.10	0.25	**0.36**	0.47	-0.25	-0.44
Seed Crowding Index	**-0.30**	**0.39**	0.22	0.17	0.33	-0.01
100 seed weight	-0.11	0.18	**-0.48**	0.39	0.05	-0.36
Cumulative Proportion (%)	35	57	75	89	95	99

Table 4. Mean ± standard errors of proximate composition of the eight cowpea accessions collected in southwestern Nigeria, in 2002.

Serial No	Accession	Crude protein (g/100g dm^3)	Ether extract	Crude fibre (g/100g dm^3)	Mineral ash fibre (g/100g dm^3)
1	OY/Vu01	21.1±0.17	1.69±0.05	6.9±0.07	3.9±0.07
2	OY/Vu02	20.3±0.26	1.94±0.01	4.2±0.10	5.18±0.89
3	OS/Vu01	22.9±0.05	1.43±0.25	4.6±0.03	3.37±0.14
4	OS/Vu02	23.2±0.32	4.69±0.69	5.7±0.88	5.2±0.02
5	EK/Vu01	21.8±0.50	0.65±0.06	5.4±0.05	5.0±0.19
6	EK/Vu02	20.5±0.13	1.58±0.29	4.0±0.01	4.3±0.20
7	EK/Vu03	24.9±0.13	1.49±0.10	5.6±0.05	4.3±0.13
8	OD/Vu01	22.06±0.01	1.81±0.01	3.9±0.37	3.4±0.19



a **b**

Plate 1. Vigna unguiculata L Walp. (*Solojo*) (a) Dark-ash seeded type (b) reddish brown seeded type. These accessions were collected from Saki, Oke-Ogun part of Oyo State Nigeria in August, 2002.

a **b**

C

Plate 2. Vigna unguiculata (L.) Walp. (*Ereguru*) (a) light brown-seeded type collected from Ado-Ekiti (Ekiti State), Akure (Ondo State), Ile-Ife (Osun State) and Ikire (Osun State) (b) black-seeded type from Ido-Ekiti (Ekiti State) and (c) White-seeded type from Ado-Ekiti (Ekiti State)

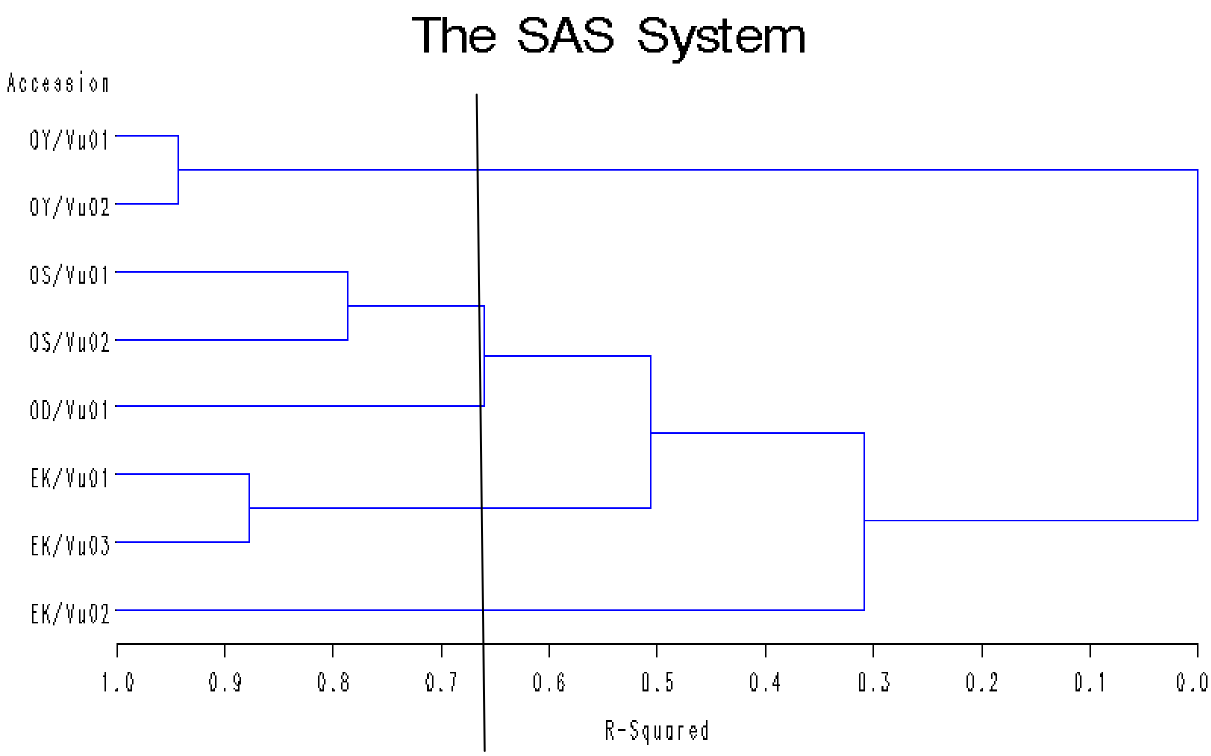


Figure 1. Genetic diversity assessment of the eight accessions of underutilized cowpea cultivars collected from 4 southwestern States of Nigeria using Ward's minimum variance cluster on the standardized values of the twelve morphological and phenological traits.