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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF SELECTED COWPEA CULTIVARS FROM BENIN, GHANA AND NIGERIA

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Abstract: Sixty cowpea (*Vigna unguiculata* [L.] Walp.) landraces and varieties were morphologically and genotypically characterized, with the aim to elucidate the genetic relationships and diversity among these cultivars from Ghana, Benin and Nigeria. Thirty eight agro-morphological traits and nine Amplified Fragment Length Polymorphisms (AFLP) primer pairs were used in the investigation. Analysis of the morphological and AFLP data revealed two major clusters within the West African cowpea germplasm, which may relate to two different gene pools. The morphological variation within the germplasm was further evaluated using the molecular data to detect duplicates and genetically similar cultivars within the collection. Genetic relatedness did not depend on geographical origin of the cultivar, suggesting extensive movement and adaptation of cowpea genetic materials across the West African sub-region.

Keywords: Diversity, cluster analysis, phenotype, genotype, gene pool.

INTRODUCTION

Cowpea, *Vigna unguiculata* (L.) Walp is one of the most ancient and important human food source and forage legume crop in the world (Wamalwa *et al.,* 2016). It is a self-pollinating dicotyledonous crop plant belonging to the family *Fabaceae* and native to Central Africa. Its diploid, 2n = 2x = 22 (Nameirakpam and Khanna, 2018).

Insight into the genetic diversity and relationships among accessions is of utmost importance. Characterisation of genetic diversity among cultivated cowpea helps to improve the available genetic resources in any hybridisation programme (Mafakheri *et al.,* 2017; Nameirakpam and Khanna, 2018). Additionally, characterisation helps to eliminate duplications within the germplasm and select representative samples for utilisation and conservation in genebanks.

Therefore, genetic diversity studies helps in crop breeding by analysing the genetic variability in cultivars, identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection as desirable genes are introgressed from diverse germplasm into available genetic base. Hence a very important step to breed for

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agronomic and economically desired traits such as time of maturity, photoperiod sensitivity, plant type, seed quality and resistance to major diseases, insect pests or parasites that afflict adapted cultivars (Nameirakpam and Khanna, 2018).

The objective of this study was to assess the genetic variation existing among indigenous West African cowpea for potential use in cowpea breeding and improvement programmes in the West African sub-region.

MATERIALS AND METHODS

Morphological characterization:

Morphological characterization for forty seven cowpea genotypes out of sixty was done due to field experimental difficulties with thirteen. The experimental plot for morphological characterization was established under natural field conditions at the research fields of CSIR-Crops Research Institute, Fumesua-Kumasi (Longitude 6º41'N, Latitude 1º28'W) in Ghana. The temperatures during experiment ranged from 22.3°C to 30.2°C while rainfall totaled 431.5 mm. The vegetation of the site is semi-deciduous forest with a bi-modal rainfall regime. The soil type was Nta series (Gleyic Arenosol, FAO-UNESCO Soil classification) – imperfectly drained sandy loam on lower slopes below Akroso series (Dystric-Haplic Nitisol, FAO-UNESCO Soil classification). The randomized complete block design with three replications was used for the experiment. The genotypes were planted in four 5 m rows per plot with spacing of 0.6 m between rows and 0.2 m within rows. Plots were spaced 1m apart. Two hand weedings (2-3 and 5-6 weeks after planting) were carried out. Pesticides, Karate 2.5 % EC (*Lambda-cyhalothrin)* and Cymethoate (Cypermethrin + *dimethoate*) were applied following the manufacturer's instructions at 30-40 and 50-55 Days after planting (DAP) respectively. There was no fertilizer application.

The International Board for Plant Genetic Resources (IBPGR) Cowpea Descriptor (1989 Edition) was used for scoring and measuring the selected qualitative and quantitative traits of all cultivars. Thirty-eight selected morphological characters were measured from 10 randomly selected plants of each genotype and the mean calculated for each quantitative trait. Statistical analyses were carried out using Genstat 13th edition (VSN International Ltd, Hemel Hempstead, UK, 2010). The data was standardized prior to analysis. The data standardization involved the determination of the standard deviation and mean for each character measured. For each character of the accessions, the deviation from the mean was divided by the standard deviation to get the z-score. The new data set of z-scores for each character per accession constituted the standardized data which was used for all analysis. Pearson's correlation analysis was conducted between the selected qualitative and quantitative traits of the accessions. The correlations were tested for significance using the student t-test at a probability of 0.01. Principal components analysis (PCA) was used to detect the extent of variation present in the germplasm and the contribution of the characters measured. The hierarchical cluster analysis was done using Euclidean distances with Unweighted Pair Group Method of Arithmetic Mean (UPGMA) algorithm as implemented in PAST version 1.94b (Hammer *et al*., 2001).

Molecular Characterization

Fifty-four accessions were successfully grown in three pots per accession until they reached the fourth leaf stage (fourteen days after planting). Two samples of 1 cm^2 fresh young leaf material were collected from seedlings of each accession for DNA extraction. The DNA extraction was done using KingFisher Flex (Thermo Scientific) protocol. Reagents for the KingFisher DNA extraction were obtained from AGOWA Genomics (Germany). The AGOWA Sbeadex Maxi Plant kit was used according to the protocol of the supplier (AGOWA Genomics). The DNA concentration was measured using the Nanodrop (Isogen).

Molecular analysis using Amplified Fragment Length Polymorphism (AFLP)

The landraces were fingerprinted using AFLP (Vos *et al*, 1995). About 250 ng DNA was used for the restriction/ligation reaction. The DNA was digested with *EcoR*I and *Mse*I. Pre-amplification was carried out with non-selective primers E01/M02 to generate secondary template DNA to be used in selective amplification. Following the pre-amplification, selective amplification was carried out with E44M59, E39M59, E39M60, E33M60, E35M59, E35M50, E32M59, E33M59 and E32M62 primer pairs (Vos *et al*, 1995), which were labeled with the Li-cor IRD700 or IRD 800 dye. The Polymerase Chain Reaction (PCR) products were separated on a 6.5% (w/v) polyacrylamide gel on a Li-cor 4200 Global system. Fragments were scored as present (1) or absent (0) using the Quantar software (Keygene, the Netherlands) and entered into a binary data matrix (Excel spread sheet). From the AFLP data set, a similarity matrix was calculated using the Jaccard similarity coefficient (Digby and Kempton, 1987). A dendrogram was constructed using the Unweighted Pair Group Method of Arithmetic Mean (UPGMA) algorithm implemented in PAST version 1.94b software (Hammer *et al*., 2001).

RESULTS

Morphological characteristics of the accessions evaluated

The germplasm (Appendix 1) was composed of local landraces, breeding lines and improved varieties from Ghana, Nigeria and Benin. The major quantitative characteristics used in the morphological separation of the accessions are as shown in Table 1.

Table 1: Summary descriptive statistics for quantitative characters of the germplasm

Trait	Mean	Minimum	Maximum	Standard deviation	Coefficient of variation (%)
Duration of flowering(days)	11.0	8.0	13.0	1.1	10
Number of main branches	4.0	3.0	6.0	0.8	20
Number of nodes	5.0	3.0	7.0	0.9	18
Calyx lobe length(mm)	11.3	11.0	20.0	2.5	22
Days to 1st matured pod	64.0	55.0	73.0	2.7	4.2
Days to flower	45.8	38.0	61.0	4.6	10
Hypocotyl length(mm)	36.1	15.0	100.0	11.6	32.1
Peduncle length(mm)	36.7	7.0	54.0	10.5	28.6
Pods per peduncle	2.1	1.0	4.0	0.5	23.8
Raceme per plant	9.5	5.0	15.0	2.2	23.2
Seed length(mm)	7.4	5.0	9.0	0.8	10.8
Seed thickness(mm)	4.6	3.5	5.0	0.5	10.9
Seed width(mm)	6.0	4.0	7.5	0.7	11.7
100 seed weight(g)	10.2	6.0	14.0	2.0	19.6
Standard length(mm)	22.5	15.0	26.0	2.3	10.2
Stipule width(mm)	4.6	2.0	7.0	1.1	23.9
Stipule length(mm)	12.1	5.0	17.0	3.0	24.8
Terminal leaf length(mm)	82.4	31.0	135.0	26.8	32.5
Terminal leaf width(mm)	48.7	14.0	95.0	19.6	40.2

Variation within the germplasm as expressed by quantitative characters is showed in Table 1. The most variable trait was terminal leaf width with a CV of 40.2 % whereas the least variable was number of days to first matured pod having a CV of 4.2 %.

The extent of variation due to the qualitative traits is as expressed by their range of CV. Leaf colour and seed splitting had the lowest (8.3 %) and the highest (374 %) CV respectively (see Table 2).

Table 2: Summary of scores for qualitative characters of the germplasm

Character	Minimum score	Maximum score	Standard deviation	Coefficient of variation (%)
Growth habit	1	6	1.4	42.5
Immature pod pigmentation	0	4	1.6	148.7
Terminal leaf shape	1	5	1.2	37.0
Twining tendency	3	7	1.7	36.1
Branches pigmentation	0	7	1.8	41.2
Eye colour	0	5	1.6	44.3
Eye pattern	1	6	1.5	40.9
Flower colour	1	2	0.5	26.2
Flower pigmentation pattern	0	4	1.6	54.2
Leaf colour	5	7	0.6	8.3
Leaf texture	1	3	0.7	29.9
Petiole pigmentation	0	7	1.6	45.7
Seed crowding	0	5	1.7	182.8
Seed shape	1	5	1.9	56.0
Number of pods per peduncle	1	4	0.5	21.1
Stem pigmentation	0	7	2.1	65.9
Testa texture	1	5	1.1	68.6
Seed splitting	0	1	0.3	374
Raceme position	1	3	0.7	41.1

Principal Component Analysis of agro-morphological characters

Table 3 shows the major agro-morphological traits that were combined for principal component analysis. Seventeen out of 38 variables accounted for 42 % of the total phenotypic variation within the cowpea germplasm.

Table 3: Principal component analysis for the major agro-morphological traits of the cowpeas

	PC 1	**PC 2**	**PC 3**	**PC 4**
Latent roots	199	160	145	103
Percentage variation	14	11	10	7
Cumulative percent variation	14	25	35	42
Latent vectors (loadings)				
Stipule length	0.3767	0.0906	-0.1099	-0.0637
Stipule width	0.3435	0.2395	-0.0618	-0.0298
Stem pigmentation	-0.2931	0.2200	0.0235	-0.0981
Hypocotyl length	0.2886	-0.1648	-0.0092	0.0422
Growth habit	-0.2530	-0.1121	-0.0162	0.2162
Petiole pigmentation	-0.2407	0.0853	0.1800	-0.0327
Raceme per plant	0.0195	0.3488	0.1681	-0.0541
Terminal leaf length	0.1174	0.3474	0.0859	-0.1750
Seed shape	0.0727	0.2701	-0.0259	0.0310
Terminal leaf width	0.1159	0.2505	0.1002	0.2636
Flower pigmentation pattern	-0.0972	0.1282	-0.3643	0.1030
Flower colour	-0.1608	0.1973	-0.3301	0.1622
Testa texture	0.1368	-0.1542	0.3339	-0.0047
Seed width	0.1005	0.1784	0.0804	0.3881
100 seed weight	0.1431	0.2029	0.1294	0.3702
Seed thickness	0.1304	0.0546	0.0614	0.3352
Flower standard length	0.1486	0.1111	0.0379	0.2510

The degree of association among the morphological characteristics used to measure the phenotypic variation within the germplasm is as shown in Table 4. The traits correlated significantly to help detect the extent of morphological variation.

Table 4: Correlations among key characteristics of the cowpea germplasm

Trait 1	Trait 2	Correlation coefficient (r)
Stipule length	Stipule width	0.78
Days to flower	Days to 1st mature pod	0.66
Flower colour	Flower pigmentation pattern	0.81
Terminal leaf length	Terminal leaf width	0.70
Stem pigmentation	Branch pigmentation	0.59
Petiole pigmentation	Branch pigmentation	0.52
Seed thickness and	Seed width	0.52
Growth habit	Peduncle length	-0.62
Growth habit	Stipule length	-0.56
Growth habit	Stipule width	-0.60
Flower colour	Testa texture	-0.69
Pod attachment	Testa texture	0.62

$p \leq 0.01$

Cluster Analysis of cowpea accessions using agro-morphological characters

The relatedness within the germplasm was analyzed by Cluster analysis using the agro-morphological data. The resulting dendrogram is based on all thirty-eight phenotypic (qualitative and quantitative) traits measured for forty seven accessions and shown in Figure 1 below.

Bootstrapping of the clustered phenotypic traits generally showed very low values except for the split between A and B (Fig.1) of which the latter contained the varieties IT-82D-716 and TVx3236. Group A is divided into several subgroups with very low bootstrap values, making the reliability of these groups weak .

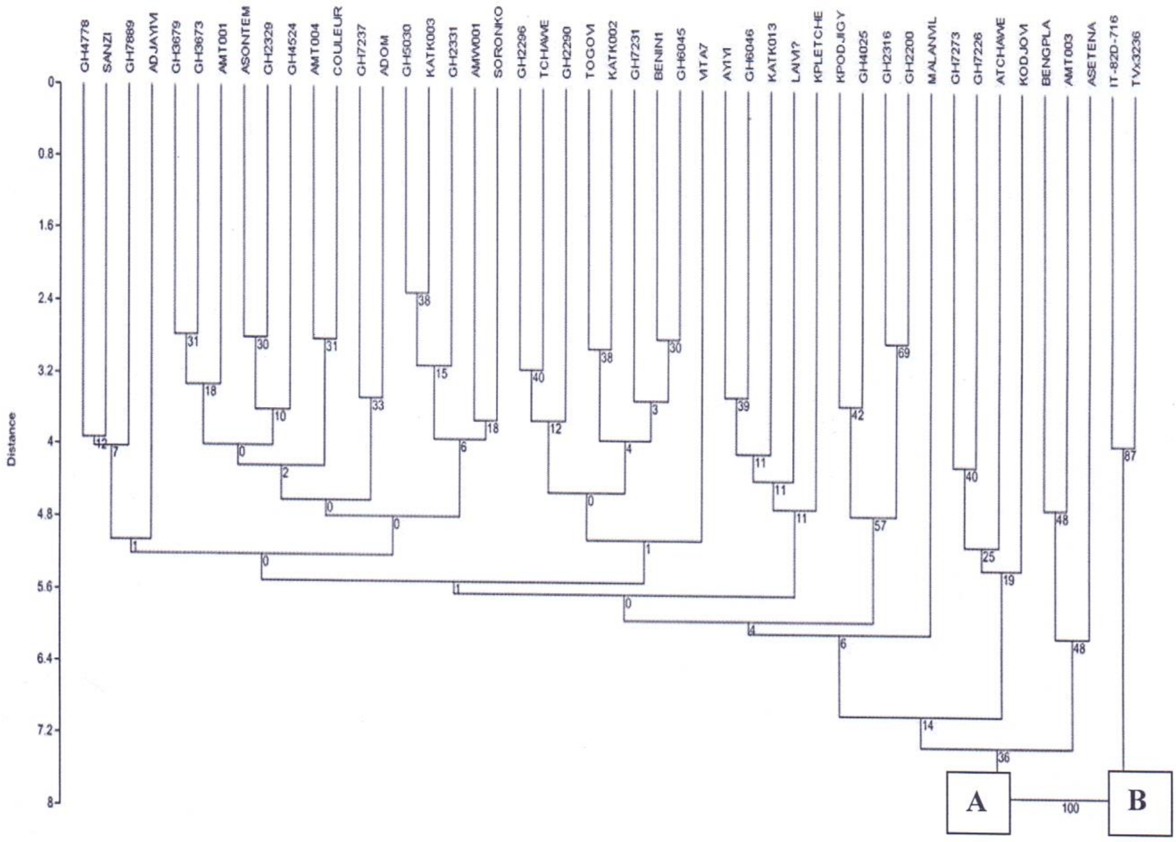

Fig 1: 1000 bootstraps (Cophenetic correlation coefficient 0.7438) of clustered (using Euclidean distance) morphological data of forty-seven accessions.

Molecular characterisation

The cluster analysis, based on 228 polymorphic markers, showed that there were two major groups present in the study germplasm (Fig. 2). Within the two groups (A and B) similar and almost identical accessions were found.

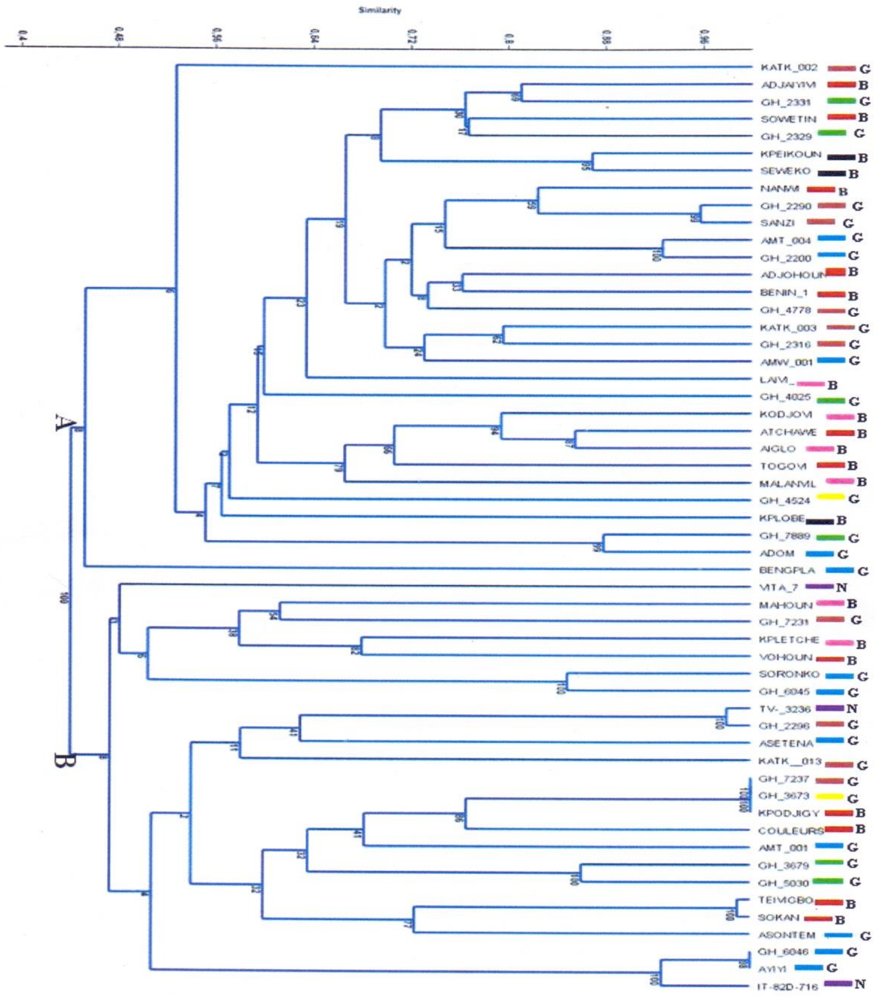

Fig. 2: Jaccard cluster analysis, 1000 bootstraps of the AFLP data for fifty-four cowpea accessions, cophenetic correlation coefficient = 0.882. Brown, green, blue and yellow coloured bars denote Ghana (G) Guinea savannah, forest, forest-transition and coastal savannah agro-ecological zones respectively. Red, pink and black for Coastal and sandy, Transitional Sudan Guinea and Ferralitic soil zones respectively in Benin (B). The violet colour denotes Nigerian (N) genotypes whose agro-ecological origin is unknown.

DISCUSSION

In this study, morphological and molecular data were used to elucidate the genetic variability among cowpea cultivars from Ghana, Benin and Nigeria.

Variation among landraces based on morphological characters

The quantitative and qualitative agro-morphological characters showed different coefficients of variation (CV) ranging from 4.1 % to 40.2 % and 8.3 % to 374 % respectively. The high CV ranges indicate a wide variation for a given trait among the different landraces and /or varieties. A low CV (< 5% for traits indicates similarity while those with high CV (> 35%) suggests some level of dissimilarity indicating the existence of phenotypic variation within the germplasm. A large variation existed for seed splitting, immature pod pigmentation, seed crowding, stem pigmentation, seed shape, testa texture, raceme position, eye colour and pattern, growth habit, petiole pigmentation, flower pigmentation pattern, terminal leaf length and width, hypocotyl length, peduncle length, stipule length and width, number of main branches and raceme per plant. The influence of these traits on plant architecture contributes directly or indirectly to the variation among these cultivars. Other traits like stipule length and width, terminal leaf length and width, days to flower and days to 1st mature pod and flower colour and flower pigmentation pattern correlated significantly. The level of variation and extent of associations between these key agro-morphological traits reveal the inherent genetic diversity of the study germplasm. The high CV for these traits in the cowpea landraces indicate phenotypic variation which can be exploited by breeders in cowpea improvement programmes. Principal component analysis also identified morphological characters relevant for elucidating the variation among the accessions within the cowpea germplasm. This investigation revealed that the first four principal axes accounted for 42 % of the phenotypic variation among the cowpea accessions. Aremu *et al*., (2007) reported that six principal axes accounted for 63.6 % of the total variation among thirty-one cowpea accessions collected from seven West African countries including Ghana, Benin and Nigeria. Similar to this study, the major characters as revealed by the analysed data, the first four principal axes were of the vegetative (pre-flower), flower and maturity stages of the crop. Contrary to this, Padulosi *et al*., (1995) identified length and number of branches, days to flower and maturity as the major characters for phenotypic variation in cowpea. In this study, stipule length and width, stem and petiole pigmentation, hypocotyl length and growth habit accounted much for the observed phenotypic variation.

Beyond the variation accounted for, the unexplained portion (58 %) of the variability within the germplasm may be due to limitations in the phenotypic descriptions and many other characters (flower colour, testa texture, seed width, 100 seed weight, flower standard length) contributing small quotas. The dendrogram (Fig. 1) resulting from the hierarchical clustering of thirty-eight phenotypic characters revealed two major clusters (A and B). However, bootstrap values were generally low for all groups except at the separation between clusters A, B and the two members of cluster B. The low bootstrap values indicate a poor reliability of the sub-clusters formed by the phenotypic descriptors used in evaluating genetic variation within the germplasm. The use of molecular method to address this shortcoming is therefore highly relevant (Musvosvi, 2009).

Relationship among landraces based on AFLP markers

The use of DNA markers to explore variability among accessions instead of phenotypic characterization has the advantage of eliminating environmental influence on morphological traits. The molecular analysis revealed two distinct clusters, A and B as shown in Fig. 2. These clusters are supported by a 100% bootstrap value. This strongly suggests that the germplasm from the three West African countries has two major gene pools present. This agrees with the report by Yann *et al.,* (2012) who also identified two major clusters for twenty cowpea landraces collected across Benin. These two clusters suggest the existence of two major gene pools in the West African germplasm, which may possibly be linked to the African and Asian gene pools as reported by Fang *et al.*,(2007). Oppong-Konadu *et al.,* (2005) identified three clusters among sixty Ghanaian cowpea accession analysed by the SDS-PAGE method. The cultivars used in this study do not overlap according to their names but there could be similarities due to free exchange of planting materials between farmers and cowpea trade in Ghana. The dendrogram (Fig. 2) shows some level of diversity within the germplasm notwithstanding the presence of identical and closely related landraces. Similarly, Zannou *et al.*,(2008), using RAPDS, showed a high genetic diversity among 70 cowpea accessions from Benin. They indicated that, a higher number of accessions from geographically worldwide origins can result in higher genetic diversity. The variation found in this study may be related to this, as the collection came from a wide geographical region across West Africa. The variation in this study is much wider than was observed by Asare *et al.,* (2010), using simple sequence repeat (SSRs) markers to study a collection of Ghanaian accessions from the national genebank collected across all cowpea growing areas in Ghana.

In this study, the following pairs of landraces, Adom – GH 7889, Aiglo – Atchawe-tola, Kpeikoun-Sewekoun, GH 2290 – Sanzi and AMT 004 – GH 2200 within cluster A had similarity coefficients ≤ 0.85 and with 95-100 % bootstrap values and therefore these can be considered genetically closely related. Within cluster B, the pairs; Soronko - GH 6045, and GH 3679 - GH 5030 are closely related. The similarity between these landraces is likely due to common ancestry or duplication. The pairs of landraces; Teivigboto - Sokan, TVx 3236 - GH 2296, Ayiyi-GH 6046 and GH 7237 - GH 3673-Kpodjigyegye with 100 % bootstrap value and a similarity coefficient of > 0.95 may be considered as genetically identical (Arens *et al.*,1998; Li *et al.,* 2001;Vosman *et al.*,2004). The accessions GH 3673 and GH 7237 possess ash brown mosaic seed testa colour, erect growth habit, no stem pigmentation and small hilium. These identical accessions have different names and origin, which may have come about due to free exchange of planting material among farmers and other users including cowpea traders. This is due to the absence of barriers in the flow of genetic materials as a result of undeveloped seed industry and free trade in Ghana and possibly other West African countries (Oppong-Konadu *et al*., 2005). TVx 3236 has cream seed testa colour and an intermediate growth habit but GH 2296 though closely related has a prostrate growth habit and red seed testa colour. There is extensive stem pigmentation in GH 2296 but no stem pigmentation in TVx 3236. Such differences may arise from different selections from a common ancestor. Bengpla (IT-83S-818) was clearly distinct from all other accessions analyzed. It is the product of multiple crosses, e.g. (TVx 33-1J X TVu 6203) X TVx 33-1J) X TVx 6332) (Li *et al*., 2001). The aggregation of different sets of genes from the different parents likely resulted in this dissimilarity.

Geographical origin and the genetic relationship among landraces

The West Africa region, considered a centre of diversity for cultivated cowpea (Baudoin and Marechal, 1985), may account for the large variation observed in this study. The wide CV ranges for the phenotypic characters and the extent of clustering of the molecular data at a cophenetic correlation coefficient of 0.882 on the dendrogram reveal much variation within the germplasm. The result shows a mix of the different landraces and accessions in the various sub-clusters irrespective of country or agro-ecological zone of origin. This clearly indicates that geographic origin (agro-ecological zone or country) of the West African cowpea cultivars does not offer genetic relatedness among members of a given cluster. This is in agreement with Oppong-Konadu *et al*., (2005) who reported that accessions from different collections clustered together are not due to geographic origin or year of collection but their pedigree. Hence these cultivars widely adapted to the different agro-ecological zones, allow free exchange of planting materials between farmers and researchers.

CONCLUSIONS

The study has shown genetic variation by the existence of two major gene pools with an appreciable level of diversity not structured by geographic origin but wide adaptation indicating extensive exchange of these genetic materials across the West African sub-region. Hence the potential utilisation of these diverse and widely adapted cultivars in cowpea breeding and improvement programmes.

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