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COMPLETE STERILITY STUDIES IN THREE MUTANTS OF COWPEA (*VIGNA UNGUICULATA* (L.) WALP

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Abstract

Three completely-sterile cowpea mutants IT85D-3625, IT85D-3628, IT85D-3641 obtained from spontaneous mutation and gamma irradiation were characterized. Reciprocal crosses between them and fertile plants failed to set pods. These lines showed significant differences with respect to various traits such as number of pollen grain per anther, anther length and width, plant height, anther indehiscence, unopened flower buds, and premature abortion of pods and seeds. The major cause of sterility was chromosome aberrations. Complete sterility in each of the three lines was conditioned by a simple recessive gene pair. Sterility in each of the three mutants was associated with floral aberrations. The symbols cs_1, cs_2 and cs_3 are being assigned to IT85D-3625, IT85D-2628 and IT85D-3641 respectively. The three mutants were homogeneous with reference to sterility inheritance.

Introduction

Male-sterility is a condition in crop plants in which the male reproductive function is inhibited (Allard, 1960). The efficiency of female reproductive parts in male sterile systems may be impaired or somewhat reduced depending on the mutant (Graybosch & Palmer, 1984). In general, there are two common types of sterility namely completely-sterile (male and female) and male-sterile (Martin & Crawford, 1951; Kaul, 1988).

Chromosome aberations generally result in chromosome imbalance in various microspores. This is a major cause of sterility in plants (Jha & Singh, 1987). Olorode, Fatunle & Adegoke (1982) stated that a combination of cross-over events within and outside the inversion loop may result in complete-sterility. Sinha & Sinha (1980) observed that in reciprocal translocation, adjacent segregation during meiosis I leads to the production of gametes which will be duplicated for some genes and deleted for others resulting in unbalanced gametes and sterility.

Male-sterility in self-pollinating species have proved useful for increasing genetic recombination (Jain & Suneson, 1963; Kaul, 1988) for accelerating recurrent selection (Brim & Stuber, 1973; Ramage, 1977) and for economizing labour and time in hybridization programmes (Rachie & Gardner, 1975; Singh & Ikehashi, 1981; Rao, Devi & Arundhaji, 1990). Completely-sterile mutants are useful for cytogenetic studies and help in the preparation of linkage maps (Kaul, 1988; Rao, Devi & Arundhaji, 1990).

In recent times, breeders of self-pollinating crops have shown considerable interest in increasing the efficiency of breeding methods with a view to improving yield potential and other quantitative characters. Consequently, the search for various types of male-sterility is on the increase (Graybosch & Palmer, 1984). In cowpea, relatively few male-sterility genes have been reported compared to other self-pollinated crops. These include ms_1, ms_2 *cp*, *crpt* (Apparao & Reddy, 1976; Sen & Bhowal, 1962; Rachie *et al.*, 1975). The present authors have been searching and also trying to induce different types of sterility which might be of use in cowpea improvement. In this process, three completely-sterile mutants were discovered.

In general, literature on complete-sterility (male

and female) is scanty. In cowpea, Rachie *et al.* (1975) only mentioned the observation of complete-sterility. The present paper reports three completely-sterile mutants in cowpea.

Experimental

Materials used for the study were three sterile cowpea mutants, IT85D-3625, IT85D-3628 and IT85D-3641. Genotypes IT85D-3625 and IT85D-3628 resulted from spontaneous mutation in a back-cross population derived from TVu 300 × IT82E-60[4], while IT85D-3641 was obtained from the irradiation of an élite cowpea line IT82D-716 with 15 kr. gamma rays. The progenies from normal plants selected from segregating rows of the three mutant lines were grown at IITA, Ibadan, for 2 years to study their mode of inheritance of sterility. At the onset of flowering, plants showing abnormalities such as empty or virtually-empty anthers were tagged as sterile plants. Data were taken on 20 plants for plant height at maturity, petal length and width, number of anthers and anther length. Young buds from sterile plants were fixed in acetic-ethanol (1:3 v/v) fixative and acetocarmine smear were prepared. Pollen grains were mounted in one per cent acetocarmine to determine pollen fertility, at a magnification of × 400 using an ocular micrometer. The number of pollen grains per anther was determined by carefully removing them from the anther lobes with dissecting needles.

Acetocarmine smears were prepared and a glass cover divided into four quadrants placed on the smear. The number of pollen grains in each quadrant was counted and added together to get the total per anther. Female-fertility was tested by crossing 10 randomly selected sterile plants with pollen from fertile plants. Reciprocal crosses were also made by using pollen from sterile plants (when available). Two hundred crosses including reciprocals were made per mutant. Chromosome pairing behaviour at meiosis I was observed in smears prepared from pollen mother cells (from 1-2 mm size flower buds). The smears were mounted in FLP orcein (Olorode, 1974) at a magnification of × 1000 by using a Leitz phase contrast microscope. Photomicrographs of chomosomes were then taken. The frequency of fertile and sterile plants within each segregating progeny row was recorded at maturity. The chi-square test of goodness-of-fit was used to determine the mode of inheritance of sterility. The heterogeneity chi-aquare was used to find out whether the three mutants are homogeneous with reference to sterility inheritance.

Results and discussion

The three mutants were found to be completely-sterile (male and female sterility) since reciprocal

TABLE 1

Mean values for vegetative and floral characters of three cowpea mutants

Mutant	Floral characteristics									
	Plant height at maturity (cm)		Petal length (cm)		Petal width (cm)		No. of anthers		Anther length (mm)	
	F	S	F	S	F	S	F	S	F	S
IT85D-3625	63.60	69.60	2.52	1.40	1.86	1.00	10	9.8	1.49	0.65
IT85D-3628	56.40	54.60	2.70	2.52	2.10	1.80	10	10	1.58	1.10
IT85D-3641	68.60	72.50	2.78	2.14	2.02	1.28	10	11.3	1.60	0.96
Mean	62.67	65.57	2.67	2.02	1.99	1.36	10	10.4	1.56	0.90
SE ±	2.51	5.6	0.12	0.85	0.91	0.61	0.03	0.20	0.29	0.83

F = fertile; S=sterile

crosses between them and fertile plants failed set to pods. The three lines were all determinate in growth habit and remained green at maturity but had differences in either vegetative or floral characteristics (Table 1).

In line IT85D-3625, the number of anthers per flower in the sterile plants was variable, ranging from 8 to 12 with a mean of 9.8 ± 0.8. Sterility in this mutant was associated with flower buds which do not open even when they attained expected size at anthesis. This attribute distinguished IT85D-3625 from the other two mutants. The anther did not dehisce by the time the flowers dropped. In common bean, Wyatt (1984) observed a mutant with indehiscent anthers which is consistent with observations in this mutant. Pollen stainability was zero for the mutant compared to 98.4 per cent for fertile plants (Table 2). Pollen number per anther ranged from 430 to 965 and 710 to 1010 respectively for the mutant and fertile plants, Pollen number and size were generally smaller for the mutant than for the fertile plants (Table 2). This finding agrees with the work reported by Childers & Mclennan (1960). Cytological investigations showed some meiotic aberrations in pollen mother cells (PMCs) of the sterile plants. Mean chomosome-association from 20 PMCs observed for IT85D-3625 was 5.10I + 8.40II, with the number of univalents ranging from zero to 22. Observation of univalents in metaphase 1 of meiosis suggests asymmetrical distribution of chromosomes resulting in imbalance in various microspores. This is a major cause of sterility in crop plants and more so in interspecific hybrids (Patil & Singh, 1976; Hadley & Openshaw, 1980; Fatokun, 1987).

In line IT85D-3628, flower buds from this mutant opened at anthesis and when crossed or selfed, pods were not formed in most cases. In some cases, pod development was initiated, however, these aborted within 3-5 days. Sterility in this mutant was associated with pod aberrations. This distinguishes it from IT85D-3641. When the aborted pods were examined, they contained poorly developed seeds. Pollen stainability for the mutant was 35.5 per cent compared with 98.3 per cent for fertile plants (Table 2). Total number of pollen for the fertile plants was 1873, while the sterile plants had 1070. The average size of the pollen from the fertile plants was 68 μm and 31 μm for the sterile mutants.

Pod abortion and poor development of seeds could be attributed to genetically imbalanced gametes resulting from chromosome aberrations (Fatokun & Singh, 1987) or physiological abnormalities (Kaul, 1988). If pod abortion was due to

TABLE 2
Mean values for pollen characters in fertile and sterile cowpea plants

	IT85D-3652		*IT85D-3628*		*IT85D-3641*	
Parameter	*S*	*F*	*S*	*F*	*S*	*F*
No. of stained pollen	0	1186	380	1841	0	1170
No. of unstained pollen	486	14	690	32	0	16
Percent stained pollen	0	98.4	35.5	98.3	0	98.6
Size of stained pollen (μm)	-	72	-	68	-	75
Size of unstained pollen (μm)	30	40	31	45	-	39
Total number of pollen per anther	628	980	640	963	-	875

F = Fertile; S= Sterile

physiological abnormalities, then embryo rescue technique could be used to enhance seed development. In cowpea, Fatokun & Singh (1987) used embryo rescue to culture hybrid embryos. Mean chromosome associations from observation of 16 PMCs were 4.20I + 8.88II with the number of univalents ranging from zero to 12. The observation of univalents at metaphase I in both IT85D-3625 and IT85D-3628 agrees with the work done by Riley (1996), Hadley & Openshaw (1980) and Fatokun (1987), who attributed the occurrence of univalents at metaphase I to either the absence of synapsis during prophase I or precocious separation of chromosomes which could lead to unequal distribution of chromosomes to the poles. Rees (1961) and Riley (1966) reported that there are some recessive genes which when homozygous, cause the production of univalents at metaphase I.

TABLE 3

Frequency of fertile and sterile plants in segregating progeny rows in cowpea

Mutant	*Frequency of plants*			
	F	*S*	$\chi^2(3.1)$	*P*
IT85D-3625	318	118	0.99	0.10-0.56
IT85D-3628	313	118	0.30	0.10-0.50
IT85D-3641	802	254	0.51	0.10-0.50

F = Fertile; S = Sterile

Mutant plants from line IT85D-3641 had variable number of anthers per flower, ranging from nine to 13 with a mean of 10.3 ± 1.0. Anthers were devoid of pollen grains and indehiscent. There were no meiotic aberrations in line IT85D-3641. Over 90 per cent of the PMCs observed had 11 bivalents. The absence of pollen grains might be due to degeneration of microspores soon after the quarter stage as observed in soybean (Patil & Singh, 1976) and in alfalfa (Childers & Mclennan,

TABLE 4

Heterogeneity chi-square testing agreement among the three cowpea mutants

Mutant	*F*	*S*	$\chi^2(3.1)$	*P*
IT85D-3625	318	118	0.99083	0.10-0.50
IT85D-3628	313	118	1.30008	0.10-0.50
IT85D-3641	802	254	0.50505	0.1-0.50
	DF	$\chi^2(3:1)$ *value*	*P*	
Deviation	1	0.23730	0.50-0.90	
Heterogeneity	2	2.55866	0.10-0.50	
Total	3	2.79596		

1960). This could be an example of genic sterility resulting from differences in gene arrangements in the parental chromosomes (Kaul, 1988).

The sterile plants of all the three mutants continued to grow vegetatively even after flowering. This agrees with reports by Sen & Bhowal (1962) and Rachie *et al.* (1975) in cowpea, who explained that lack of pods on the sterile plants may have allowed the plants to have enough photosynthate for continuous vegetative growth. The three mutants may be good for fodder production due to their continuous vegetative growth.

The results of the inheritance study are presented in Tables 3 and 4. The frequency of fertile to sterile plants in the segregating progenies fitted very well to 3:1 ratio as expected on the basis of monogenic inheritance. The pooled data are presented in Table 3. The heterogenity chi-square test indicated that the three mutants agree with each other in showing a 3:1 ratio (Table 4). Thus, sterility in each of the three mutants is conditioned by a single recessive gene pair.

Conclusion

The results of the present study lead to the following conclusion.

1. The major cause of sterility in IT85D-3625 and IT85D-3628 was chromosome aberrations.

2. Sterility in each of the three mutants was associated with morphological aberrations.

3. The continuous growth of the three mutants after anthesis makes them useful materials for fodder production.

4. Complete sterility in each of the mutants was conditioned by a simple recessive gene pair. Gene-symbols cs_1, cs_2 and cs_3 are being assigned for IT85D-3625, IT85D-3628 and IT86D-3641 respectively.

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