FURTHER OBSERVATIONS ON THE HYBRIDS OF *Sesamum indicum L.* AND *Ceratotheca sesamoides L.*

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ABSTRACT

Completely sterile plants were observed in the interspecific hybrids between *Sesamum indicum* and *Ceratotheca sesamoides.* This was attributed to meiotic abnormalities such as univalent and multivalent associations, non-disjunction bridges and presence of fragments and micronuclei. The low pollen viability (38-48%) and the poor seed set(0-25%) are explained on the basis of these irregular events. Results from this study also showed that hybridization may be important in the population dynamics of the genus *Sesamum.*

Keywords; *Sesamum indicum Ceratotheca sesamoides* non-disjunction bridges, univalent and multivalent associations, fragments and micronuclei

INTRODUCTION

The genus *'Sesamum'* belongs to the family pedaliaceae. It consists of about 36 species out of which the commonly recognized is *Sesamum indicum* (Perseglove, 1974). Other related species are S. *angolense,* S. *radiatum,* S. *capense,* S. *Alatum,* S. *laciniatum,* S. *schekiti,* S. *occidentate,* S. *prostratum* and *Ceratotheca sesamoides* most of which have been reported in Africa (Salunke and Desai, 1986). The most common species in Nigeria is S. *indicum.* It is an important oil seed crop in many parts of the country (Falusi and Salako, 2001). *Ceratotheca sesamoides* on the other hand is a species endemic in Nigeria. It is closely related to *Sesamum indicum* and is commonly referred to as "false sesame". It is characterized by many medium hairs on stem and petiole, dentate leaf margin, pink flowers and sub-erect growth habit (Van Rheenen, 1970).

Successful inter-specific hybridization between *Sesamum* species has been reported by various authors (Joshi 1961; Aiyadurai, 1963 and Falusi *et al,* 2001). Falusi *et al* (2001) reported partially sterile plants from the cross between S. *indicum* and *Ceratotheca sesamoides.* Sterility in inter-specific hybrids of plants is one of the most commonly observed phenomena. However, in many cases, the nature and mode of action involved in causing this sterility is not understood (Stebbins, 1950). In most cases, the occurrence of such sterility has great significance in evolution and the determination of inter specific relationship. Therefore observations on such cases are always desirable. The aim of this study was to carry out meiotic observations on the inter-specific hybrids between S. *indicum* and *Ceratotheca sesamoides* with a view to further ascertaining the probable cause of sterility in them.

MATERIALS AND METHODS

The experimental materials were obtained from the National Cereals Research Institute, Badeggi, Nigeria. The identities of these materials are summarized in Table 1. They were identified as *Sesamum indicum* and *Ceratotheca sesamoides* by the morphological description of Hutchinson and Dalziel (1963) and Van Rheenen (1970). The seedlings were raised in plastic buckets containing sand compost fertilizer mixture. At maturity crosses were made reciprocally between *Sesamum indicum* and *Ceratotheca sesamoides*. This was done by carefully opening and emasculating flower buds (i.e. removing the corolla with the stamens and covering the pistil with a small piece of drinking straw closed at the outer end) one day before anthesis. The flower buds were recognized by their characteristic size. The following morning after emasculation, pollen was placed on the stigma with a pair of forceps after which the pistil was covered with a piece of drinking straw closed at the outer end to exclude unwanted pollen.

Parental and F 1 seedlings were also raised to maturity in plastic buckets. Flower buds (5-6 days old) from them were harvested for meiotic studies. The flower buds were harvested between 9a.m. and 10a.m. and fixed in ethanol acetic acid (3.1 v/v of 95% ethanol and 99.5% acetic acid mixture) for twenty-four hours before squash preparation. For squash preparation, the flower buds were rinsed in water and hydrolyzed in 18% hydrochloric acid for two minutes. The hydrolyzed flower bud was rinsed in water and one anther at a time was squashed in FLP orcein (Olorode, 1973). Chromosome associations were scored at Diakinesis and photomicrographs of suitable cells were taken for illustration. Pollen grain viability was estimated by staining in cotton blue in lactophenol and pollen diameter was measured at x 400. Pollen grains that were deeply stained and that had spherical shape were considered viable.

Table 1: Identity of the plants used for this study

S/N	Code Number	Source	Suspected Species	Description
1.	NSG-KG-02	N.C.R.I Badeggi	*S. indicum*	Erect plants with few long hairs in stem and petiole Creamy white seeds 2-3mm length.
2.	NSG-NG-02	N.C.R.I. Badeggi	*Ceratotheca sesamoides*	Sub erect plants with many long hairs in stem and petiole. Black seeds 2-3 mm length
3.	NSG-KN-03	N.C.R.I. Badeggi	*S. indicum*	Erect plants with few long hairs in stem and petiole. Creamy white seeds 2.2-3.5 mm length.
4.	NSG-KD-03	N.C.R.I. Badeggi	*Ceratotheca sesamoides*	Sub erect plants with many long hairs in stem and petiole. Brownish seeds 2.5-3.5 mm length
5.	NSG-KD-04	N.C.R.I. Badeggi	*S. indicum*	Erect plants with few long hairs in stem and petiole. White seeds 2.5-3 mm length

RESULTS AND DISCUSSION

Percentage successes observed in the interspecific crosses were low (Table 2). Most capsules dropped off at an early stage of development. The capsules obtained from NSG-NG-02 X NSG-KD-04 cross, produced only shriveled and non-viable seeds, which probably resulted from lack of fertilization. Seeds obtained from other crosses had low germination percentages (Table 2). The percentage pollen viability was very high in the parent plants ranging between 64% and 82% (Table 3) while it was low in all the hybrids. Variation in pollen size was also high as shown by the coefficient of variation (Table 3). The low pollen viability observed suggests that micro sporogenesis has been impaired in the hybrids. This strongly implies that the two species are relatively cross-sterile. Nevertheless, the present results still indicate that these crosses can occur in nature and result in new cultivars.

Chromosome associations at Diakinesis for all the plants studied are summarized in Table 4. The frequencies of univalent and quadrivalents were very high in all the hybrids compared with the parents. The hybrids also showed lower frequencies of bivalents and high frequencies of meiotic irregularities. Plate 1 is a diakinesis cell in NSG-NG-02 X NSG-KD-04 plant with 2 IV + 10 II + 1 I while plate 2 is an anaphase I cell in NSG-KN-03 X NSG-KD-03 plant showing clumping of chromosomes. Non-disjunction bridges were observed at anaphase II in all the hybrids. The frequencies of occurrence of these non-disjunction bridges were high (plate 3). Other abnormalities such as malformation of the tetrad stage (plate 4) fragments and micronuclei (plate 5) were common in the hybrid plants. These are consistent with the low pollen viability and the high variation in pollen size observed in the hybrids

The occurrence of univalents, bivalents and quadrivalents chromosome associations in parents and hybrid plants is indicative' of reciprocal translocations involving non-homologous chromosomes (Aliyu and Awopetu, 2007) This kind of chromosomal association and segmental interchange suggests hybrid origin for parental plants and homeology of the ancestral genomes. It is an indication that natural hybridization may be important in the population dynamics of these species. Clumping and multivalent associations usually arise from stickiness and residual affinities of the chromosomes (Raman *et al.*, 1964; Ekanem and Osuji, 2006). All the abnormalities observed may lead to unequal distributions of chromosomes to the anaphase poles resulting in pollen grain inviability (sterility) and variable pollen sizes as recorded in this study for NSG-KN-02 x NSG-KD-02 plants (Table 3 and Table 5).

Table 2: Percentage Pollination success and F_1 seed germination

Cross combination	No. of Flowers Crossed	% Pollination success	No. on which estimate was based	% germination of F_1 seeds
NSG-KG-02 X NSG-NG-02	50	18.2	20	12
NSG-KN-02 X NSG-KD-02	50	8.5	45	25
NSG-NG-02 X NSG-KD-04	50	4.4	20	0
NSG-KD-03 X NSG-KN-03	50	12	50	20

Table 3: Pollen data of parents and hybrids plants of *Sesamum indicum* and *Ceratotheca sesamoides*

Parent Plant	Pollen Number	Pollen Viability	Mean Pollen Sizes (pm) based on 30 Measurements	C.V.%
NSG-KG-02	750	82	25.50±2.15	8.24
NSG-NG-02	770	64	27.60 ± 1.85	10.12
NSG-KD-03	900	65	23.55 ± 1.84	6.95
NSG-KN-03	850	68.2	26.10 ± 2.30	9.36
NSG-KD-04	750	75	25.50 ± 2.26	7.46
HYBRIDS				
NSG-KG-02 X NSG-NG-02	700	38.7	26.25 ± 4.10	10.20
NSG-KN-03 X NSG-KD-03	700	44	25.75 ± 4.38	11.50
NSG-KD-03 X NSG-KN-03	700	48	26.20 ± 4.20	13.10

Table 4: Diakinesis chromosome association in parent and hybrid plants

Plant	Univalent I	Ring Bivalent II	Rod Bivalent II	Rod Quadrivalent IV	Rod Hexavalent VI
NSG-KG-02 2n=26	0.26 (0-2)	5.56 (1-7)	2.7 (1-6)	1.2 (1-3)	0.3 (1-3)
NSG-NG-02 2n=32	0.6 (2-4)	5.2 (2-10)	3.5 (2-5)	0.9 (1-2)	0.4 (1-3)
NSG-KD-03 2n=32	0.4 (2-4)	4.5 (2-9)	5.3 (2-8)	0.4 (0-1)	0.3 (0-1)
NSG-KN-03 2n=26	0.2 (0-2)	4.6 (2-9)	4.5 (2-6)	0.6 (1-4)	0.4 (1-2)
NSG-KD-04 2n=26	0.5 (2-6)	6.9 (3-10)	4.2 (2-8)	0.16 (0-3)	0.1 (1-2)
NSG-KG-02 X NSG-NG-02	0.3 (1-3)	3.8 (2-6)	5.3 (2-8)	1.2 (2-3)	0.16 (1-2)
NSG-KN-03 X NSG-KD-03	0.2 (0-2)	3.2 (2-4)	4.4 (1-6)	1.2 (1-3)	0.4 (1-3)
NSG-KD-03 X NSG-KN-03	0.4 (0-3)	4.8 (3-7)	5.1 (1-12)	0.8 (1-3)	0.1 (1-2)

Fig. 1

Plates 1-6 and Figure 1: Meiotic chromosomes in parents and hybrids of *Sesamum indicum* and *Ceratotheca sesamoides;*

Plate 1: Diakinesis cell in NSG-NG-02 x NSG-KD-04 plant showing 2IV + 10II+ 11.

Figure 1: Interpretative drawing of Plate 1, (arrow indicates quadrivalent chromosomes while dotted arrow indicates a univalent chromosome)

Plate 2: Anaphase 1 cell in NSG-KN-03 x NSG-KD-03 plant showing clumping of chromosomes

Plate 3: Anaphase II cell in NSG-KD-03 x NSG-KN-03 plant showing non-disjuntion bridge.

Plate 4: Triad in NSG-KN-03 x NSG-KD-03 plant.

Plate 5: Anaphase II cell in NSG-KD-03 x NSG-KN-03 plant showing fragment and micronuclei.

Plate 6: Anaphase 1 cell in NSG-KN-03 plant showing normal separation.

Table 5: Fruit and seed set in F_1 plants of *Sesamum indicum* and *Ceratotheca sesamoides*

Cross	No. of Flowers Produced	Percent Fruit Set	Average No. of seeds per fruit	% Good Seeds per Fruit
NSG-KG-02 X NSG-NG-02	30	0.5	30	0.32
NSG-KN-03 X NSG-KD-03	30	0	0	0
NSG-KD-03 X NSG-KN-03	35	0.15	16	0.38

ACKNOWLEDGEMENT

The authors are grateful to the management of National Cereals Research Institute, Badeggi, Nigeria for support and permission to publish this paper.

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