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Genetic Diversity in Cowpea (*Vigna unguiculata* (*L.*) *Walp*) under Two Growing Conditions*

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Abstract

This study explores the use of genetic variability for advancing the genetic improvement of Cowpea (*Vigna unguiculata* (*L.*) *Walp*), particularly in response to insect infestation stress. Over a period spanning 2015 to 2017, forty accessions of cowpeas were evaluated to determine their variability under both insecticide spray and no insecticide spray conditions at the Teachings and Research Farms, Federal University of Agriculture, Abeokuta. The experimental design was a randomized complete block design in three replicates. The accessions were evaluated for plant height, leaf length, leaf width, number of days of 50% flowering, number of pods per plant, pod length, number of seeds per plant, 100-seed weight, and seed yield. Data collected were subjected to principal component and single linkage cluster analyses. Principal axis I (PCA1) accounted for 39% and 35% under insecticide spray and no insecticide spray respectively to the total variation in the accessions. Plant height with a factor score of 0.38, leaf length (0.41), number of leaves (0.37), and 100-seed, weight (0.30) was related to PCAI under insecticide spray while leaf width (0.32). Pod length (0.37) and number of seeds/plant (0.38) were significant to PCA1 under no insecticide spray. Notably, accessions such as SAMPEA6, SAMPEA10, IFE-Brown, and IFE-BPE exhibited consistent performance across both conditions, while others displayed condition-specific attributes. For instance, NGB1063, NGB1152, and NGB1093 demonstrated distinct traits under insecticide spray, while NGB1146 and NGB1124 exhibited notable characteristics under no insecticide spray conditions. Therefore, identifying these forty accessions with desirable traits hold promise for future genetic improvement efforts of cowpea cultivation in Nigeria and beyond.

Keywords

Cowpea, Vigna Unguiculata, Genetic Diversity, Cowpea Accessions

*Breeding program to improve cowpea genetics.

1. Introduction

Cowpea (*Vigna unguiculata*) is an important crop in many countries of tropical Africa, Asia, and South America. The grains and leaves are rich and cheap sources of protein. They are used alongside cereal and root vegetables in tropical Africa [1] [2]. On average cowpea grains contain 23% - 25% protein and 50% - 67% starch on dry matter bases [3]. Cowpea has several products such as immature pods, and immature and mature seeds from a single planting. Careful and positive attention to cowpeas would support 850 million people in sub-Saharan Africa with high incidences of undernourishment [4] [5]. Also, cowpea has many beneficial and desirable horticultural characteristics usually non-food associated. It is also an efficient nitrogen-fixing, heat and drought-tolerant plant.

In most African countries, cowpea is either grown alone or intercropped with various other crops such as maize, millet, sorghum, beans, pigeon peas, and cotton [6] [7]. In intercropping production systems, when grown alongside other crops, cowpeas spread out and help stop weeds from growing, protecting the soil from washing away. In addition, some types of cowpeas stop a harmful plant called *Striga hermonthica* from growing on cereal crops [8].

Genetic diversity is particularly important for agricultural productivity and development [9]. Despite its importance, genetic diversity within cowpea remains relatively narrow, highlighting the need for comprehensive studies to elucidate the genetic variation and potential for improvement [7]. This paper aims to investigate the genetic diversity and characterize cowpea accessions to facilitate breeding programs for enhanced crop performance and adaptation.

Genetic diversity refers to the diversity within species and is the foundation of the genetic improvement of crops. Genetic diversity serves as a way for crops to adapt to changing environments. Lately, there have been attempts to save and study this variety to improve breeding programs, but more research is still needed [10]. The study on genetic diversity in cowpeas is essential for accessing and exploiting genetic variation within the species, thereby justifying introgression and ideotype-breeding programs to enhance crop performance [11]. The first step of any breeding program is to identify crop plants that exhibit exploitable variation for the traits of interest [12]. A quantitative assessment of the genetic divergence among germplasm and the contribution of different traits to genetic divergence provides an essential and effective contribution of different traits to genetic divergence provide essential and effective information to a breeder in his hybridization program and thereby genetic improvement of yield.

The primary objectives of this study are to determine the genetic diversity among forty accessions of cowpea under two growing conditions (Insecticide and Non-Insecticide) and to identify the characters contributing to genetic variation among these accessions.

2. Literature Review

Cowpea (*Vigna unguiculata. Walp*) is an important food and fodder legume in the world cultivated in the tropics and subtropics and it is a major food crop in Africa, Latin America, and India because it is high in protein, which is good for improving plants [13]. The average yield of cowpeas in West Africa was estimated as 483 kg/ha [14]. World production 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 [15].

Germ-plasm assessment is very important for gene bank managers since it allows more efficient sampling of available resources for crosses and also removes obvious duplicate materials. Diversity in the available gene pool is the foundation of all plant improvement programs. Cowpea is a particularly valuable component of low-value input farming system for resource-poor-farmers because of its productivity and high yield stability in the face of abiotic stress (drought, heat, low soil fertility), and the ability of the crop to enhance soil fertility for succeeding cereal of tuber crops grown in rotation with its greater tolerance to heat, drought, and low soil fertility [16]. Nevertheless, the potential production yield is still below 50%, despite its widespread cultivation. There remains untapped potential for yield improvement and adaptation to environmental stresses. Genetic diversity assessment is crucial for efficient sampling of available resources and forms the foundation of all plant improvement programs.

2.1. Origin, Domestication and Distribution

Cowpea, among the oldest crops, has sparked debates about its origin. Some people believe that cowpeas originated from West Africa because both wild and cultivated species abound in the region. Others believe that it originated in Southern Africa. Its production has spread to East and Central Africa, India, Asia, and South and Central America [17]. Cowpeas are grown in more than 100 countries around the world, and Nigeria is the top producer, making 6.5 million metric tons each year [13].

Nowadays, it is widely cultivated globally and consumed by a lot of people [18]. A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia, and South America as origin. Some literature indicates that cowpea was introduced from Africa to the Indian subcontinent approximately 2000 to 3500 years ago, at the same time as the introduction of sorghum and millet [19]. While others suggest it reached Europe and North Africa from Asia before 300 BC. Speculations are that the Northern part of the Republic of South Africa (former Transvaal region) was the center of speciation of *V. unguiculata,* determined by the presence of the earliest primitive wild varieties. They further hypothesized that the species moved northwards from the Transvaal to Mozambique and Tanzania, where the subspecies pubescence evolved. Cowpea now thrives in various climates, nourishing about 110 million people worldwide. The species *V. unguiculata* includes domesticated forms, i.e., *V. unguiculata ssp. unguiculatavar.* unguiculata, wild annual forms, i.e., spp. unguiculata var. spontanea (Schweinf.), and 10 wild perennial subspecies [20] [21].

Domesticated cowpea includes five cultivar groups Morphological analysis contrasted evolved cultivars and more primitive cultivars according to seed size, on the one hand, and early and late flowering (under inductive conditions) on the other hand. The latter character was markedly correlated with the ovule number. Photosensitive and early-flowering photo-independent cultivars had 11 - 17 ovules per pod and late-flowering photo period-independent cultivars had 16 - 25 ovules per pod [3]. However, this organization of the domesticated gene pool was poorly correlated with isozyme data, which only showed more diversity in primitive cultivars [22].

The African origin of cowpea was suggested by Richard as early as 1847 [23], and since Piper discovered the wild prototype of cowpea in 1913 [24], no one has contested it since wild cowpea plants are found only in tropical Africa and Madagascar, but not in Asia [25]. However, where the crop was first domesticated is still uncertain, and different centers of diversity and origin of the cowpea have been proposed, i.e., Ethiopia [22] [26] [27], West Africa [28]-[33], and Eastern and Southern Africa [34]. "Diffuse" domestication in the savanna after the dispersal of cereals was also hypothesized [2] [10] [35]. Cowpea's tough nature, able to thrive even in poor soils and partial shade, makes it a favorite for farmers who mix it with cereals and rotate crops [36].

2.2. Classification of Cowpea

Verdcourt [37] and Marechal *et al*. [30] classified cowpea as follows;

Kingdom: Plantae

Order: Fabales

Family: Fabaceae

Subfamily: Faboideae

Genus: Vigna

Species: *V. unguiculata*

Cowpea belongs to the genus Vigna and is classified under the Fabaceae family. The Vigna genus includes multiple species, yet the precise count varies according to different authors. All cultivated cowpeas are grouped under *V. unguiculata*, which is further divided into four semigroups: Unguiculata, Biflora, Sesquipedalis, and Textiles [30] [38] [39].

2.3. Uses

Cowpea seeds serve as a nutritious dietary staple for both humans and livestock. Bressani reported nutrient content of cowpeas includes protein (24.8%), fat (1.9%), fiber (6.3%), carbohydrate (63.6%), thiamine (0.000074%), riboflavin (0.000042%) and niacin (0.002 81%) [40]. Cowpeas can also gain advantages by using their wild relatives that can breed with them, similar to what was seen with common beans [18].

Vigna unguiculata ssp. sesquipedalis offers a special type of vegetable pod that's vine-like, keeps growing, and stays fresh for a long time [41]. The young green leaves serve as a vital food source in Africa and are cooked and eaten like spinach. The undeveloped pods are utilized much like snap beans, often mixed into various foods. The green cowpea seeds are commonly boiled and enjoyed as a fresh vegetable, or they can be preserved through canning or freezing processes. Furthermore, the dry, mature seeds of cowpeas are suitable for cooking and canning purposes alike. In many areas of the world, cowpea is the only available high-quality legume hay for livestock feed. Cowpeas may be used in green or as dry fodder. It is also used as a green manure crop, a nitrogen-fixing crop, or for erosion control [42]. It is very good for quick growth and establishment, and for increasing organic matter and improving soil structure. It has excellent heat tolerance and good drought tolerance. It can also be used for intercropping with other main crops like pearl millet (*Pennisetum glaucum*) or sorghum (*Sorghum bicolor*).

2.4. Cowpea Characterization

The trade of both seeds and processed cowpea foods presents lucrative opportunities for individuals in both urban and rural settings to generate steady income. Beyond its importance for food and feed, the spreading indeterminate or semi-erect bushy cowpea varieties provide ground cover, thus suppressing weeds and providing some protection against soil erosion.

The role of the crop in soil fertility restoration as well as its compatibility with many crop mixtures, has made it a common component of most cropping systems of the savanna zone of tropical Africa. The roots and root nodules decay to enrich the soil for the benefit of the subsequent crop. As a result of this, cowpea forms an important component of most cereal-legume cropping systems.

Another important feature of cowpeas is that they fix atmospheric nitrogen through symbiosis with nodule bacteria (*Brady rhizobium spp.*). In so doing, it provides 80% - 90% of its nitrogen requirements and allows adequate yields in nitrogen-deficient soils where non-nodulated crops such as cereals fail. Besides, cowpea is a drought-tolerant crop, capable of producing reasonable yield where other legumes and cereals fail.

Cowpeas yields are low because the environments where they are produced are characterized by various abiotic and biotic stresses. However, even under optimal conditions, the yields are variable and unpredictable, partly due to variability in the growth and development of individual plants. Understanding the extent, distribution, and nature of this variation would be useful in the development of cowpea genotypes within the increased yield potential and improved adaptation to environmental stresses. Phenotypes and genetic diversity can be evaluated using morphological characters, and biochemical or molecular markers (DNA markers).

3. Materials and Methods (Experimental Materials)

Forty (40) accessions of cowpeas collected from the Germ-plasm Unit of the National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan, were used for the study in **Table 1**. The genotype included four cultivated materials.

Table 1. Species of the cowpea accessions used in the study.

Species and Assessions	
Accessions	Species
NGB964	V. spontanea
NGB1070	V. spontanea
NGB1132A	V. spontanea
NGB1152	V. spontanea
NGB963	V. spontanea
NGB1044	V. spontanea
NGB1058	V. spontanea
NGB1105	V. spontanea
NGB1115	V. spontanea
NGB1126	V. spontanea
NGB1090	V. spontanea
NGB1109	V. spontanea
NGB1027	V. spontanea
NGB1028	V. spontanea
NGB1094	V. spontanea
NBG1127	V. spontanea
NGB1163	V. spontanea
NGB1014	V. spontanea
NGB1113	V. spontanea
NGB1118	V. spontanea
NGB1140	V. spontanea
NGB1069	V. spontanea
NGB1088	V. spontanea
NGB1093	V. spontanea
NGB1160	V. spontanea
NGB1162	V. spontanea
NGB1063	V. spontanea
NGB952	V. spontanea
NGB1087	V. spontanea

Continued

NGB1124	V. spontanea
NGB1153	V. spontanea
NGB1116A	V. spontanea
NGB1146	V. spontanea
NGB1164	V. spontanea
NGB1168	V. spontanea
NGB1173	V. spontanea
Ife BPE	V. spontanea
Ife Brown	V. spontanea
SAMPEA10	V. spontanea
SAMPEA6	V. spontanea

a. The information presented in Table 1 was collected and compiled as part of the cowpea experiment.

3.1. Experimental Site and Field Design

The experiment was carried out at the Research Farm of the Federal University of Agriculture, Abeokuta (FUNAAB). Abeokuta is located in a forest transition zone and the experimental site falls on latitude 3˚23'E and longitude 7˚20'N. The experimental field, 30 m × 40 m (1200 m), was cleared manually with the use of cutlasses and hoes and plowed manually with the use of a hoe. It was laid out in a randomized complete block design with three replicates. Each block, 7 m × 40 m was divided into two groups of 40 single-row plots. The plots were 2.5 m long, separated at 0.75 m, and were assigned randomly and independently within the groups. One group was controlled for insect pests while the other was not. The groups were 2 m apart within the blocks.

Field experiment was carried out and seeds of the accessions were planted per hill along the rows at 0.50 m apart and the emerging seedlings were thinned to one plant per hill at 2 weeks after sowing. A total of 5 plants were maintained per plot. Metaforce at 60 ml /20L of water was sprayed at 2 weeks after planting and subsequently. No fertilizer application was done and weeding was done as necessary.

3.2. Character Evaluation

At 50% flowering till maturity, the three inner plants along the row were measured for yield and yield-related characters. Eleven characters were evaluated and they included plant height, leaf length, leaf width, number of leaves, days to 50% flowering, number of pods per plant, pod length, number of seeds per pod, seeds per plant, 100-seed weight, and overall seed yield in Table 2. Measurement was done accordingly using meter rule vernier calliper and sensitive electronic weighing balance.

Table 2. Eleven quantitative characters were evaluated in the cowpea accessions.

Element Quantitative measure	
Elements	Measurement
Plant height (cm)	Measured from ground level to the shoot apex of the plant.
Leaf length (cm)	Measured from the tip of the leaf to the point of attachment to the petiole.
Leaf width (cm)	Measured as the widest part of the leaf.
Number of leaves	Was counted.
Days to 50% of flowering	Days from sowing till the appearance of flowers on 50% of the plants per plot.
Number of pods per plant	Was counted at maturity.
Pod length (cm)	Measured from the tip of the pod to the point of attachment to the pedicel.
Number of seeds per pod	Was counted on matured pods.
Number of seeds per plant	Was estimated as the product of the number of seeds per pod and the number of pods per plant.
100-seed weight (g)	Weight of 100 seeds.
seed yield (g)	Weight of total seeds harvested per plant.

b. The data presented in Table 2 was collected and analyzed as part of the cowpea experiment.

3.3. Data Analysis

The values observed were averaged over a single plant basis per plot. Data collected were considered independently as a factorial design under plots with insecticide and without insecticide. Analysis of variance was used to determine significant differences among the cowpea accessions for the characters evaluated.

Principal component analysis was used to determine characters that contributed significantly to variation among the cowpea accessions and bi-plots between the first and second principal component axes were used to describe the genetic diversity among the accessions. Also, single linkage cluster analysis was used to generate a dendrogram that summarizes the diversity among the accessions into a single cluster.

4. Results

Factor scores of the characters evaluated in the cowpea accessions under two growing conditions are presented in Table 3. Principal axis I (PCA1) accounted for 39% of the total variation of the cowpea accessions under insecticide spray and 35% under no insecticide spray. Considering the first three principal axes, 70% and 72% of the observed variation in the accessions were revealed under insecticide spray and no spray respectively. Characters contributing significantly to the variation under the two growing conditions include several pods per plant and several seeds per pod. In addition, high factor scores (>3.00) were observed in plant height (0.38), leaf length (0.41), number of leaves (0.37), and 100-seed weight (0.30) under insecticide spray and leaf width (0.32), pod length (0.37) and number of seeds per plant (0.38) under no insecticide spray.

Table 3. Factor scores from the first three principal axes of characters evaluated in forty cowpea accessions under insecticide and no insecticide spray.

Character	Insecticide/No Insecticide					
	PCA1		PCA2		PCA3	
Plant Height	0.38	−0.11	−0.31	0.26	−0.12	0.49
Leaf length	0.41	−0.08	−0.29	0.37	−0.03	0.25
Leaf width	0.27	−0.32	−0.36	0.21	0.20	0.25
Number of leaves	0.37	−0.28	−0.35	0.33	−0.01	0.25
Number of pods/plant	0.32	0.42	0.15	0.17	0.34	−0.12
Pod length	0.01	0.37	0.27	0.18	0.33	0.27
Number of seeds/pod	0.33	0.43	0.27	0.15	0.02	−0.32
Number of seeds/plant	0.20	0.38	0.39	0.30	0.11	−0.15
Days to 50% flowering	0.26	0.29	0.24	0.26	0.51	0.25
100-seed weight	0.30	0.19	0.32	−0.45	−0.4	0.45
Seed yield	0.27	0.20	0.30	−0.44	−0.44	0.44
Eigen value	4.33	3.84	2.11	2.37	1.31	1.71
Variation	0.39	0.35	0.19	0.22	0.12	0.16
Cumulative	0.39	0.35	0.59	0.56	0.70	0.72

c. Principal component axis (PCA) analysis was employed in the generation of these factor scores derived from the evaluation of characters in forty cowpea accessions under insecticide and no insecticide spray conditions.

Leaf width (0.32) number of seeds per plant (0.39) and seed yield (0.30) contributed significantly to 19% of the variation observed in the accessions under insecticide spray in PCA 2. Whereas leaf length (0.37), number of leaves (0.33), 100-seed weight (0.45), and seed yield (0.44) contributed more to the 22% variation observed in the accessions under no insecticide spray in PCA 2. Days to 50% flowering were not related to any of the first three principal axes.

4.1. Discussion

The existence of genetic variation in available germ-plasm is a prerequisite for the improvement of desirable characters through selection and breeding [41]. The genetic variability of cowpeas in response to environmental stress was considered in the study. This is necessary to identify superior genotypes for breeding programs.

Genetic diversity exists among the cowpea accessions under the two growing conditions. Ousters observed among the genotypes indicated that the cowpea genotypes responded differently within the growing conditions. However, it was observed that some of the genotype's responses to the two growing conditions were similar. These genotypes were Extinct and consistently grouped as similar genotypes under the two growing conditions. The genotype included the improved and cultivated materials, SAMPEA6, SAMPEA 10, IFE-Brown, and IFE-BPE.

Also distinct among the genotypes were NGB1063, NGB1152, and NGB1093 under insecticide spray, and NGB1146 and NGB1124 under no insecticide spray. As revealed foam, the principal component axes NGB1152 can be selected for a higher number of leaves and leaf width while NGB1063 and NGB1152 are desirable for several seeds/plant, 100-seed weight, and seed yield under insecticide spray. NGB1124 is desirable for leaf width, 100-seed weight, and seed yield while NGB1146 can be selected for higher leaf length, number of leaves, and number of seeds/plant under no insecticide spray. The cultivated genotypes are superior for leaf length, number of leaves, number of pods/plants, number of seeds/pod, 100-seed weight under insecticide spray, and 100-seed weight and seed yield under no insecticide spray.

4.2. Conclusion

Exploiting genetic diversity in different environmental stresses will help to identify and select promising crop plants for breeding programs. The selected genotypes can be used to develop improved and adaptable genotypes across the environment. Additionally, exploring how cowpea seeds look and their colors, along with studying their genes, gives a deeper understanding of cowpea diversity. This is crucial for pinpointing desirable traits and supporting breeding efforts [34] [42].

In the study, NGB1063, NGB1093, NGB1152, NGB1146, and NGB1124 were identified as potential cowpea genotypes that can be used for the genetic improvement of cowpea. Ultimately, this study represents a significant step towards boosting food security and advancing sustainable agriculture practices not only within Nigeria but also globally. Additionally, Leveraging local, underused cowpea varieties can expand the genetic pool, leading to stronger and more diverse cowpea varieties.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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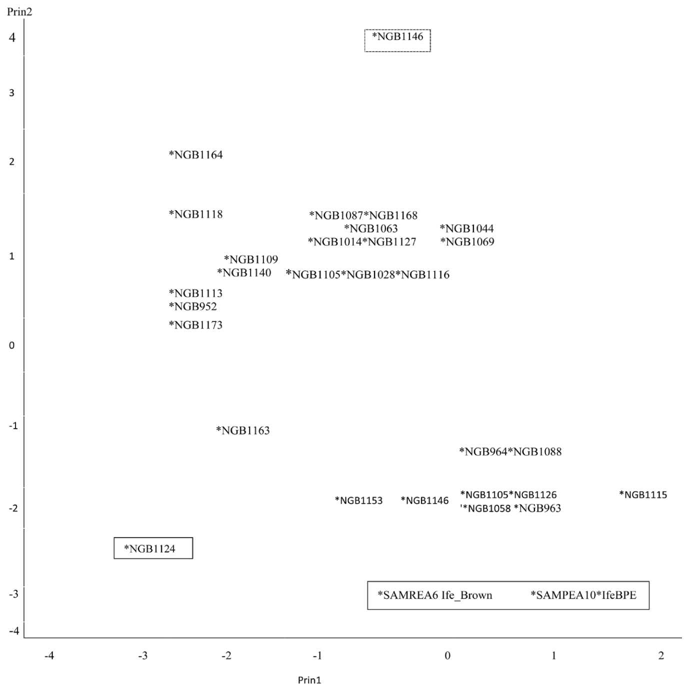
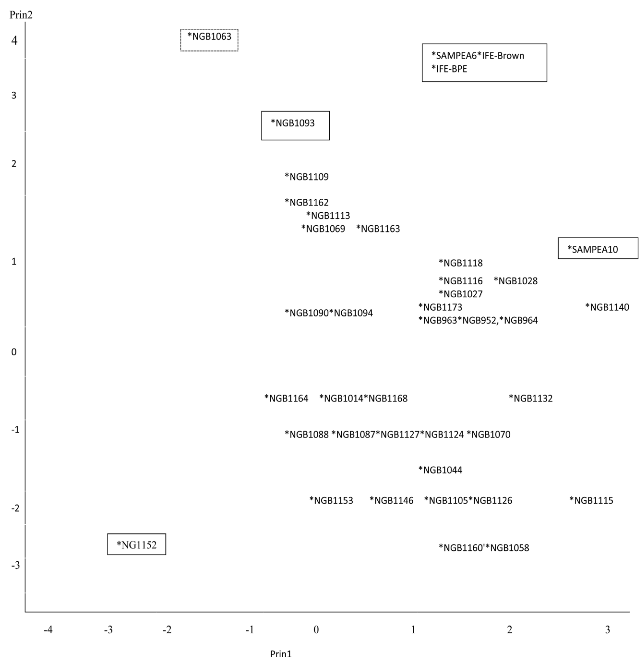
Appendix



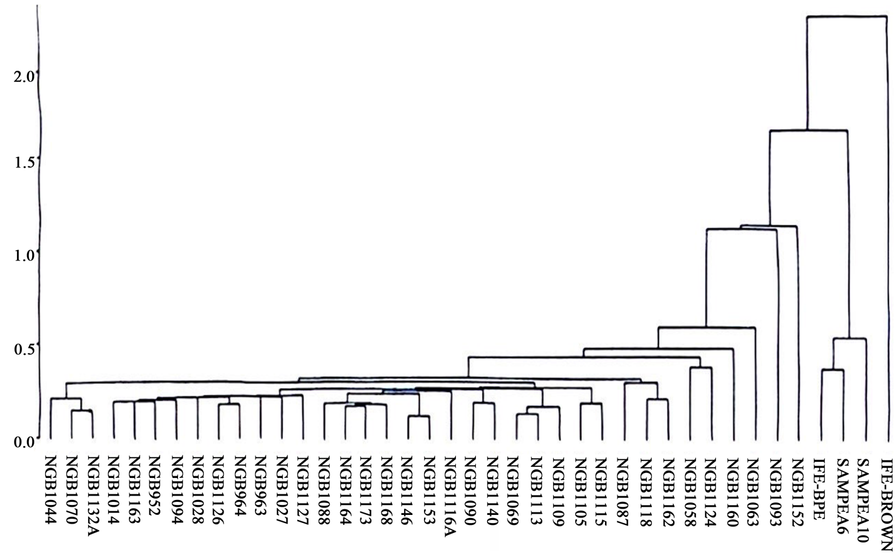
Note: Bi-plot between PCA1 and PCA2 revealing genetic diversity within the forty accessions of cowpea under insecticide spray was presented in **Figure 1**, six groups were revealed among the accessions, SAMPEA6, IFE-Brown, IFB-BPE, and SAMPEA 10 were clustered. Also, NGB1063, NGB1093, and NGB1152 were at a distinct distance from the forty cowpea genotypes.

Figure 1. Bi-plot between principal axes 1 and 2 of forty accessions of cowpea under insecticide spray.

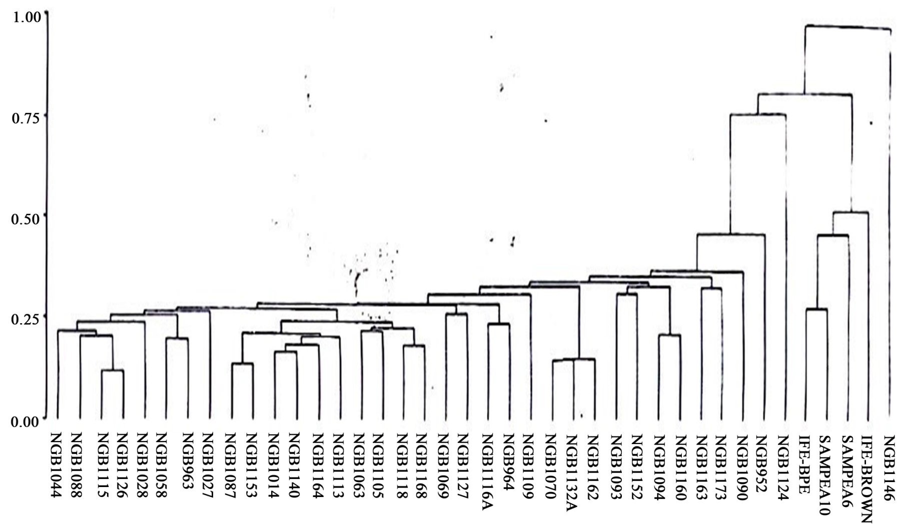


Note: **Figure 2** revealed genetic clusters among the forty cowpea accessions under no insecticide spray. Similarly, SAMPEA6, IFE-Brown, IFE-BPE, and SAMPEA1O were clustered together and distinctly from the accessions. However, NGB1124 and NGB1146 were revealed as other distinct cowpea accessions under no insecticide spray.

Figure 2. Bi-plot between principal axes 1 and 2 of forty accessions of cowpea under no insecticide spray.



(a)



(b)

Note: The dendrogram generated among the cowpea accessions under two growing conditions from single linkage cluster analysis is presented in **Figure 3**. Under the two growing conditions, no cluster was formed at 100% similarity coefficient. The first cluster was formed at an 88% similarity coefficient between NGB1146 and NGB1153 under insecticide spray and between NGB1115 and NGB1126 under no insecticide spray. The dendrogram revealed the diversity of NGB1093, NGB1152, IFE-BPE, SAMPEA6, SAMPEA10 and IFE-Brown under insecticide spray and NGB1124, IFE-BPE, SAMPEA10, SAMPEA6, IFE-Brown and NGB1146 under no insecticide spray.

Figure 3. Dendrogram of 40 accessions cowpea evaluated under insecticide spray (a) and no insecticide spray (b).