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**Effect of Crossability in Improving Some Selected Cowpea (*Vigna*
Unguiculata [L.] Walp) Varieties**

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ABSTRACT

The studies on the effect of crossability in improving some selected cowpea (*Vigna unguiculata* [L.] walp) varieties (IT87D941 - I, IT97K 499 - 35 and Dankurmi) was carried out in the screen, Institute for Agricultural Research (IAR), Ahmadu Bello University. Seeds of three cowpea varieties were sown in a Completely Randomized Design. Pollen viability studies and cytological study were conducted. The result of the study showed that fertile hybrids were produced which were vigorous and intermediate between the parent varieties with respect to many morphological characteristics. Meiotic analysis of parents and F₁ hybrids revealed normal meiosis with 11 bivalents and normal viable pollen were found in high percentage ranging from 90% to 98% in both parents and F₁ hybrids. It is concluded that fertile hybrids were produced through cross combinations. Hybridization is quantitatively an effective method to improve cowpea genotypes using quantitative traits. This study recommended that hybridization using cowpea varieties with high receptivity for pollen due to high fertility and compatibility could improve local varieties with low pollen contents which will in turn improve the quality and quantity of cowpea genotypes (varieties).

Key words: cowpea, pollen, segregation, varieties, viability

1.0 INTRODUCTION

Cowpea (*Vigna unguiculata*) is an essential grain legume in tropical and sub - tropical regions. It provide inexpensive proteins, feeding people and their livestock (Singh and Adu - dapaah, 1998). It therefore contributes significantly to food security in the African continent. Cytological studies revealed that cowpea is a diploid plant species (2n = 22) (Pasquet, 1999). This legume according to Pasquet (1999) is composed of cultivated cowpea (ssp. *unguiculata*) and 11 wild / weedy forms. The wild / weedy forms of cowpea are only encountered in the African continent (Pasquet, 1999). Cultivated plants and wild relatives represent an interesting system from agricultural and evolutionary points of view.

A drought-tolerant and warm-weather crop, cowpeas are well-adapted to the drier regions of the tropics, where other food legumes do not perform well. It also has the useful ability to fix atmospheric nitrogen through its root nodules, and it grows well in poor soils with more than 85% sand and with less than 0.2% organic matter and low levels of phosphorus (Singh, 2003). The history of cowpea dates to ancient West African cereal farming, 5 to 6 thousand years ago, where it was closely associated with the cultivation of sorghum and pearl millet (Davis *et al.*, 1995). Cowpea is therefore one of the most ancient crops known to man, with its center of origin and subsequent domestication being closely associated with pearl millet and sorghum. Now it is a broadly adapted and highly variable crop, cultivated around the world primarily as a pulse, but

also as a vegetable (both for the greens and the green peas), a cover crop, and for fodder (Quinn, 1999). Cowpea is an important source of dietary protein and nutritious fodder in the semiarid tropics, particularly in West and Central Africa. The protein in cowpea seed is rich in the amino acids, lysine and tryptophan, compared to cereal grains; however, it is deficient in methionine and cystine when compared to animal proteins. Therefore, cowpea seed is valued as a nutritional supplement to cereals and an extender of animal proteins (Davis *et al.*, 1995).

Most local Cowpea varieties are late maturing and dwarf in height and are generally affected by photoperiod and production rate is at local subsistence level. There is the need for improving these varieties through hybridization of quantitative traits using improved varieties due to the frequent use of the local varieties by farmers. Beside these, the massive increase in price of fertilizer coupled with economic melt-down of the recent years has made the purchase of Nitrogen fertilizer out of the scope of local farmers. Therefore the need for growing economic crops that have less demand for Nitrogen deemed necessary. As a legume fixes its own nitrogen, cowpeas do not need nitrogen fertilizer (Quinn, 1999). Cowpeas do fix nitrogen without the addition of Rhizobium, as long as these bacteria are present in the soil from previous soyabeans cultivation.

The important role of hybridization on the plant populations is well documented (Hugues *et al.*, 2007). Gene flow and natural hybridization between plants species are established to be of significant consequences in the evolution and ecology of the plant species (Arnold, 2004). It can also result to the evolvement of the new plant species (Rieseberg *et al.*, 2003). With the concern of bio safety for genetically modified crops, there is progressively more and more interest in crop \times wild and wild \times crop hybridization. This simply because gene flow can be an avenue for transgene escape (Ellstrand and Holfman, 1990). To understand the dynamic of hybridization and introgression within *Vigna unguiculata*, three wild subspecies with the cultivated cowpea and estimated one main

component of fitness of offspring that is the pollen viability.

Several staining techniques have been used to estimate pollen viability in plant species. Aceto carmine stain (Asghari, 2000), Potassium - Iodine stain and Alexander's stain (Asghari, 2000) are the most widely used staining techniques for pollen viability estimation. The central principal of these reactions is due to the affinity of some acid in plant cells with basic dyes, colouring pollen (Asghari, 2000). Data on fertility of pollen is an important means to differentiate the potential hybrid and parental plant. The level of hybrid viability might provide a few signals of the extent of heritable connection among its parents. Therefore, data on pollen viability for any plant species is essential for plant breeding. Karyotypic studies of a several wild species of the genus *Vigna* were examined for their chromosome numbers and mitotic metaphase plate configurations. The results obtained revealed the presence of somatic chromosome numbers of 20 and 22 in a number of wild African species and one from East Africa (FrahmLiveld, 2004). Thirty six cultivars of *V. unguiculata* and *V. sesquipedalis* of various tropical and subtropical sources were investigated, together with wild or spontaneous types of *V. unguiculata* and some closely allied forms. The diploid chromosome numbers of 22 and 24 have been mentioned (Frahm Le Liveld, 2004).

Mukherjee (1968) studied the pachytene chromosomes and observed that the 11 bivalent complement consisted of 1 short (19 μ m), 7 medium (26-36 μ m), and 3 long (41-45 μ m) chromosomes. He noted that the chromomeres were not distributed uniformly along the arms. But while studying induced tetraploid forms of *V. unguiculata*, Gottschalk and Wolff (2012) reported marked cultivar differences in morphologic traits, pollen sterility, fruit setting, and meiotic irregularities including the formation of quadra, trivalents, and univalents and presence of Laggards and unequal distributions in the anaphase stage. They concluded that pollen sterility was the factor most limiting to the development of commercially tetraploid cultivars. Venora and Padulosi (1997) carried out a karyotypic analysis of mitotic Chromosomes of 11

Wild taxa of *Vigna unguiculata*. From a Karyological view point however, little is known about *Vigna* species mainly because of the extremely small size of their chromosomes.

This paper aimed at studying the effect of crossability in improving some selected cowpea (*vigna unguiculata* [L.,] walp) varieties (IT87D941 - I, IT97K 499 - 35 and Dankurmi). The objective of this paper is to determine the effect of Segregation and Pollen Viability in the improvement of some selected quantitative traits in three Cowpea varieties.

2.0 MATERIALS AND METHODS

2.1 Study Site

The Pollen Viability studies were conducted at the screen house of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. IAR is located at the latitude 11° N and longitude 11° 40' E and on altitude 660m above sea level.

2.2 Sources of the Seeds Genotypes

Seeds of three cowpea varieties: IT87D 941-1, IT97K 499-35 and Dankurmi were collected from the Institute for Agricultural Research, Ahmadu Bello University, Zaria.

2.3 Experimental Design

Completely Randomized Design was used. The Seeds of the three cowpea varieties (IT87D 941-1, IT97K 499-35 and Dankurmi) were sown in Plastic pots and are arranged with six replications.

2.4 Cytological Study

Young flower buds were collected from randomly selected plants of the three genotypes (varieties) and fixed into separate vials containing carnoy's fluid (1:3:6 glacial acetic acid, chloroform, absolute alcohol) (Choudhary and Joshi, 2000) for meiotic studies. Meiosis was studied using pollen mother cells (PMC's) from flower buds collected by the arcetoorcein method (Choudhary and Joshi, 2000). Anthers from the three plant genotypes (varieties) were fixed in carnoy's fluid (glacial acetic acid, ethanol, chloroform 1:3:6). After 24 hours at room temperature, it was transferred to 70% absolute alcohol and stored at 4°C until visualize under

microscope. For analysis, fixed anthers were stained in arcetoorcein (2g orcein dissolved in 300ml of 45% acetic acid at 60°C (Choudhary and Joshi, 2000). A clean cover slip was used to crush the anthers by gentle tapping on the slide. Photomicrographs were taken from the temporary smeared preparations using the resolution objectives (×10) of a compound microscope. For pollen viability, ripe but undehisced flower buds were collected. Anthers were removed from flowers and stained with 1% arceto-orcein for observation under a microscope. Pollen (male) viability was indirectly estimated by considering round and well stained pollen grains as viable while those that were either abnormally small or large and stained only partially or not at all were counted non-viable (Adu-Dapaah *et al.*, 1999) based on differential staining of viable and non-viable pollen grains (Tello *et al.*, 2018). The viable and non-viable pollen grains were counted on three slides, at ten locations per slide. Pollen viability was expressed in percentage of total pollen and percentage pollen viability was calculated from stained pollen/total pollen counted X 100 (Odeigah *et al.*, 1996). The female viability (seed setting ability) of the parental types was also recorded (Odeigah *et al.* 1996) using seed setting in the crossability study.

2.5 Data Analyses

Data for pollen viability were subjected to one way Analysis of Variance (ANOVA) at 5% level of significance. Duncan's Multiple Range Test (DMRT) was used to separate the means. Chi - Square was used to determined the F₁ and F₂ with the three cowpea varieties (IT87D941 - I, IT97K 499 - 35 and Dankurmi) using SAS V. 9.2 (2008).

3.0 RESULTS AND DISCUSSIONS

3.1 Segregation Pattern for Plant Height and Pod Length

The segregation patterns for plant height and pod length in different populations derived from the crosses between the three varieties, IT87D, 941-1 IT 97K 499-35 and Dankurmi are presented in Tables 1a and 1b. All the F₁ plants of Dankurmi were short while all the F₁ plants of IT87D 941-1 as well as the plants of IT97K – 499-35 were tall. The mean plant height of Dankurmi was 19.7 cm while that of

IT87D – 941-1 was 41.0 cm and that of IT97K 499-35 was 42.7 cm. The F₁ plants were close to the tall parents in height, with a mean plant height of 38.0 cm indicating dominance of the tall varieties (IT87D 941-1 and IT97K 499-35). The F₂ Population segregated into 33 tall to 9 short plants giving a close fit to a 3 tall: 1 short ratio. The backcross F₁ plants involving Dankurmi segregated into 1 tall to 1 short giving a relative 1:1 ratio even though back cross gave very small population size. All the plants of the backcross involving IT87D 941-1 as well as the backcrosses involving IT97K 499-35 were tall with mean plant height of 39.5 cm. These results revealed that plant height was controlled by a single dominant gene.

The mean pod length of the short pods was 9.1 cm while the mean pod length of the long pods was 15.5 cm. The F₁ plants had pods close to the short pods in length, with a mean pod length of 10.9 cm indicating dominance of the short pods over the long pods. The F₂ population segregated into 29 short pods to 13 long pods giving a close fit to the ratio of 3:1 Short/Long pods. The backcross F₁ plants involving the short podded Dankurmi segregated into 1 short pod to 1 long pod giving a relative 1:1 ratio (even though population size is small). All the 2 plants of the backcross involving the long podded (IT87D941 -1 and IT97K 499-35) were short podded with a mean pod length of 11.2cm. The segregation pattern for pod length and plant height showed dominance of the short podded plants and also dominance of the tall plants. The F₂ segregation for both the pod length and plant height is in consonance with works done by Ishiyaku and Singh (2001) involving F₂ segregation in plant height in cowpea. Singh and AduDapaah (1998) in F₂ segregation for normal to partial sterile mutant in cowpea. Oluwatosin (2002) also obtained a good fit in F₂ segregation in a study involving inheritance of genes for leaflet shape and leaflet shape modifier in cowpea. In studies involving inheritance of genes controlling seed coat color in cowpea, Oluwatosin (2002) obtained a similar result in the F₂ generation indicating that a single gene differentiated the two parents. The back cross data is similar to the work of Ishayaku and Singh (2001) in backcross data in cowpea plant height.

3.2 Pollen Characteristics and Cytology

Cytological studies of the parents and F₁ showed that they exhibited normal chromosome pairing at pachytene stage and during diakinesis and metaphase-I (Plate I- IV). Eleven bivalents were always observed with predominance of the ring bivalents and rare occurrence of rod bivalents. Anaphase-I showed regular separation (dysjunction) of chromosomes with equal number of chromosomes to each pole. No higher chromosome associations were noticed in the parents and F₁ generation. Cytological investigation of pollens was presented in Table 2. The pollen grains from the three cowpea varieties and the resultant F₁ hybrids appeared normal in size (Plate V - VIII) but the mean percentage of stained pollen grains was 97.7% in the parent varieties and 92.6 % in the F₁ hybrids respectively. The percentage normal pollen viability of stained pollen grains was 97.1% for Dankurmi compared to 98.3% for IT 97K 499-35 and 97.9% for IT 87D 941-1. Apparently, the pollen viability in the local variety Dankurmi was less than that of the improved varieties in the F₁ hybrids. The F₁ IT97K 499-35 X Dankurmi had the highest percentage pollen viability of 95.7% while the F₁ IT87D 941-1 X Dankurmi had the lowest percentage pollen viabilities of 90.0%. The Meiotic analysis of parental genotypes and hybrids exhibited the presence of 11 bivalents in the genotypes (varieties) studies. This is in consonance with the work of Singh (2005) on cowpea and Reddy *et al.* (2000) on pigeon pea (*cajanus*). Bivalent association in cytological studies is an indicator of genetic homology between the respective pairs of chromosomes. The variation obtained in some morphology and vegetative characteristics of the cowpea genotypes (Varieties) used as parents in the study may be due to physiological reasons or influence of cytoplasm rather than the non-homology between the nuclear genes of the genotype. Normal Meiosis in most diploid species of *Helianthus* Species was reported by Sexena *et al.* (1998) and Geogieva- Todorova (1990). High pollen viability was observed in parents and F₁ hybrids. This is in consonance with the work of Singh and AduDapaah (1998) who reported high pollen viability in normal cowpea plants. Reddy *et al.* (2001) also reported high pollen fertility in

pigeon pea cultivar. Atlagic. The high pollen viability observed in the parents and F₁ hybrids may be due to the occurrence of normal meiosis in the pollen mother cells. The variability in pollen could be due to reasons other than gross chromosomal abnormalities. It is assumed that the chromosome homology between IT87D941-1 and IT97K499-35 was much greater than that between Dankurmi and

either of IT87D 941-1 or IT97K 499 – 35 and so had a higher compatibility, this is in line with the findings of Adamuet *al.* (2001). The expression of a given character is therefore the reflection of a relatively small amount of the total heritable material of a plant often obscured by the environment as stressed by Van Hintum (1992).

Table 1a: Segregation Pattern For Plant Height And Pod Length In Populations Derived From Crosses Involving Three Selected Cowpea Varieties

Cross and generation	Number of plants			Tested Ratio	Chi-square	Probability
	Tall (>26cm)	Short (<25.5cm)	Total			
Dankurmi	0	0	15			
IT 87D 941-1	18	0	18			
IT 97K 499-35	17	0	17			
F ₁	35	0	35			
F ₁ X Dankurmi	1	1	2	1:01	1	>0.25
F ₁ X IT87D 941-1	2	0	2			
F ₁ X IT87K 499-35	2	0	2			
F ₂	33	9	42	1:03	0.27	>0.50

Table 1b: Segregation Pattern Forplant Height And Pod Length In Populations Derived From Crosses Involving Three Selected Cowpea Varieties

Cross and Generation	Number of plants			Tested Ratio	Chi-square	Probability
	Short Pod	Long pod (>12cm)	Total			
Dankurmi	12	0	12			
IT 87D 941-1	0	12	12			
IT 97K 499-35	0	12	12			
F ₁	24	0	24			
F ₁ X Dankurmi	1	1	2	1:01	1	>0.25
F ₁ X IT87D 941-1	1	0	1			
F ₁ X IT87K 499-35	1	0	1			
F ₂	29	13	42	1:03	0.8	>0.25

Table 2: Pollen Viability Of Selected Cowpea Varieties

Genotype	% Pollen Viability
IT 87D 941 – 1 (A)	97.9
IT 97K 499-35 (B)	98.3
Dankurmi (C)	97.1
F ₁ (A X B)	92.2
F ₁ (A X C)	90
F ₁ (B X A)	92.4
F ₁ (B X C)	95.7

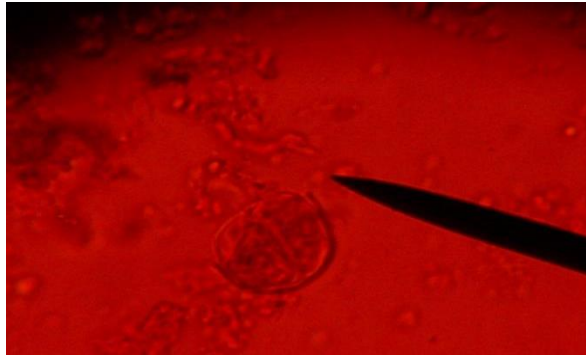


Plate I: Anaphase I In Variety It 97k 499-35

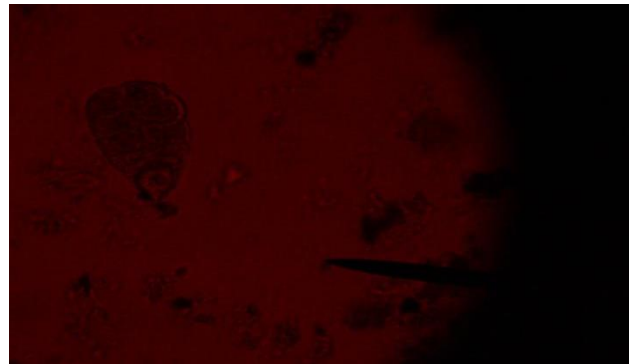


Plate II: Metaphase I In Variety Dankurmi

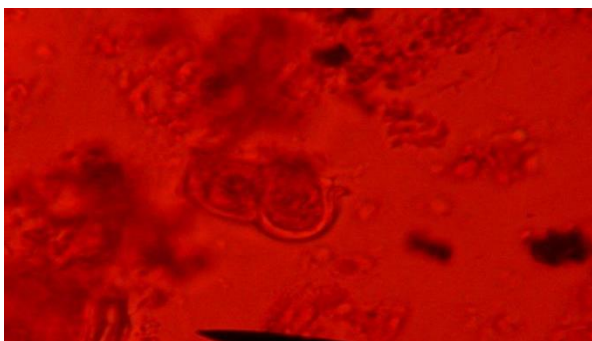


Plate III: Anaphase I In Variety It 87d 941-1

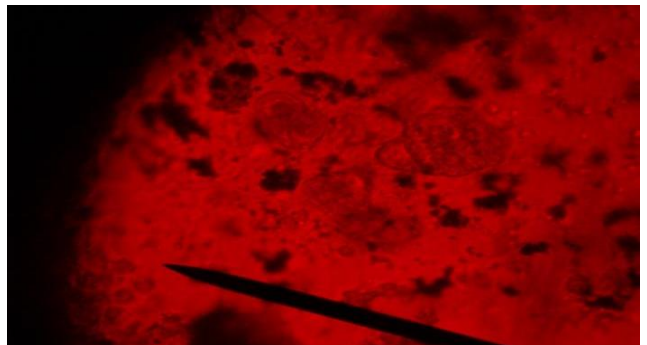


Plate IV: Metaphase I In F₁ It87d 941-1 X Dankurmi

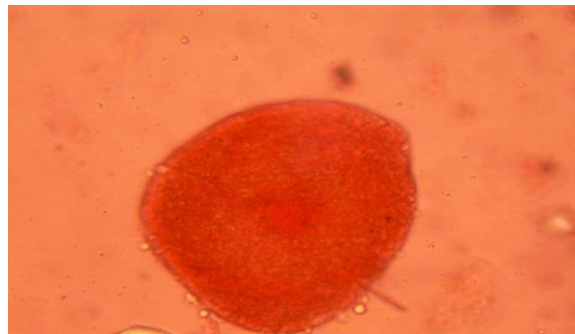


Plate V: Well Stained And Viable Pollen In Variety It97k 499-35

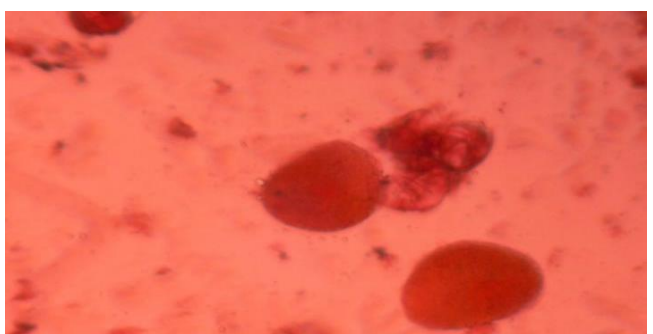


Plate VI: Well Stained And Viable Pollen In F₁ It87d 941-1 X It97k499-35

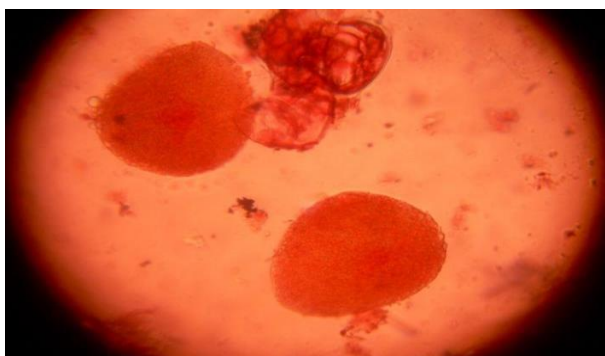


Plate VII: well stained and viable pollen in It8787d 941-1



Plate VIII: well stained and viable pollen in variety Dankurmi

4.0 CONCLUSIONS

Fertile hybrids were produced through cross combinations. Hybridization is quantitatively an effective method to improve cowpea genotypes using quantitative traits. The seed set data indicated that cross compatibility was lower in crosses involving Dankurmi, which may be due to low pollen contents and the high seed set in IT87K 499-35 suggest high receptivity for pollen. This may be due to high fertility and compatibility. The findings of this research concludes that cross combination through the use of hybridization would improve the quality and quantity of genetic traits which would in turn improve the quality and quantity of cowpea genotypes (varieties). Hybridization using cowpea varieties with high receptivity for pollen due to high fertility and compatibility could improve local varieties with low pollen contents which will in turn improve the quality and quantity of cowpea genotypes (varieties).

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