WIDE CROSSES IN COWPEA: AN APPRAISAL OF SOME RESISTANCE AND SUSCEPTIBLE LINES

* Bolarinwa K. A.[1], Ogunkanmi L. A.[1], Adekoya K. O.[1], Oboh B. O.[1] and Ogundipe O. T.[2].
[1]Department of Cell Biology & Genetics, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
[2]Department of Botany, Faculty of Science, University of Lagos, Akoka, Lagos, Nigeria
*Corresponding author: princekenny4u2003@yahoo.com

Abstract

Wide crosses of twelve different genotypes of cultivated cowpea Vigna unguiculata were made with a view to transferring useful genes to obtain hybrids with new traits that can be of immense contribution to the yield of cowpea in the world. The expected hybrids are intended to be used for mapping population for further cowpea breeding programs. A total of 102 attempts were made but only eight out of all the crosses were successful. Out of the eight successful crosses only three were viable and germinated when planted. All the twelve genotypes studied were examined for their morphological characters which were later used to construct a dendrogram. The dendrogram showed that the genotype with accession number IT845-2246-4 is genetically more diverse compare to others. At 0.40 similarity coefficient three clusters were formed. These clusters contain sub-clusters which vary from each other. However, at a very high similarity coefficient of about 0.72 there exist relationships between genotypes 2 and 8. A combination of the morphological and breeding works can be used to identify various kinds of genotype and as a reference point for further studies.

***Keywords**: Vigna, Wide crosses, Genotypes, Dendrogram, Variability.*

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp.) is one of the world's dicotyledonous leguminous food crops and a major food crop of millions of people in the developing countries (Summerfield *et al.*, 1974). Cowpea as a pulse crop is of major importance to the livelihoods of millions of relatively poor people in less developed countries. It is valued because it is highly nutritious, has the ability to tolerate drought and also has the ability to fix atmospheric nitrogen, allowing it grow and improve on soil with poor fertility (Senff *et al.*, 1992).

Drastic improvement have been made in the production of cowpea globally with an increase in the yield from about 1.1 million tons in 1974 to about 4.5 million tons in 2004 with the help of breeding work that has taken place over the last decades resulting in the act of creating different varieties of cowpea which has the ability to resist different diseases, insects pest and parasitic weeds. More than 5.4 million tons of dried cowpeas are produced worldwide, with Africa producing nearly 5.2 million. Nigeria, the largest producer and consumer, accounts for 61% of production in Africa and 58% worldwide. Africa exports and imports negligible amounts (IITA, 2012).

Cowpea is a self pollinated crop and this is facilitated by the arrangement of the floral parts. However, out crossing has been reported and frequency of occurrence depends on the genotype involved and the environment where grown (Fatokun and Ng, 2007). Lelou and Van (2006) reported some barriers in intra specific crosses made between some wild African germplasm of *V. unguiculata* and cultivated cowpea. They observed that in most of the reciprocal crosses, the growth of the pollen tubes was arrested in the stigmatic tissue resulting to a large failure of the intra specific crosses which they attributed to lack of fertilization and the unfertilized ovules.

Some barriers have been identified to plague sexual reproduction in cowpea. The barriers could be pre zygotic, post zygotic or hybrid sterility. Chen *et al.*, (1983) identified pollen-still incompatibility as the major prezygotic barrier. Researches by Van Tuyl and De Jeu, (1997) pointed to slow rate of pollen elongation in the stylar tissue and/or distorted pollen tube. Generally, pollen is said to be incompatible when the pollen tube fails to grow down the style to effect fertilization.

Van and De Jeu (1997) used bud pollination and style grafting methods successfully to overcome pre-fertilization barriers. Also, in vitro methods like embryo culture at condition that maintains optimal vitality have been used to overcome post fertilization barriers. Chen *et al.* (1983) reported that F1 inter specific hybrids are obtainable using embryo culture medium technique. Cross incompatibility problems can also be surmounted by end of season pollination, in vitro culture of interspecific hybrid embryos, hormonal treatments of flower buds prior to pollination, polyploidization using cultural methods and pollination at low temperature. Luo *et al.* (2005) reported that an individual is viable if it can survive the adult stage which indicated that the late stage embryo mortality is the main cause of seed sterility. Rieseberg (2001) also reported that embryological disturbances could be caused by genetic factors. Adverse climatic effects can probably disturb the normal embryo development which varies considerably in different localities and species (Hall, 2003).

The present study is a re-assessment of the morphological characters of both the quantitative and qualitative features of the plant with a view to providing concrete information on the classification of the species. Breeding characters were also investigated so as to study the cross compatibility between the twelve different genotypes of cowpea species.

MATERIALS AND METHODS

All the twelve cowpea genotypes (Table 1) used for this research were from the Genetic Resources Unit, International Institute of Tropical Agriculture (IITA), located in Ibadan, Oyo State, Nigeria.

Table 1: List of Cowpea genotypes used in the breeding work.

CODE	ACCESSION NUMBER	RESISTANT/ SUSCEPTIBLE
1	IT98K-205-8	DROUGHT TOLERANT
2	IT98KD-288	DROUGHT TOLERANT
3	IT86D-719	DROUGHT SUSCEPTIBLE
4	IT81D-994	BRUCHID RESISTANT
5	IFE BROWN	BRUCHID SUSCEPTIBLE
6	TVX 3236	BRUCHID SUSCEPTIBLE
7	IT845-2246-4	BRUCHID RESISTANT
8	(IA4B45)244	VEGETABLE COWPEA
9	IT97K-499-35	STRIGA RESISTANT
10	(IA4B55)257	VEGETABLE COWPEA
11	IT90K-277-2	STRIGA SUSCEPTIBLE
12	IT98K-452-1	STRIGA RESISTANT

Morphological Analysis

Morphological Studies

The morphological evaluation was carried out using a modified method of Padulosi *et al.* (1993). Quantitative and qualitative assessment of the parent plant that produced the hybrids was carried out and include the following features: Plant habit, Plant height, Terminal leaflet shape, Terminal leaflet length, Terminal leaflet width, Terminal leaflet/width ratio, Leaf petiole length, Terminal leaflet petiole length, Leaf rachis length, Terminal leaflet base shape, Terminal leaflet top shape, Pigmentation (plant parts and intensity), Hairiness of plant, Number of branches per plant on main stem, Total number of nodes on main stem, Pod position within canopy, Pod length, Pod width, Pod stripes, Total number of pods per peduncle, Total number of pods per plant, Total number of leaves per plant, Seed length, Seed width, Seed thickness.

The evaluation was done with naked eyes, numbers of plant parts were determined by counting and measurements were taken with the use of a ruler and vernier caliper.

DATA ANALYSIS

The values of both quantitative and qualitative morphological characters obtained were used to compute pair wise distance (similarity) matrices using sequential, hierarchical and nested (SAHN) clustering option of the NTSYS-pc version 2.02j software package (Rohlf, 1993). The program generated dendrogram, which grouped the test lines on the basis of Nei genetic distance (Nei, 1972) using unweighted pair group method with arithmetic average (UPGMA) cluster analysis. The groupings were used to determine the genetic diversity of each of the genotypes.

Breeding Experiment

The research was carried out in a screen house within the Botanical Garden of the University of Lagos. The screen house was to protect the plants from insect and other forms of pest attack.

The plants were crossed using emasculation and bud pollination methods. This was done when the flower bud ready for emasculation had attained its maximum unopened size. The bud selected for emasculation was firmly held at the base with gloves to prevent contamination and to prevent the fragile basal attachment between the bud and raceme. A finely pointed forcep was used to make a cut at the center of the unopened size bud and this expose the reproductive organ of the plant. Since petals mature before the stamens, the immature stamens are removed with the help of the forcep to prevent self pollination. The forcep is dipped into 70% ethanol regularly in other to maintain aseptic conditions and also to prevent the pollination of a newly emasculated bud with pollen from a previous cross.

Bud pollination is an act in which a mature flower is taken and the inner most petals are removed to expose the mature pollen. The mature pollen of our choice is then dusted on the stigma of the emasculated buds in order for pollination to occur. The pollinated buds are then left open and tagged.
The crosses made were such that different strains were crossed with each other to see if they are compatible since they are from the same species. Reciprocal crosses were also made.

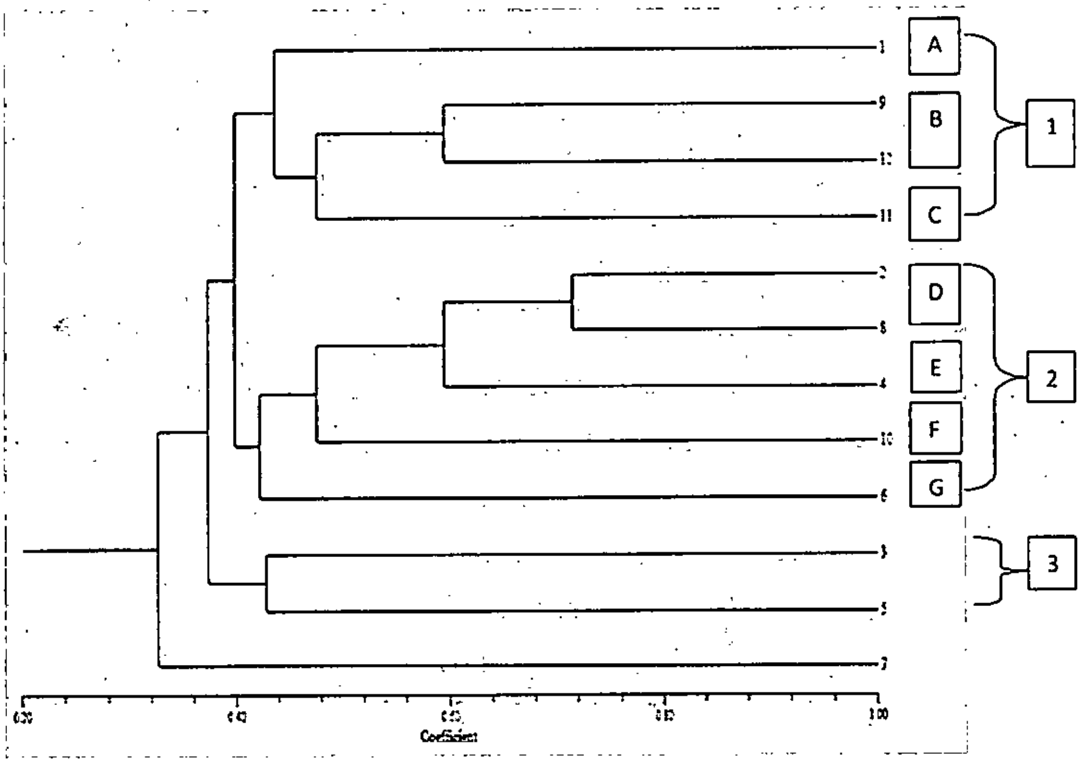
RESULTS

A total of twenty six morphological characters which included eight qualitative and eighteen quantitative characters were compared among the genotypes. The dendrogram reveal that genotype IT845-2246-4 (Bruchid resistant) is a very distinct strain from all others, since it did not form any cluster with any of the genotypes except at 0.20 up to similarity of coefficient 0.32. The dendrogram also shows that at truncated line 0.40 similarity coefficient, three distinct clusters were observed. The first cluster include genotype IT98K-205-8 (Drought tolerant), IT97K-499-35 (Striga resistant), IT98K-452-1 (Striga resistant) and IT90K-277-2 (Striga susceptible), second cluster include genotypes IT98KD-288 (Drought tolerant), (IA4B45)244 (Vegetable cowpea), IT81D-994 (Bruchid resistant), (IA4B55)257 (Vegetable cowpea) and TVX 3236 (Bruchid susceptible) and the third cluster include just genotypes IT86D-719 (Drought susceptible) and Ife Brown (Bruchid susceptible).

The first cluster produced three sub-clusters which are A, B and C, with A having only one genotype (1), B having two genotypes (9 and 12) and C having only one genotype (11). Cluster II produced four sub-clusters which are D, E, F and G with D having two genotypes (2 and 8), E, F and G having one genotype each 4, 10 and 6 respectively.

At a very high coefficient of about 0.72 it was observed that genotypes 2 and 8 are in the same cluster.

Figure 1: A UPGMA dendrogram showing the relationship among *Vigna* strains used in this study based on both qualitative and quantitative data.



KEY:
1 = IT98K-205-8 (DROUGHT TOLERANT)
2 = IT98KD-288 (DROUGHT TOLERANT)
3 = IT86D-719 (DROUGHT SUSCEPTIBLE)
4 = IT81D-994 (BRUCHID RESISTANT)
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6 = TVX 3236 (BRUCHID SUSCEPTIBLE)
7 = IT845-2246-4 (BRUCHID RESISTANT)
8 = (IA4B45)244 (VEGETABLE COWPEA)
9 = IT97K-499-35 (STRIGA RESISTANT)
10 = (IA4B55)257 (VEGETABLE COWPEA)
11 = IT90K-277-2 (STRIGA SUSCEPTIBLE)
12 = IT98K-452-1 (STRIGA RESISTANT)

Breeding Compatibility

A total of 102 crosses were made between different strains of cowpea in other to see if they are compatible with each other. These crosses range from crosses between drought tolerant and susceptible, bruchid resistant and susceptible, striga resistant and susceptible and also several crosses were made between different traits e.g. striga and drought, vegetable and bruchid, bruchid and striga, drought and bruchid.

DISCUSSION

Morphological Study

Before now many researchers have used morphological traits to characterise cowpea such as plant pigmentation, plant height, pod traits, seed traits etc. These traits were all found to be of great importance to assess level of genetic variability and have lead to a better classification of cowpea species (Apte *et al.*, 1987; Fery and Dukes, 1994;

Karkannavar *et al.*, 1991; Ogunbodede, 1988; Uguru and Uzo, 1991; Fawole, 1988; Roquib and Patnaik, 1990).

Emebiri (1989) also characterized cowpea cultivars using their flower size and style length, and reported that both characters were highly heritable. As in previous studies, morphological traits (quantitative and qualitative) evaluated in this studies also proved that they are still valuable tools for cowpea genetic diversity studies.

The cluster analysis substantiated the existence of high level of diversity among the twelve genotypes for the morphological traits studied. The clustering pattern shows that the genotype with accession number IT845-2246-4 (bruchid resistant) is genetically more diverse from the other genotypes.

However, at a very high similarity coefficient of about 0.72 there exist relationships between genotypes 2 and 8 which are IT98KD-288 (Drought tolerant) and (IA4B45)244 (Vegetable).

This means that even though both of them are extremely distinct types of genotype they still have very high genetic inter-relationship between them.

Cowpea Breeding

Based on the natural concept that closely related species interbreed with each other, all the genotypes used in this study are from the closely related species and also belong to the same sub-species which are supposed to be cross compatible i.e. gene exchange should be easily achieved. However, the fact that high failure rate was recorded in the crosses made suggests the existence of some barriers and limitations to gene flow and that gene transfer between different genotype of *Vigna* is not easily achieved (Fatokun, 1991).

The unsynchronized nature of flowering observed among the various genotype used is one of the limitations observed. For example, all the genotypes flowered earlier except for the vegetable genotype, out of which only vegetable strain with accession number (IA4B55)257 flowered but vegetable strain with accession number (IA4B45)244 did not flower throughout the experiment so there was no cross made with it. Consequently, the cross compatibility of this genotype could not be studied.

Another limitation might be barrier that may hinder successful fertilization and propagation of hybrid embryos which could have prevented the success of most of the crosses made. These sexual barriers result from incompatibility. Successful fertilization could have been prevented by factors such as pollen-pistil incompatibility (Ogundipe and Ogukanmi, 2000) which could have resulted in failure of foreign pollen to germinate and penetrate the stigma or failure of foreign pollen tube to penetrate the style. Such prefertilization barriers arise from the breakdown in the cascade of inter- and intracellular events and genetic programs necessary for the successful completion of the various stages ultimately lead to successful fertilization. Furthermore, post fertilization barriers which might be as a result of the absence of endosperm, failure of the endosperm to develop, breakdown and abortion of flowers could have hampered the success of some of the crosses made.

Only 1 out of 4 reciprocal crosses between bruchid resistant and bruchid susceptible was successful. Between striga resistant and striga susceptible reciprocal crosses the success rate is about 10%. Seven per cent success rate was recorded between drought tolerant and drought susceptible reciprocal crosses. Crosses were also attempted between different strains e.g. Striga x Bruchid, Drought x Vegetable, Striga x Drought, Striga x Vegetable, Bruchid x Vegetable, Bruchid x Drought and the result is about 16.5%.

The unidirectional nature in drought strains which success was only observed when the drought tolerant was used as the female parent could arise from pollen-pistil incompatibility. This observation has been reported by Dana (1966), Ahn and Hartmann (1978), and Ogundipe and Ogunkanmi (2000). Ogundipe and Ogunkanmi (2000) suggested a link between pollen size, pollen tube growth rate and pistil length in a way that short pollen tube from small sized pollen are not able to grow through long pistils of other species to achieve fertilization. Nucleocytoplasmic interaction in reciprocal combination has also been suggested in unidirectional cases (Chowdhury and Chowdury, 1997; Chen *et al.*, 1983).

The bidirectional failure observed in 5 out of 8 of the F_1 hybrids are in agreement with those made by Fatokun (1991) who failed in all attempts to obtain F_1 hybrid from crosses between *V. vexillata* and various cultivated and non cultivated cowpea. But in this research the success rate is about 2.67% F_1 hybrids resulting in them producing F_2 hybrids for further studies.

The success recorded implies the possible transfer of the gene(s) into the hybrid seeds. These transfer of gene(s) may be a means of improving the grain yield of cowpea in Nigeria and the world in general.

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