Interspecific Hybridization between *Sesamum indicum L.* and *Ceratotheca sesamoides* Endl.

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Summary

Sesamum indicum *L. (2n = 26) and* Ceratotheca sesamoides *Endl. (2n = 32) were crossed to investigate their genomic relations and feasibility of gene exchange. Results showed that crossability between the two species was low. Pollen viability was drastically reduced in the F1 hybrids. This was attributed to meiotic abnormalities such as univalent and multivalent associations, non-disjunction bridges, and presence of fragments and micronuclei. Findings from this study also imply that gene exchange between* Sesamum indicum *L. and* Ceratotheca sesamoides *Endl. is possible. The implications of these results in the species evolution are discussed.*

Résumé

Hybridation interspécifique entre *Sesamum indicum* L. et *Ceratotheca sesamoides* Endl.

Sesamum indicum *L. (2n = 26) et* Ceratotheca sesamoides *Endl. (2n = 32) ont été croisés pour évaluer leurs relations génomiques et la faisabilité d'échanges de gènes. Les résultats obtenus montrent que la réalisation de croisement entre ces deux espèces est difficile. La fertilité du pollen est réduite drastiquement chez les hybrides F1. Ceci a été attribué à des anomalies méiotiques telles l'apparition d'univalents et de multivalents en prophase I, la mauvaise disjonction des chromosomes, la présence de fragment de chromosomes et la production de microcytes. Les résultats obtenus mettent en évidence la possibilité d'échanges de gènes entre* S. indicum *et* C. sesamoides. *L'implication de ces résultats dans l'évolution de l'espèce est discutée.*

Introduction

Sesame (Sesamum indicum L.*)* also known as Beniseed, Gigelly, Simsim, Til, Ajonjoli and Kunshunt is an important edible oil seed crop in many regions of the world. It is believed that the crop originated from Africa where the greatest diversity of the genus *Sesamum* and its family, Pedaliaceae is present (9). Currently the crop is cultivated in the tropical and subtropical regions of Africa, South America, North America and Asia principally for its seeds which contains about 50-52% oil, 17-19% protein and 16-18% carbon hydrate (10). It is regarded as one of the world's most important oil seed crop grown preferentially in developing countries by small holders (4). In Nigeria, the crop is grown majorly in Igbira area of Kogi State, Kwali area of Federal Capital Territory, Tiv and Idoma areas of Benue State, parts of Plateau State and Bali area of Gongola State (1). Some of the local names of the crop in Nigeria are ("Ridi": Hausa) ("Ishwa": Tiv), ("Igorigo": Igbira), ("Yamati or Eeku": Yoruba) and ("Doo": Jukun).

Ceratotheca sesamoides Endl., on the other hand is a species endemic in Africa. 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 with purple or brown dots and sub erect growth habit (5, 11). Successful interspecific hybridization between *Sesamum* species has been reported by various authors (2, 3, 6). Joshi (6) stated that viable seeds were obtained from the cross *S. indicum* X *C. sesamoides.* Van Rheenen (11) also mentioned that viable seeds were obtained from the cross *C. sesamoides* X *S. indicum*. Wild species are important because they could be sources of useful genes for the improvement of Sesame. The aim of this study was therefore to make interspecific crosses between *S. indicum* and *C. sesamoides* so as to establish their genomic relationships and determine the feasibility of gene transfer between them.

Material and methods

The experimental materials were obtained from parts of central and north Western Nigeria. They were collected in September 1998 when farmers were harvesting the crop. The identities of these materials are summarized in Table 1. They were identified as *Sesamum indicum*

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Table 1
Identity of the plants used for this study

S/N	Code number	Source	Suspected species	Description
1.	BE-01	Otukpo Benue State	*S. indicum*	Erect plants with few long hairs in stem and petiole, compound leaf margin and white flowers with few red dots. White seeds 2-3 mm in length.
2.	KG-01	Okene Kogi State	*S. indicum*	Erect plants with few long hairs in stem and petiole, compound leaf margin, creamy white flowers with few red dots. Creamy white seeds 2-3 mm length.
3.	KD-02	Kafanchan Kaduna State	*Ceratotheca sesamoides*	Sub erect plants with many hairs in stem and petiole, dentate leaf margin pink flowers with purple brown dots. Brownish black seeds 2.5-3.5 mm length.
4.	KD-04	Kafanchan Kaduna State	*S. indicum*	Erect plants with few long hairs in stem and petiole, compound leaf margin and white flowers with few brownish red dots. White seeds 2.5-3 mm in length.
5.	KN-02	Janguza Kano State	*S. indicum*	Erect plants with few long hairs in stem and petiole, compound leaf margin and creamy white flowers with many red dots. Cream white seeds 2.5-3 mm in length
6.	NA-01	Doma Nessarawa State	*S. indicum*	Erect plants with few long hairs in stem and petiole, compound leaf margin and brownish white flowers with few red dots. White seeds 2-3 mm in length.
7.	NG-01	Bida, Niger State	*Ceratotheca sesamoides*	Sub erect plants with many hairs in stem and petiole, dentate leaf margin pink flowers with purple dots. Black seeds 2-3.5 mm in length.
8.	KD – 03	Kafanchan Kaduna State	*Ceratotheca sesamoides*	Sub erect plants with many hairs in stem and petiole, dentate leaf margin pink flowers with purple brown dots. Brownish red seeds 1.5-2.8 mm in length.

and *Ceratotheca sesamoides* by the morphological description of Hutchinson and Dalziel (5) and Van Rheenen (11). The seedlings were raised in plastic buckets containing sand compost fertilizer mixture. At maturity, crosses were made reciprocally between *Sesamum indicum* and *Ceratotheca sesamoides* Endl. 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 F1 seedlings were 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 9 a.m. and 10 a.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 hydrolysed in 18% hydrochloric acid for two minutes. The hydrolysed flower bud was rinsed in water and one anther at a time was squashed in FLP orcein (7). Chromosome associations were observed 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.

Results

The four successful crosses recorded were KG- 01 X NG – 01, NG – 01 X KD – 04, KN – 02 X KD – 02 and KD – 02 X KN – 02. Pollination successes were generally low (0.0-15%) (Table 2). Most capsules dropped off at an early stage of development. The capsules obtained from NG – 01 X KD – 04 cross produced only shrivelled and non viable seeds. All the F1 plants were morphologically normal but had reduced pollen viability (Table 3). Hybrids KG – 01 X NG – 01, KN – 02 x KD – 02 and KD – 02 X KN – 02 had 38.7%, 44% and 48% pollen viability respectively compared with 82%, 73%, 68.3% and 64% which were recorded for KG – 01, NG – 01, KN – 02 and KD – 02 parent plants respectively. Most of the pollen were deformed shrunken and variable in size (Plate 1 Table 3).

The percentage fruit and seed set in the F1 hybrids were very low (Table 4). Majority of the seeds obtained from them were only empty developed ovules. Meiosis in these plants showed a large number of cells with deviations from normal course of meiosis. Table 5 gives some indication of the nature and extent of these deviations. Various frequencies of univalent, bivalent and multivalent chromosomes were observed both in parents and hybrids plants. The hybrids however showed higher frequencies of univalent and quadrivalent chromosome associations with corresponding lower frequencies of bivalents. Plate 2 is a diakinesis cell in NG – 01 X KD – 04 plant with 2 1V + 10 II + 1I = 29. Other abnormalities such as non-disjunction bridges (Plate 3) and malformation of the tetrad stage (Plate 4) were common.

Table 2
Percentage pollination success and F1 seed germination

Cross Combination	No of flowers crossed	% Pollination success	No on which estimate was based	% Germination of F1 seeds
KG – 10 X NG – 01	45	15.4	25	10
KG – 01 X KD – 03	40	-	-	-
BE – 01 X KD – 02	45	-	-	-
BE – 01 X KD – 03	40	-	-	-
KN – 02 X KD – 02	45	6.5	50	20
KN – 02 X KD – 03	40	-	-	-
NA – 01 X NG – 01	50	-	-	-
KD – 04 X NG – 01	50	-	-	-
KD – 04 X KD – 03	40	-	-	-
NG – 01 X KG – 01	40	-	-	-
NG – 01 X KD – 04	45	2.4	25	0
NG – 01 X NA – 01	45	-	-	-
KD – 02 X BE – 01	45	-	-	-
KD – 02 X KN – 02	45	10	50	18
KD – 03 X KN – 02	40	-	-	-
KD – 03 X KD – 04	40	-	-	-
KD – 03 X KG – 01	40	-	-	-
KD – 03 X BE – 01	40	-	-	-

Table 3
Pollen data of parents and hybrids plants

Plant	Pollen Number	Pollen Viability	Mean pollen sizes (µm) based on 30 measurements	C.V. %
KG – 01	750	82	25.50 ± 2.15	8.24
BE – 01	850	85	25.16 ± 2.85	8.10
KD – 04	750	75	25.50 ± 2.26	7.46
KN – 02	850	68.2	26.10 ± 2.30	9.36
NA – 01	750	73	24.75 ± 2.45	7.48
NG – 10	770	64	27.60 ± 1.85	10.12
KD – 02	900	65	23.55 ± 1.84	6.95
KD – 03	800	60	25.12 ± 2.30	7.50
HYBRIDS				
KG – 10 X NG – 10	700	38.7	26.25 ± 4.10	10.20
KN – 02 X KD – 02	700	44	25.75 ± 4.38	11.50
KD - 02 X KN – 02	700	48	26.2 ± 4.20	13.10

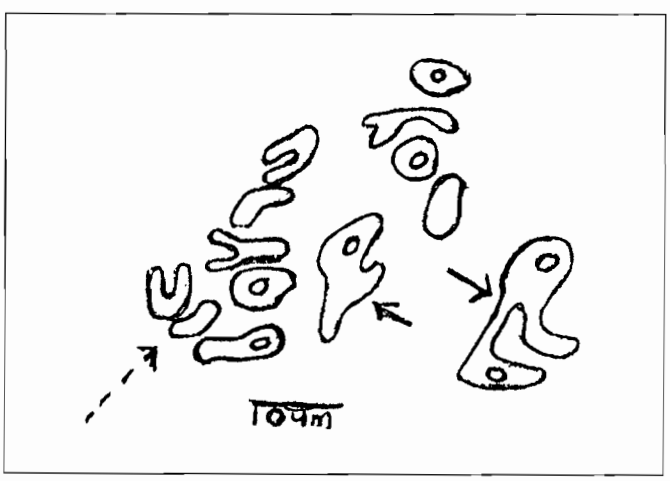

Figure 1: Pollen and meiotic chromosomes in hybrids between *Sesamum indicum* and *Ceratotheca sesamoides.*

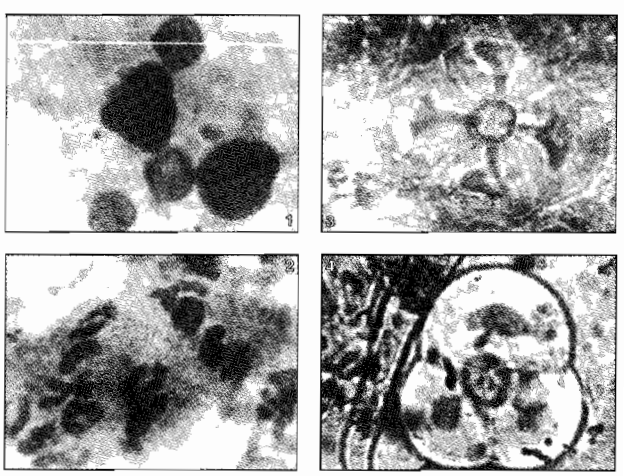

Plate 1: Pollen grains in NG – 01 x KD – 04 plant with two sized classes.
Plate 2: and Figure 1: Diakinesis cell in NG – 01 x KD – 04 plant with 2 IV + 10 II + 1I.
Plate 3: Non disjunction bridge in NG – 01 x KD –04 plant.
Plate 4: Triad in KN – 02 x KD – 02 plant.
(Arrows indicate quadrivalent chromosomes, while dotted arrow indicates a univalent chromosome)

Table 4
Fruit and seed set in F1 plant

Cross	No of flowers produced	Percent fruit set	Average no of seeds per fruit	Average no of good seeds per-fruit	Percent good seeds set per fruit
KG – 01 x NG – 10	25	0.4	25	7	0.28
KN – 02 x KD – 02	30	0	0	0	0
KD – 02 x KN – 02	28	0.12	14	5	0.32

Table 5
Nature and extent of deviations from the normal course of meiosis

Stages of division	No. of cells total	Abnormal	Nature of abnormalities
Prophase I	50	32	Centric or acentric chromosome, stickness.
Metaphase I	100	64	Condensed chromatin in the cytoplasm, univalent, multivalents, fragments.
Anaphase I	21	4	Bridges, lagging, fragments.
Metaphase I	60	48	Micronuclei.
Anaphase II	40	38	Bridges fragments distributed at random.
Tetrads	80	50	Micronuclei irregularly distributed.

Discussion

The low fertilization success observed coupled with the poor viability of the F1 seeds strongly implies that there is difficulty in crossing between *Sesamum indicum* and *Ceratotheca sesamoides*. This corroborates the report of Van Rheenen (11) that the two species are relatively cross sterile. Seedlessness may arise from lack of fertilization or, if fertilization occurs, from a ceassation of seed development soon afterwards, resulting in fruits containing aborted and in inviable seeds, as in many incompatible crosses. However, the successful crosses recorded coupled with F1 viability and fertility show that hybridization is possible in nature between *Sesamum indicum* and *Ceratotheca sesamoides* and this could probably be responsible for the occurrence of a wide variety of sesame plant types in cultivation. The possibility of gene exchange between the two species seems to indicate that they both should be classified in a same genus.

The occurrence of univalent, bivalents and other multivalent associations in parent and hybrid plants are indicative of reciprocal translocations involving non-homologous chromosomes. This kind of chromosomal association and segmental interchange suggest hybrid origin for the parental plants and homoeology of the ancestral parental genomes. Multivalent association usually arise from stickness and residual affinities of the chromosomes (8). The frequent occurrence of a bridge and a fragment during anaphase II suggests a paracentric inversion accompanied by a single cross over in the interstitial region. These irregularities are expected to lead to deficiencies, duplications and consequently low pollen fertility and high standard deviation in pollen sizes as recorded for the hybrids in this study.

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