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Breeding for long pod trait in Cowpea (*Vigna unguiculata* L. Walp)

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ABSTRACT

In this study, a cowpea breeding programme was set up to develop varieties with long pods that may combine early maturing and pest resistance. The strategies were to combine the early maturing characters of cultivated cowpea (Vigna unguiculata ssp. unguiculata cv-gr unguiculata) with long pod characters of yard long bean (V.unguiculata ssp. unguiculata cv-gr sesquipedalis) genotypes. Efforts were also made to cross pest resistance of wild cowpea V.unguiculata ssp. dekindtiana var. pubescensor V.unguiculata ssp. dekindtiana var.protracta with the yard long genotypes. Out of sixty one crosses attempted, only 1 success was recorded. This revealed a very low success rate of the crosses (2.94%), which is probably due to specific cross combinations, genetic divergence and environment. The only successful cross was between V. unguiculata ssp. unguiculata cv-gr unguiculata and V. unguiculata ssp. unguiculata cv-gr sesquipedalis and this was achieved when the former was used as a female parent. Morphological evaluation of the parents from the successful cross revealed that the cultivated cowpea lines had larger leaves while the long pod cowpea had conspicuously longer pods. However, the F_1 seeds from the successful hybrid failed to germinate when planted for hybrid testing.

Keywords: *Vigna unguiculata, breeding, long pod, hybrid*

INTRODUCTION

Cowpea is a highly nutritious grain legume that is important to the livelihoods of millions of people living in the tropics and subtropics. It is widely cultivated for its leaves, pods and seeds (Ng, 1992). Being highly rich in protein and carbohydrate, cowpea is used as a nutritious fodder for livestock while its pods, leaves and grains are eaten as vegetables (Singh *et al*., 2003; Hall *et al.*, 1997; Nielsen *et al*., 1997).Sales of cowpea products serve as a source of income for rural households in the Western part of Africa especially northern Nigeria (Langyintuo *et al*., 2003).

Cowpea shows considerable variation in pod habit. Some varieties of cowpea (*V.unguiculata* ssp. *unguiculata* cv-gr *sesquipedalis*) have remarkably longpods that can grow between 35 and 75cm (Ehlers and Hall, 1997). These varieties are commonly known as yard long cowpea and are majorly grown for their long succulent pods which are eaten in fresh form as vegetables (Singh *et al*., 2003).

Most breeding programs are aimed at improving the economic worth of crop plants and one way by which this can be achieved is by improving the grain yield of such crops (Chahal and Gossal, 2002). Grain yield is strongly correlated with pod length and number of pods per plant. Thus, increase in pod length and number of pods results in a corresponding increase in yield and subsequently increase in protein content (Hazra *et al.*, 2007; Ogunkanmi *et al*., 2007).

Wide crosses have been widely used to transfer agriculturally important characters, such as pest resistance, from one gene pool to another within the genus *Vigna* (Ehlers and Hall, 1997; Hall *et al.*, 1997; Singh *et al*., 1997). Attempts have also been made to transfer genes between the cultivar group or cv-gr *sesquipedalis* (yard long cowpea), cv-gr unguiculata (cultivated cowpea) and wild cowpea within the subgenus *Vigna*, however low success has been reported (Kongjaimun *et al*., 2012; Hazra *et al.*, 2007; Fatokun *et al.*, 1997). This strongly suggests the existence of sexual barriers preventing such intrageneric gene transfer (Fatokun *et al.*, 1997).

In this research therefore, wide crosses were made with the aim of creating hybrid varieties that possess long pods with plenty seeds which will help to improve grain yield in cowpea breeding and also help in further cowpea breeding programs such as marker assisted selection.

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MATERIALS AND METHODS

Three or fourviable seeds from each accession of the three types of cowpea, *Vigna unguiculata* ssp. *unguiculata*cv- gr*sesquipedalis* (yard long cowpea), *Vigna unguiculata* ssp. *unguiculata* cv-gr *unguiculata* (cultivated cowpea), and *Vigna unguiculata* ssp. *Dekindtiana* var.*protracta* (wild type), designated 1, 2, and 3, were planted in separate plastic flowering pots (25 cm in diameter) which were pre-filled with loam soil. The planting pots were already placed in a screen house built at the Botanical Garden of the University of Lagos, Nigeria. Each accession was planted in three replicates.

Environmental conditions in the botanical garden were as follows: temperature of *27°C + 2°C (day) and 18°C + 1°C (night), a relative humidity of 82% + 2% (day) and 78% +2% (night), and a photoperiod of 12hr light supplied by sunlight.*

Plants were crossed using emasculation and bud pollination methods. Cowpea study under the screen house environmental conditions showed that a flower bud ready for emasculation had attained its maximum unopened size and had started to pale slightly from the normal deep green color (Ogunkanmi *et al*., 2007). Bud selected for emasculation was firmly but gently held at the base so as to prevent any form of stress at the fragile basal attachment between the bud and raceme. Finely pointed forceps was to used make a cut at the center of the straight edge of the selected bud and about two-thirds of the width of the bud was removed to expose the reproductive organs.

The immature stamens were removed with the forceps to prevent self pollination. Forceps was dipped into 70% ethanol intermittently after successive crosses so as to maintain aseptic conditions and also prevent the mistake in pollination of a newly emasculated bud with pollen from a previous cross. Pollens obtained from a mature unpollinated flower were dusted on the stigma of the emasculated buds. Pollinated buds were tagged with small paper tags that carried the detailed information (crosses made, date and time) about a cross. Plants were watered every other day between 8 and 9 am. Weeds were controlled by hand pulling when necessary.

Morphological evaluation involving both qualitative and quantitative assessment of the parent plants used in producing the hybrid was carried out. Parameters such as pod length, terminal length and width of terminal leaflet, petiolule and petiole length, rachis length, and pulvin length were quantitatively measured using a measuring rule while other parameters such as top shape and base of the terminal leaflet were assessed by visual observation.

RESULTS

A: Morphological Assessment

Table 1 shows results obtained from the qualitative and quantitative assessment of leaves and pods of the parent plants that produced the hybrid. The morphological studies of the two parents that led to the emergence of the hybrid revealed that *unguiculata* (TVu 1) had higher mean value in all characters evaluated except for three characters. Leaflet top and base shapes were similar but the pod length in *sesquipedalis* (TVu 223) was more than six times the pod sizes of the *unguiculata*, hence the aim of the work to transfer this trait to the second parent.

Table 1: Morphological attributes of *Vigna unguiculata* ssp. *unguiculata cv-gr unguiculata* (Tvu 1) and *Vigna unguiculata ssp. unguiculata cv-gr sesquipedalis* (Tvu 223).

Characters	***V. unguiculata* ssp. *unguiculata* cvgr. *sesquipedalis***	***V. unguiculata ssp. unguiculata* cvgr. *unguiculata***	**Hybrid**
Terminal leaflet length (cm)	9.64	11.04	DNO
Terminal leaflet width (cm)	5.76	6.74	DNO
Petiolule length (cm)	2.9	3.42	DNO
Petiole length (cm)	9.16	13.26	DNO
Rachis length (cm)	12.2	12.2	DNO
Pulvin length (cm)	0.9	0.72	DNO
Terminal leaflet top shape	Attenuate	Attenuate	DNO
Terminal leaflet base shape	Sub-hastate I	Sub-hastate I	DNO
Pod length (cm)	37	6	DNO

DNO: Data Not Obtained

B. Cowpea Hybridization

Table 2 shows the types of crosses attempted alongside their corresponding frequencies, as well as the number of pods obtained from each cross combination and the results obtained from the hybrid testing.

Out of a total of 61 wide crosses attempted, only a cross between *V. unguiculata*ssp. *unguiculata* cv-gr *sesquipedalis*× *V. unguiculata*ssp. *unguiculata* cv-gr *unguiculata* (Tvu 1× Tvu 223) was successful. The cross was successful only when Tvu 1 (*V. unguiculata* ssp. *unguiculata* cv-gr *unguiculata*) was used as the female parent (See plate 1). The reciprocal crosses attempted failed and all similar crosses attempted on subsequent occasions either resulted in pods that degenerated or ovules that aborted. Hybrid seeds also failed to germinate when planted.

Table 2: Results of wide crosses showing the type of cross made, number of attempts, percentage success and success rate of the hybrid obtained.

Cross combination	**No. of flowers crossed**	**No. of pods obtained (%)**	**No. of seeds sown**	**No. of seeds germinated (%)**	**No. of plants obtained**
1×2	34	1 (2.94)	3	0	0
1×3	27	0	0	0	0

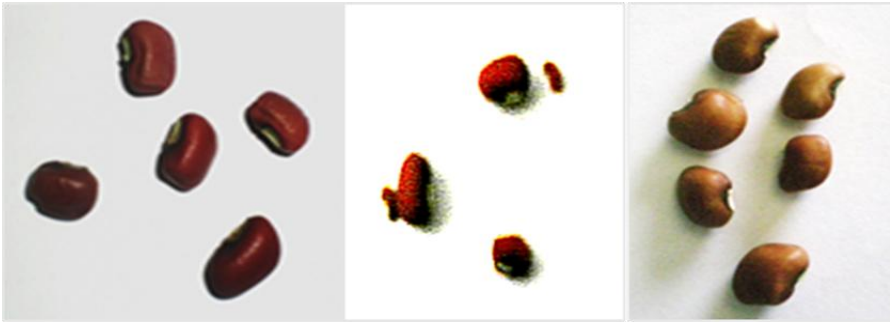

Plate 1: Images of seeds of parent plants and hybrid plant from the Cowpea breeding programme conducted in this study. (a) TVu 1 (b) hybrid between TVu1 and TVu 223 (c) TVu 223.

DISCUSSION

Crosses between genotypes of *sesquipedalis* and *unguiculata* revealed low success (2.94%). Also, crosses between *sesquipedalis* and *dekindtiana* genotypes recorded zero success. Information obtained through personal conversation with Prof MichealTimko of the University of Virginia, USA also agreed to the low success rate obtained in earlier crosses between *V. unguiculata* ssp. *unguiculata* cv-gr *unguiculata* and *V. unguiculata* ssp. *unguiculata* cv-gr *sesquipedalis.*

While the reasons for failures of interspecific crosses in *Vigna* are poorly understood (Chen *et al*., 1983), several factors, such as specific cross combination, genetic divergence and environment, could have influenced the low success rate. Furthermore, pre-fertilization and post-fertilization barriers such as pollen-pistil incompatibility (Chowdhury and Chowdhury, 1977), embryo abortion (Ahn and Hartmann, 1977; Fatokun *et al*., 1997), and hybrid failure and breakdown (Chen *et al*., 1983) could have also been responsible for the low success rates and failure of crosses.

Hazra *et al*. (2007) revealed that partial dominance of short and light pods of *unguiculata* and *cylindrical* over long and heavy pods of *sesquipedalis* genotypes may have also contributed to cross incompatibility or hybrid failure. He suggested that advancement of genotypes of highly heterotic*unguiculata* × *sesquipedalis* or *dekindtiana* × *sesquipedalis* hybrids could have been modified by backcross-pedigree method which proved successful (Hazra *et al*. 2007). The observed failure of germination of the hybrid seeds could have resulted from loss of seed viability which could have been caused by series of factors such as poor seed preservation technique, unfavourable germination conditions, and genetic factors like the presence of lethal alleles in hybrid genotype (Saccardo *et al*., 1992). Egawa (1988) reported that all the 56 seeds from a *V. mungo* × *V. radiata* cross and 5 seeds from a *V. umbellate* × *V. radiata* cross failed to germinate.

Chances of success should therefore be increased by using *in vitro* pollination and fertilization followed by embryo rescue (Fatokun and Singh, 1987; Chen *et al.*, 1977). Pollination methods such as cut-style and style grafting should also be employed alongside the bud pollination technique (Van Tuyl and De Jeu, 1997).

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