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Inheritance of a spontaneous male sterile mutant in cowpea plant population.

O. B. Oluwatosin

Genetics and Molecular biology Research Laboratory, Department of Botany and Microbiology, University of Ibadan, Ibadan, Oyo State, Nigeria.

ABSTRACT: A male sterile plant was observed in a segregating population of a cross between two varieties of cowpea (*Vigna unguiculata* [L.] Walp.), Ife Brown and Sel. 4992. The plant had an exposed stigma, normal sized floral petals and anthers with complete pollen sterility. The inheritance of this spontaneous male sterility that did not appear consistently in other F_2 population of this and other crosses, was studied in crosses involving the mutant and fertile Ife brown and Sel. 4992 varieties. The F_1, BC_1F_1, BC_2F_1, F_2 and F_3 generations were evaluated. The test of allelism between this male sterile line and some previously reported male sterile lines in cowpea indicated that this new mutation occurred at a new locus with respect to those previously reported. The results suggest that this spontaneous male sterility is heritable and is conditioned by a single recessive gene.

Running title : Male sterility in cowpea.
Key words: Cowpea, *Vigna unguiculata,* Male sterility, Inheritance.

INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp) is an important and widely cultivated grain legume in Nigeria. It is an important source of protein and other essential nutrients for a large segment of the populace in most developing countries. Cowpea is normally self-fertilized. However, considerable outcrossing has been reported in natural populations through bees and thrips (Rachie and Roberts 1974).

The male sterile line reported in cultivar Poona by Sen and Bhowal (1962) was observed to be more vigorous, with reduced floral parts and small-undeveloped anthers of a whitish or yellow color. They did not shed pollen even when fully developed nor were fruits produced unless when outcrossed with pollen from fertile plants. Sen and Bhowal (1962) proposed the symbol *ms* for the male sterility. Rachie *et al.*, (1975) described male sterility in the cultivar Prima that was similar to the sterility reported by Sen and Bhowal (1962). The anthers are small, poorly formed and do not produce viable pollen. Outwardly, the flower development is identical with that of a fertile plant. However, without hand or insect pollination they do not produce fruits but remain green and continue growing and flowering as long as condition remain favorable. Ladeinde *et al.*, (1980) studied the allelic relationship between the genetic male sterility reported by Rachie *et al.*, (1975) in TVx 391 and two other male sterile lines, TVx 4-4c and TVu 2920 in selected crosses. They observed that the three sources of male sterility investigated were controlled at three different loci.

MATERIALS AND METHODS

The present study was carried out at the University of Ibadan Botanical Nursery and Screen-house, Ibadan, Nigeria. 180 F_2 plants of the cross between Ife brown x Sel. 4992 were planted in hybridization blocks in the first season of 1995/96. One plant, Sel. M95, with abnormal appearance was observed in the segregating population. The flower had white translucent anthers which, were devoid of pollen grains. The stigma was also exposed, but the sizes of the floral parts where the same as those of normal plants. These observations suggests that the new male sterile line may be different from previously reported ones in cowpea (Sen and Bhowal 1962; Rachie *et al.*, 1975; Ladeinde *et al.*, 1980) which, were reported to have reduced flowers. The inheritance of this mutant phenotype was analyzed in crosses with the parents Ife brown and Sel. 4992. Forty randomly selected fertile F_2 plants were advanced to the F_3 generation. All segregants which failed to produce viable pollen, were classified as male sterile in all the generations tested. The male sterile F_2 plants were kept for further studies. A test of allelism between the male sterility gene in the mutant observed in this study and those previously reported by Ladeinde *et al.*, 1980 were conducted in the screen house and evaluated on the field in the early and late planting seasons of

1998. The crosses listed below were made in the screen house:

- Sel. M95 x Ife brown
- Sel. M95 x Sel. 4992
- (TVx 391 x Ife brown) x (Sel. M95 x Ife brown)
- (TVu 2920 x Ife brown) x (Sel. M95 x Ife brown)
- (TVx 4-4c x Ife brown) x (Sel. M95 x Ife brown)

The origin of the male sterile and normal lines used in this study is presented in Table 1. The male sterile line reported in cultivar Poona (Sen and Bhowal 1962) was not available for this and previous studies (Rachie *et al.*, 1975; Ladeinde *et al.*, 1980), presumably because it is no longer extant.

Table 1: Name, origin and pollen characteristic of the lines used in this study.

Name	Origin	Pollen characteristic
Ife Brown	I. A. R. & T.	fertile
Tvx 391	IITA	sterile
Tvx 4-4c	IITA	sterile
Tvu 2290	IITA	sterile
Sel. 4992	U. I	fertile
Sel. M 95	U. I	sterile

U. I : University of Ibadan, Ibadan, Nigeria.
IITA: International Institute of Tropical Agriculture, Ibadan, Nigeria.
I. A. R. & T.: Institute for Agricultural Research and Training, Ibadan, Nigeria

RESULTS AND DISCUSSION

The cross of the male-sterile mutant and its fertile counterparts were successful, suggesting that the male-sterile plant was female-fertile. The mutant produced about 5 seeds per pod when artificially pollinated. This is however less than the average of 8 seeds per pod when source lines were selfed. In the F_1, twenty plants were grown which were fertile and had normal anther phenotype. The segregation pattern of anther morphology observed in the backcross to the male-sterile parent and the F_2 generation in both crosses (Table 2) indicated that the male-sterile trait was heritable. Good fits were obtained to the 3 normal: 1 mutant and 1 normal: 1 mutant anther phenotypic ratios in the F_2 and backcrosses to the mutant parent respectively. The 24 progenies of the backcross to the fertile parents were male fertile and had normal anther morphology. These results suggest that a single recessive gene governed the male sterility. Similar findings have been reported in *Cajanus cajan* (Reddy *et al.*, 1978, Verulkar and Singh 1997), *Pisum sativum* (Singh and Singh 1995) and *Vigna unguiculata* (Sen and Bhowal 1962; Rachie *et al.*, 1975; Ladeinde *et al.*, 1980).

The male sterile plants were grown in the first (April - July) and second (September - December) planting seasons. The plants expressed male sterility in both seasons suggesting that the gene for the observed male sterility is not affected by the season of planting.

A test of allelism was conducted to compare the behavior of the male sterility gene observed in this study with those previously reported in *V. unguiculata* (Table 3). If the F_1 x F_1 progeny from separate crosses of two male sterile lines with a parent, Ife brown {(e.g. Tvx 391 x Ife brown) x (Sel. M95 x Ife brown)}, segregates 3 fertile to 1 male sterile, then the male sterile sources are allelic. However, if the sources are non-allelic the F_1 x F_1 progeny should be all male-fertile. The absence of male sterile progeny in the F_1 x F_1 progeny of each of the three crosses is consistent with an interpretation of non-allelic male sterility loci. The F_1 segregated at the two male sterile loci producing three different expected segregation results in the F_2: 1/4 all fertile, 1/2 segregating 3 fertile to 1 male sterile, and 1/4 segregating 9 fertile: 7 male sterile in the F_2 generation. The results also showed consistencies among the crosses investigated indicating that the four sources of male sterility were controlled by separate loci and appeared to result from different mutations.

Furthermore, microscopic examination of the developing anther in both the male sterile and the fertile segregants suggested that the archesporium was formed as usual, but the pollen mother cells degenerated at the early prophase stage. Thus, early stages of prophase appeared normal, but meiotic activity did not proceed beyond diakinesis. The nuclei also appeared to be poorly differentiated at all stages of development in cells of sporogenous tissues of male sterile plants.

Sen and Bhowal (1962) designated the male sterile gene in cultivar Poona as *ms-1* while Ladeinde *et al.* (1980) designated the male sterility genes in lines TVx 391, TVu 2920 and TVx 4-4x as *ms-2, ms-3* and *ms-4* respectively. The symbol *ms-5* is proposed for the male sterility observed in this study in conformity with previously assigned symbols for male sterility in cowpea (Sen and Bhowal 1962; Rachie *et al.*, 1975; Ladeinde *et al.*, 1980).

The potential of this male sterile line is being explored in commercial production of hybrid with desirable seed coat colour in cowpea in combination with the branched peduncle cowpea mutant which has been very useful for clarifying gene interactions at the seed coat colour loci (Oluwatosin 2000).

Table 2: Crosses of male sterile mutant x male fertile parents

Crosses and generations	Number of plants		Total	Tested Ratio	X^2	P
	Normal	Male sterile				
P_1 x P_2						
F_1	20					
P_1 x F_1	11	10	21	1:1	0.05	>0.75
P_2 x F_1	24		24			
F_2	116	41	157	3:1	0.1	>0.75
F_3 families						
26S : 13NS			39	2:1	0	>0.95
F_3 pooled (1df)	568	196	764	3:1	0.13	>0.50
Heterogeneity (38 df)					19.76	>0.75
P_1 x P_3						
F_1	20					
P_1 x F_1	13	11	24	1:1	0.17	>0.50
P_3 x F_1	24		24			
F_2	121	37	158	3:1	0.21	>0.50
F_3 families						
27 S : 13 NS			40	2:1	0.01	> 0.95
F_3 pooled (1df)	622	200	822	3:1	0.2	> 0.50
Heterogeneity (39 df)					21.67	> 0.75

P_1 = male sterile line (Sel. M95)*
P_2 = Ife brown
P_3 = Sel. 4992
P = probability
S = Segregating population
NS = Non-segregating population
* : male sterile lines were established when the plants had produced flowers

Table 3: F_2 segregation in a test for allelism of Sel. M95 to existing male sterile lines of cowpea

Crosses	No. of progeny rows**	Number of plants		Total	(X^2)	Probability (d.f =1)	X^2 Hetero-geneity* (d.f)
		Fertile (Normal)	Male Sterile				
(Tvx 391 x Ife brown) x	9	85	59	144	0.45[a]	> 0.50	5.32 (8)
(Sel. M95 x Ife brown)	21	201	65	266	0.04[b]	> 0.75	3.86 (20)
	10	106		106			
(Tvu 2920 x Ife brown) x	8	91	63	154	0.51[a]	> 0.50	5.18 (7)
(Sel. M95 x Ife brown)	21	206	64	270	0.24[b]	> 0.50	10.53 (20)
	11	113		113			
(Tvx 4-4c x Ife brown) x	9	86	61	147	0.32[a]	> 0.50	6.87 (8)
(Sel. M95 x Ife brown)	20	241	85	326	0.2[b]	> 0.50	8.92 (19)
	11	116		116			

* : Heterogeneity between the segregation pattern of the progeny rows
d.f : degree of freedom
**: Seeds were planted on individual plant basis in the F_2
[a]: tested ratio = 9 : 7
[b]: tested ratio = 3 : 1
P = Probability
Sel. M95 = male sterile line. Sel. M95 is maintained from in heterozygous condition from generation to generation.

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Author's address of correspondence: : O. B. Oluwatosin, Department of Botany and Microbilogy, University of Ibadan, Ibadan, Oyo State, Nigeria. Fax : 234-2-8103043, E-mail address : fedesng@yahoo.com