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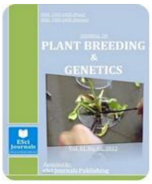
Journal of Plant Breeding and Genetics

ISSN: 2305-297X (Online), 2308-121X (Print)

http://www.escijournals.net/JPBG



COMPARATIVE STUDY OF COWPEA GERMPLASMS DIVERSITY FROM GHANA AND MALI USING MORPHOLOGICAL CHARACTERISTICS

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ABSTRACT

Cowpea improvement can be enhanced by knowledge of genetic diversity available between and within local and regional gene banks. This variability is the foundation of all cowpea improvement programs. A total of 94 accessions (47 from Ghana and 47 from Mali) was used for this study. Twelve qualitative and twenty quantitative traits such as flower color, growth habit, raceme position, seed shape, day 50% flowering, day 50% maturity, plant height, seed length and seed weight were used to assess collections. Data collected from different morphological traits were analyzed using XLSTAT 2013. Principal component analysis, scatter plot matrix and clustering separated the accessions according to some qualitative and quantitative traits. Accessions were classified based on their morphological relationships using un-weighted pair-group average Cluster Analysis. Results showed a relatively low level of genetic diversity between and within both germplasm; levels of similarity ranged between 0.95 to 0.069 for combined qualitative and quantitative data. However, some morphological traits discriminated more efficiently between the accessions than others. Few groups of the accessions were different from other accessions for some important traits. Implications of the variability in cowpea improvement are discussed.

Keywords: Cowpea, Germplasms diversity, Description of relatedness, Cluster analysis.

INTRODUCTION

Cowpea [(*Vigna unguiculata* (L.) Walp.] (2n=22) is one of the most important legume crops in the world and it is a major food crop in Africa, Latin America and India because of its high protein content (Kareem and Taiwo, 2007). Production of this crop was estimated at 11.8 million ha with 5.4 million tons of dried grains; Africa alone provides 91% of the global production thus 10.7 million ha from West Africa (FAOSTAT, 2010).

In Mali, cowpea production is between 300 kg/ha and 3,000 kg/ha; intercropped system is the most developed method and accounts for 132,800 tons against 28,538 tons produced by monocropping (DNA Mali, 2011). The main cowpea producing areas in Mali are: Kayes, Koulikoro, Mopti, Segou and Sikasso district. Cowpea is an important source of vegetable protein and minerals for over 70% of Ghana population and it is the second most important grain legume after groundnut. It is currently a food security crop with the mean production ranged from 340 kg/ha to 4,000 kg/ha (MOFA, 2010).

In these two countries, Ghana and Mali, farmers select their cowpea seeds by local names based on morphological characteristics and agronomic attributes which are of interest to breeders (such as plant morphology, seed coat colour or other visible seed/pod characteristics). Improvement of the local varieties and crop management practices can increase farmer's production and productivity, and reduce poverty. This cannot be achieved without knowing the genetic diversity available within and between local and regional germplasm collections (Hedge and Mishra, 2009; Hall, 2004).

Germplasm assessment is very important for gene bank managers, since it allows more efficient sampling of available resources for crosses and also to remove obvious duplicate materials. Diversity in the available gene pool is the foundation of all plant improvement

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programs. It can ensure long-term selection gain in genetic improvement, and promotes the rational use of genetic resources (Barrett and Kidwell, 1998; Messmer *et al.*, 1993). Moalafi *et al*. (2010) undertook Germplasm evaluation and enhancement of the development of cowpea through crosses. Genetic distance and diversity among some cowpea genotypes were assessed by Adewale *et al.* (2011) using some traits such as plant height, number of pods per plant, days to first flower etc.

Morphological characterization of cowpea germplasms from Ghana and Mali is important. Identification and differentiation of the relatedness of cowpea germplasm will be useful for breeding in the two countries. This will contribute to efforts aimed at maximizing the selection of diverse parent genotypes and to broaden the germplasm base for future cowpea breeding programs in Mali and Ghana. The objectives of this study were therefore to screen cowpea accessions from Ghana and Mali using morphological descriptors and select desirable varieties for breeding programs.

MATERIALS AND METHODS

A trial was conducted under open field condition at CSIR-Crops Research Institute (CSIR-CRI) at Fumesua in Ashanti Region (01° 36°W; 06° 43°N) from the end of the major season to the beginning of minor season, 2011 (July to early October). The location receives an average total annual rainfall about 1,727.2 mm and has a bimodal rainfall distribution. The soil belongs to the Asuansi series and is classified as Ferric Acrisol (FAO/UNESCO, 1988). It has 16-20 cm thick layer of sandy loam and slope of 1-5%.

The experiment was established with 94 genotypes. The accessions from Mali collected in cowpea gene bank of Cinzana Agricultural Research Station (SRAC) of Institut d'Economie Rurale (IER), were composed of 30 local varieties, 9 improved from the cowpea breeding program and 8 introduced varieties. Majority of Ghanaian materials from Plant Genetic Resource Research Institute (PGRRI), Bunso, was composed as follows: 39 local varieties from PGRRI, 7 improved varieties from CSIR-CRI and 1 introduced variety from IITA. The trial was established in 22nd of July 2011. The genotypes were planted in Complete Randomized Design (CRD) without replication and carried out on 338 m^2 area. Each accession was planted on one row plot 2 m long at spacing of 20 cm within row with 1.5 m space between rows. Three seeds were sown per hill and thinned to two plants two weeks after emergence. The experimental area was surrounded by one variety which became the border. Two kilograms of NPK (15.15.15) was applied at two weeks after planting and weeding, the plants were treated with Cymethoate Super EC and Pawa 2.5 EC Super (NOVA AGRO HK LT, South Africa) against thrips and aphids respectively. Using the IBPGR (1983) descriptor for cowpea, data were collected from twelve qualitative traits from 4th week and twenty quantitative traits after sowing. They were submitted for analyzing using XLSTAT Statistical Analysis Software (Kovach Computing Service 2013) based on the means of the qualitative and quantitative parameters. Morphological data were subjected to cluster analysis with XLSTAT based on SAHN (Sequential, Agglomerative, Hierarchical and Nested) clustering with un-weighted and pair-group average method.

RESULTS AND DISCUSSION

Qualitative morphological analysis: The observed flower colors of the accessions used in this study showed that about 73% of the accessions produced violet flowers, 19% produced white flowers and 8% produced white-violet which is in agreement with the observation of Cobbinah *et al.* (2011) who found in their study the higher percentage of violet flowers followed by white flowers.

Phenotypic observation of raceme position showed that 73% of the accessions had their raceme positions mostly above the canopy while 18% held the raceme positions in the upper canopy, 9% had their raceme position throughout the canopy. All raceme positions were represented. This parameter is important; varieties with racemes above the canopy are easier and cheaper to harvest than those with raceme below canopy using mechanical techniques (Bennet-Lartey and Ofori, 1999).

Seed testa texture ranged from smooth, smooth to rough and rough. Additionally, the result from Nkouannessi (2005) study showed that seed testa texture ranged from rough to wrinkled. Smooth to rough seed testa texture were reported in accessions evaluated by Adewale *et al*. (2011). Comparing to these previous studies, the difference can be based on the large number of genotypes used for actual study. Recorded at mature leaf in the 6th week after sowing, majority of the genotypes from Mali had terminal leaflet shape sub-globose and this form characterized more than 50% of the accessions studied. The second group had terminal leaf shape globose. The forms hastate and sub-hastate were predominantly from Ghana.

On the seven growth habit patterns of the International Board for Plant Genetic Resources (IBPGR) (1982) cowpea descriptor, four of them were observed in all the accessions. Germplasm from Mali had shown more than 80% semi-prostrate, 10% prostrate and 5% intermediate. Majority of Ghanaian accessions had semi-prostrate growth habit, some with intermediate or prostrate growth habit and only 1% showed erect growth habit. Touré *et al*. (1999) found some improved varieties such as PRL 22 (Djemani), PBL 74 (Yerewolo), PBL 112 (Dounafana), CZ1-94-23-1, CZ1-94-23-2 and CZ11-94-5C to be prostrate; but they showed semi-prostrate growth habit in this study. These differences may be due to the acclimatization of the genotype or genetic make up of the tested genotypes. Growth habit is very important in the cropping system of cowpea in Ghana and Mali; most farmers from these two countries use varieties in intercropping that have either prostrate or semi-prostrate growth habit.

Apart from uniform pigmentation, all characteristics of immature pod pigmentation were found in accessions studied based on IBPGR (1983). Most important pod pigmentation observed has been pigmented tips while 1% was for splashes of pigment. Pigmented valves and green sutures and splashes of pigment of pod were found in collection from Ghana. The study revealed that the pattern of pigment distribution of full grown immature pods varied; pods of most of the immature accessions in this study were not pigmented even though Porter *et al*. (1974) had reported of six different patterns of pod pigmentation.

Quantitative morphological analysis: The greatest number of days to 50% flowering has been with PRL 73 (Yerewolo) with 60 days while TVU7714 had the earliest (33 days). TVU7714 took the same days to attain 50% flowering with Milo.

Days to 50% maturing among cowpea genotypes ranged from 50 to 84 days. Milo took fewer days to mature (50 days) and was an extra early maturing genotype followed by TVU7714 and GH2329 (53 days). PRL 73 (Yerewolo) was observed as the late maturing genotype (84 days). Breeding for earliness is an important breeding objective with cowpea as breeder and farmers always require the early maturing genotypes to fit the crop to the short rainy seasons a consequence of climate change. Fifty percent of the genotypes from Ghanaian accessions matured between 50-60 days whereas 10 varieties were only from Mali. The genotypes studied showed variation in maturity between and within both germplasm. Such variation in maturity may be due to climatic conditions or genetic makeup of the tested genotypes. These results are in conformity with those of Amanullah *et al*. (2000), who studied 20 genotypes and found significant variations for days to maturity. Dugje *et al*. (2009) had classified cowpea varieties that matured in 60 days as extra- early, 61-80 days as early and > 80 days as late. Based on their classification, majority of accessions from this study were early maturing whilst PRL 73 (Yerewolo) was the only late maturing variety. The highest height of the plant at 4 weeks after planting was for GH5050 with 32 cm. Across the ninety-four accessions; the least height (8 cm) was obtained by GH2293. In accordance with Khan *et al*. (2010), genotypes with early maturity produced dwarf plants, while genotypes with late maturity showed highest plant height. The same results were reported by Ram *et al*. (1994). In current investigation, some earlier materials had high plant height and vice-versa. This variation might be attributed to the differences in the genotypes or might be due to environmental fluctuations, the period of rainfall was not at the vegetative stage of the accessions.

The lines TVU7705, Parajani, IT82D-812 and TVU7696 gave the smallest sizes for the three traits about seed length, seed width and seed thickness with 0.6 mmx10^1, 0.4 mmx10^1 and 0.3 mmx10^1 respectively. Conversely, Ejura and CZ1-94-23-1 had 1 mmx10^1, 0.7 mmx10^1 and 0.5 mmx10^1 respectively. The size of the grain is very important for a consumer, that's why the breeder selects plants with bigger seeds and at the same time with good technological traits. One variety from Ghanaian accessions (Ejura) had the highest 100 seed weight (19 g) followed by CZ1-94-23-1 (18 g). The varieties TVU7617, TVU7687 and TVU7616 recorded the smallest weight for 100 seeds (6 g). In terms of hundred seed weight, less than ten grams was given by the majority of the accessions from Malian collection. These differences in seed weight might be based on the time factor for accumulation of assimilates in the seeds or genetic makeup of different genotypes. Acclimatization factor might also be responsible for higher seed weight. This result also supports the work done by Khan *et al*. (2010) who found highly significant variation for 100 seed weight in 24 exotic cowpea genotypes.

Qualitative and quantitative traits

principal component analysis: The main reason for plant collection is to obtain raw materials that can be useful for providing germplasm pools for crop improvement. According to Johnson (1998), Principal Component Analysis (PCA) is one of the most useful statistical tools for screening multivariate data with significantly high correlations. Information from PCA may assist the plant breeder to identify limited traits for using in hybridization and selection programs. Twelve qualitative and twenty quantitative traits were analysed to establish principal components but few of them are presentated in Tables 1 and 2. The first six principal components explained a total variation of 72.05% and each of them had percentage variability greater than 7 from qualitative scoring (Table 1). The most effective characters for distinguishing among the cowpea accessions included flower color, growth habit, raceme position, testa texture, immature pod pigmentation, seed shape and seed coat color.

Table 1. Principal components for seven selected qualitative traits of cowpea.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Eigen value	1.795	1.601	1.511	1.131	1.016	0.871
Variability (%)	16.31	14.56	13.74	10.29	9.23	7.92
Cumulative of variability (%)	16.31	30.88	44.61	54.90	64.13	72.05
Squared cosines of the variables						
Flower color	0.000	0.029	0.335	0.294	0.020	0.087
Growth habit	0.032	0.048	0.269	0.072	0.358	0.000
Immature pod pigmentation	0.034	0.477	0.048	0.011	0.008	0.054
Raceme position	0.136	0.330	0.143	0.058	0.019	0.082
Seed coat colour	0.375	0.007	0.120	0.000	0.001	0.000
Testa texture	0.502	0.045	0.033	0.004	0.064	0.004
Seed shape	0.321	0.003	0.050	0.115	0.005	0.269

PC= Principal component.

Total variability was 24.45% and 19.95% respectively for PC1 and PC2, more than 70% of quantitative traits contributed for divergence between the accessions (Table 2). Some of these traits are days to 50% flowering, days to 50% matured, seeds weight, plant height, seed length, seed thickness and seed width. They allowed some lines to be different to the others. For the two morphological characters, majority of the traits contributed to divergence between and within both germplasm. Comparative study on the phenology and yield components of ten cowpea varieties by Manggoel *et al*. (2011) showed that out of the nine characters assessed, six had contributed for divergence between the accessions.

Table 2. Principal components for seven selected quantitative traits of cowpea.

	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**
Eigen value	4.890	3.990	2.410	1.553	1.208	1.037
Variability (%)	24.45	19.95	12.05	7.77	6.04	5.18
Cumulative of variability (%)	24.45	44.40	56.45	64.22	70.26	75.44
Squared cosines of the variables						
Day 50% flowering	0.601	0.022	0.263	0.047	0.003	0.008
Day 50% maturity	0.776	0.005	0.147	0.022	0.001	0.000
Plant height/cm	0.034	0.276	0.146	0.159	0.010	0.052
Seed length/mmx10^1	0.041	0.540	0.132	0.014	0.000	0.004
Seed thickness/mmx10^1	0.023	0.531	0.051	0.046	0.000	0.030
Seed width/mmx10^1	0.087	0.518	0.167	0.038	0.005	0.002
Seed weight/g	0.000	0.555	0.127	0.049	0.013	0.015

PC= Principal component.

Qualitative and quantitative scatter plot matrix: The first two principal components (PC1 and PC2) for qualitative traits explained 30.88% of the total variation and was associated with five characters that made some varieties from the study accessions distant to the others. Varieties in the first quardrat will be good candidates for genetic improvement (Figure 1). For example, KPR1-96-54 with semi-prostrate growth habit and white seed coat; TVU7624 with semi-prostrate growth habit; Ejura with intermediate growth habit and kidney seed shape; GH7233 with raceme mostly above canopy, semi-prostrate growth habit and globose seed shape; can be intersting to improve cowpea varieties which will be useful for intercropping systems.

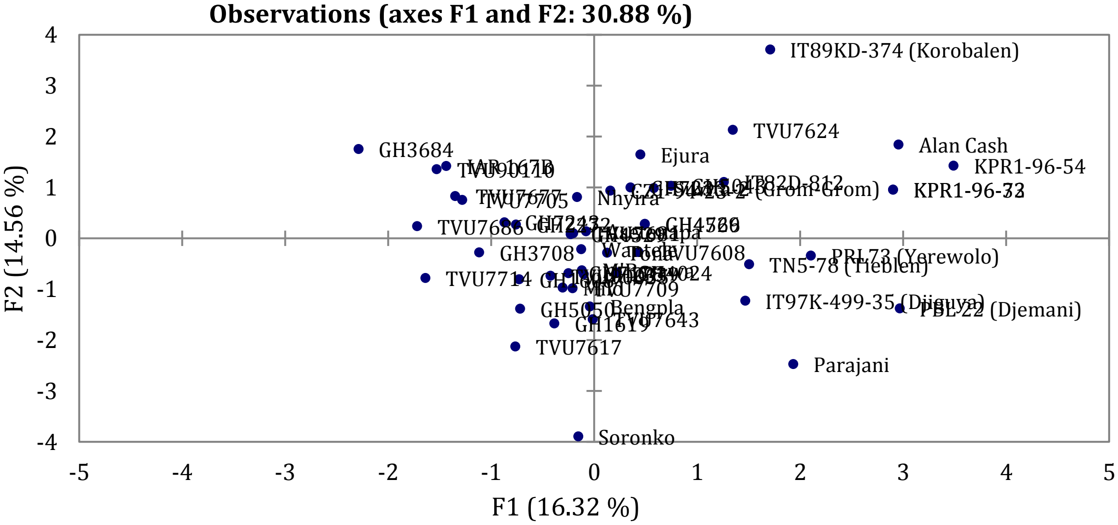

Figure 1: Distribution of varieties among accessions in PC1and PC2 for qualitative traits.

Genotypes diverged from the others based on the contribution of some reproductive traits and seed components (days to 50% flowering, days to 50% maturity, seed length, seed width, seed thickness and 100 seeds weight) from the first two principal components (Figure 2). From the first quardrat, Ejura with large seed length-seed width-seed thickness, intermediate maturity, 100 seed weight and number of main branch; PRL 73 (Yerewolo) with a number of main branch, number of pod/peduncle, large seed length-seed width-seed thickness; IT97K-499-35 with extra early maturity; can be used for crosses to improve some varieties from the two germplasms (Ghana and Mali).

The results of this study were close to those from Manggoel *et al*. (2011), and Sulnathi *et al.* (2007). These authors found that day in 50% flowering, days to 50% maturity and 100 seed weight contribute very much for the divergence between the accessions. These three characters should be considered while selecting the parents for the hybridization program in yield improvement of cowpea.

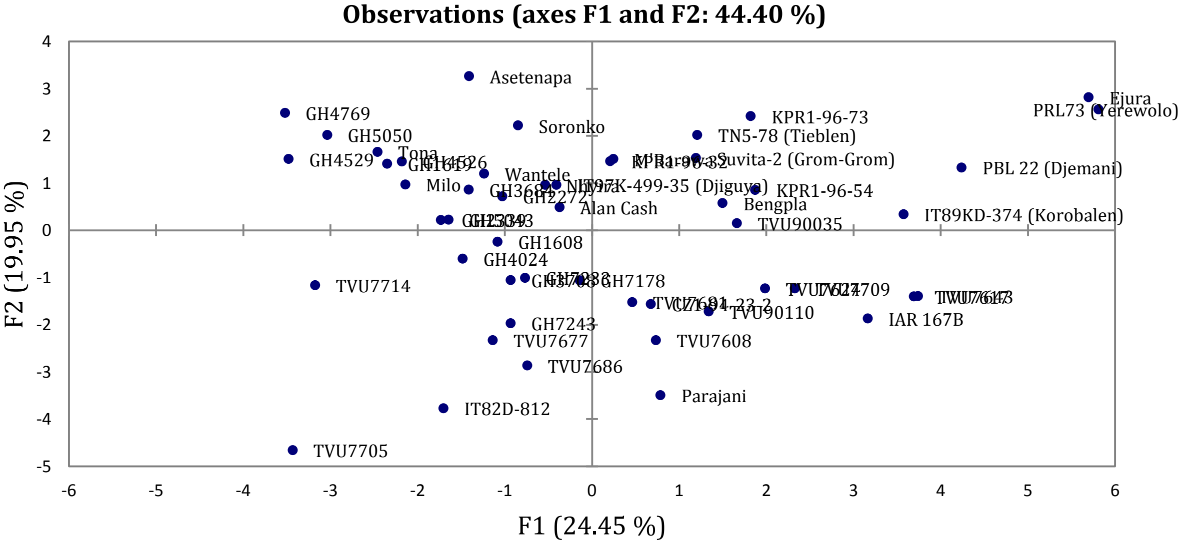

Figure 2: Distribution of varieties among accessions in PC1and PC2 for quantitative traits.

Cluster analysis: Principal Component Analysis (PCA) alone may not give an adequate character representation in terms of their contribution for the genetic divergence hence, it needs to be complemented with other techniques such as Cluster Analysis which provides more information about the relative positions of the accessions. Cluster analysis decreases the number of individual variable units by arranging into groups which are translated into a dendrogram on the basis of coefficient of similarity (Tatineni *et al.*, 1996). It determines the relationships between genotypes and hierarchical mutually exclusive grouping such that similar descriptions are mathematically gathered into the same cluster (Hair *et al.*, 1995; Aremu, 2007).

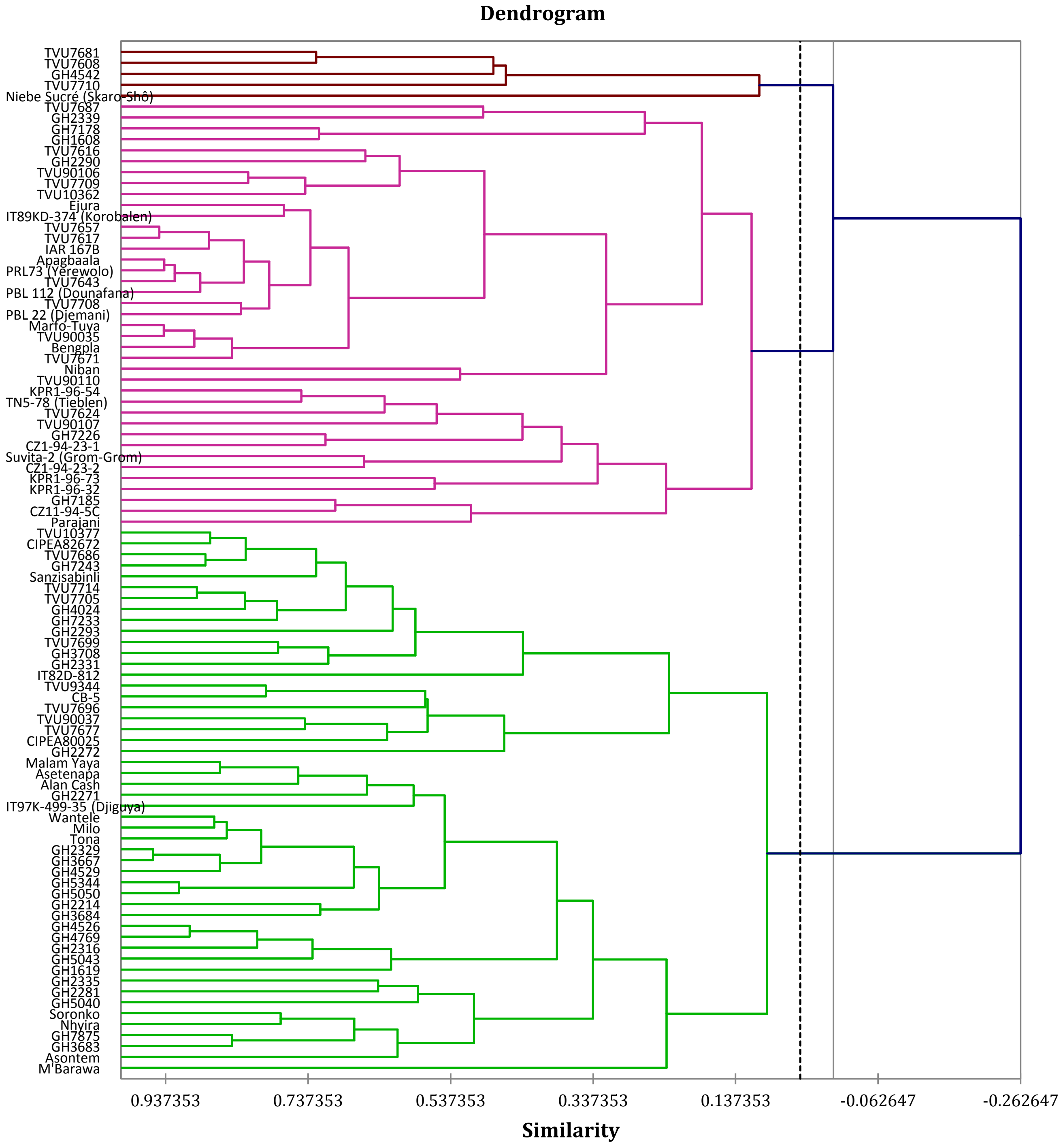

Figure 3: Dendrogram based on combined qualitative and quantitative traits of 94 cowpea accessions.

The agglomerative un-weighted pair-group average illustrated with data collected from qualitative and quantitative traits, showed the relationship among the accessions (Figure 3). At 0.97 level of similarity, almost all the 94 accessions were distinct from each other while at 0.95 levels there were similar to each other. Based on Pearson correlation coefficient, the dendrogram at similarity distance 0.069 was divided into three major clusters (A, B, C) according to the morphological characters associated with them (Table 3). Each cluster has two sub-clusters at 0.137 level of similarity; variability between clusters was 29.85% whereas it had been 70.15% within clusters. Majority of study accessions was under cluster C with more than 70% of Ghanaian materials. Cluster B was composed of 28 genotypes from Mali and 11 from Ghana whilst five accessions was belong to cluster A.

Table 3. Acessions distribution between clusters based on combined qualitative and quantitative traits.

Cluster	Similarity of coefficient	Number of accessions
A	0.069	4 from Mali (TVU7608, TVU7681, TVU7710, Niebe Sucré (Skaro-Shô)); 1 from Ghana (GH4542).
B	0.069	11 from Ghana (GH1608, GH7178, Bengpla, Parajani, Ejura, GH2339, Marfo-Tuya, GH2290, Apagbaala, GH7226, GH7185); 28 from Mali (TN5-78 (Tieblen), TVU7617, IAR 167B, PBL 22 (Djemani), TVU90110, IT89KD-374 (Korobalen), KPR1-96-32, KPR1-96-73, CZ1-94-23-2, TVU7643, TVU7624, KPR1-96-54, PRL73 (Yerewolo), TVU90035, TVU7709, Suvita-2 (Grom-Grom), TVU7671, TVU7657, CZ1-94-23-1, TVU10362, TVU90106, TVU7687, TVU7708, TVU90107, CZ11-94-5C, PBL 112 (Dounafana), Niban, TVU7616).
C	0.069	15 from Mali (TVU7705, M'Barawa, IT97K-499-35 (Djiguya), TVU7714, TVU7686, IT82D-812, TVU7677, TVU7699, CIPEA80025, TVU7696, TVU90037, CB-5, CIPEA82672, TVU10377, TVU9344); 36 from Ghana (GH5040, GH3684, GH4769, GH7243, GH5050, Nhyira, Milo, Soronko, GH2272, Asetenapa, Tona, GH1619, GH5043, GH3708, GH4529, GH4024, GH4526, GH7233, Wantele, Alan Cash, Malam Yaya, GH3683, GH7875, GH2281, GH2293, GH2331, GH3667, Asontem, Sanzisabinli, GH2335, GH2329, GH2316, GH2214, GH2271, GH5344).

CONCLUSION AND RECOMMANDATIONS

A relatively high level of similarity was observed among the accessions for most of the morphological traits (qualitative and quantitative) analyzed between and within germplasm collections from the two countries (Ghana and Mali). The current study also revealed that some morphological traits discriminated more efficiently between the accessions than others. It would be very important to identify beforehand the right agro-morphological characters (those with high discriminative capacity) before undertaking genetic diversity studies based on morphological traits. Some groups of accessions (TVU7608, TVU7681, TVU7710, Niebe Sucré (Skaro-Shô), GH4542) were different from others for some important traits such as growth habit, days to 50% maturity, days to 50% flowering, testa texture, days to 50% emergence etc.

The high similarity level in this study can be attributed to seeds exchange by traders between countries, same genotypes from different geographical zones or from the same gene bank for example IITA, or self-pollination of cowpea which realized pollination before the flower opens. These results were previously supported by Kuruma *et al.* (2008) who found low level of genetic diversity among cowpea accessions in Kenya based on morphological markers. But Musvosvi (2009) found low level of similarity between studied accessions with the most similar genotypes clustered together at 53.74 similarity level. From actual study, these high levels of similarity between and within both germplasm collections should be investigated by characterization using molecular markers. For example Kuruma *et al.* (2008) have reported generally low level of variability in cowpea after using molecular markers. Because of the importance of diversity study in cowpea germplasm, it will be important to replant the same collection during the major season and also evaluate these collections at the second location, Mali.

ACKNOWLEDGEMENT

This work was supported by grant from Alliance for a Green Revolution in Africa (AGRA). The author is grateful to Institut d'Economie Rurale (IER) Mali and CSIR-Crops Research Institute for supplying the seeds used in this study. This study was part of a Master of Agronomy (Plant Breeding) thesis of the first author at Kwame Nkrumah University of Science and Technology (KNUST), Kumasi (Ghana).

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