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Comparative Pollen Study of Three Species of the Family Cucurbitaceae (*Cucurbita moschata, Cucurmeropsis mannii, Telfairia occidentalis*)

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ABSTRACT

The presence of polyploidy in the family Cucurbitaceae has been confirmed. However, there is poor fruit set in this family compared to the prolific production of, especially, male flowers. The aim of this study is to identify the main differences in pollen characteristics of three Cucurbits, *Cucurbita moschata, Cucurmeropsis mannii* and *Telfairia occidentalis* which would contribute to the understanding of the pollen viability and poor fruit set observed. In this study, pollen fertility, morphology and size distribution within the three species belonging to the family Cucurbitaceae were carried out under light microscope (LM). Pollen fertility assessment was made with aceto-carmine glycerol jelly. The pollen sizes (µm) varied greatly among the different species. There was some correlation in the pollen size with known chromosome number of these three species. Furthermore, the pollen grain sizes of *C. moschata* on the frequency distribution graph were observed to be bimodal, while those of *T. occidentalis* and *C. mannii* were unimodal. In addition, the wide variation in pollen sizes within each of these species points to the fact that there is some measure of irregular meiosis taking place, an indication of the presence of aneuploids and polyploids. The pollen fertility for the three species was generally high. The taxon under study showed marked variations in the palynomorphs: two distinct pollen types were recognized based on shape, pores and spikes index of the pollen grains. From the pollen shapes and pollen size distribution, *T. occidentalis* and *C. mannii* are more closely related than *C. moschata*.

Keywords: Cucurbitaceae, pollen viability, *Cucurbita moschata, Cucurmeropsis mannii, Telfairia occidentalis*, bimodal.

Introduction

Cucurbitaceae, commonly known as the gourd family, comprises of some 965 tropical and subtropical species collectively known as cucurbits (Christenhusz and Byng, 2016; Schaffer and Paris, 2016). Though the Cucurbitaceae family is not large in particular compared to other vascular plant families (Willis *et al*., 2017), it contains a high proportion of economically important species, including some of the world's earliest domesticates (Lira-Saade, 1995).

Cucurbit species from at least 20 genera are grown for their culinary purposes, medicinal applications and as ornamentals (Schaffer and Paris, 2016). The Cucurbitaceae, with about 21 genera and 41 species in Nigeria, have perhaps more species in cultivation than any other family.

In most Cucurbits, the male plant flowers earlier than the female. This Family exhibits polyembryony (Esiaba, 1982; Odiyi, 2003; Onovo *et al*., 2009) and facultative apomixes (Fayeum *et al*; 2016). These phenomena have been suggested to improve productivity in the crops, but the limitation of these is the absence of genetic recombination that is only possible through meiosis and syngamy (Fayeum *et al*; 2016). Hence the inability of plant breeders to develop varieties through hybridization. Knowledge of reproductive biology, which entails floral biology, pollination mechanisms and breeding systems, is a prerequisite for plant breeding and without this progress, crop improvement through hybridization would be limited. Reproductive biology also provides information on the nature of species, adaptation and hybridization (Anderson *et al*., 2002; Neal and Anderson, 2005).

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Pollen has important biological functions in sexual reproduction including transfer of genetic material via pollinators and providing energy for pollen germination and pollen tube growth. Viable pollen is important for species dispersal, fitness and survival of the next plant generation. It is also essential for directed plant breeding and consequently crop improvement.

Pollen viability could be used as an umbrella in describing the capacity of pollen to grow, germinate or develop (Dafni and Firmage, 2000). Within species, pollen size has been shown to vary among anthers, flowers, individuals and populations and across seasons (Cruzan 1990).

Pollen morphology in Cucurbitaceae has received considerable attention (Van der Ham and Van Heuven, 2003; Barth *et al*., 2005) and molecular data support perception that pollen characters in Cucurbitaceae are relatively conserved.

According to Jeffrey (1990), the pollen of Cucurbitaceae is eurypalynous, with considerable differences in grain shape, ornamentation pattern and position of apertures between the individual genera. Among the species, fruit set is poor and not commensurate with the abundance of flowers produced, especially the male ones. This could be attributed to several reasons such as: environment, pollinators, pollen viability and floral morphology.

The presence of polyploids in the family Cucurbitaceae has also been confirmed in producing polyploid genotypes through sexual polyploidization (Singhal *et al*., 2012). An attempt was made in this study to investigate the degree of polyploid occurrence in the three species studied. To this end this study examined the pollen diameters, as well as the various pollen shapes and pollen fertility of *Cucurbita moschata, Cucurmeropsis mannii and Telfairia occidentalis*

The aim of this study is to identify the main differences in pollen characteristics of the three Cucurbits, *Cucurbita moschata, Cucurmeropsis mannii* and *Telfairia occidentalis* which would contribute to the understanding of the pollen viability and poor fruit set observed in this family.

Materials and Methods

Collection of Cucurbits

The three cucurbit varieties used for this experiment-*Cucurbita moschata*, *Cucurmeropsis mannii* and *Telfairia occidentalis),* were obtained from established plots of the Rivers Institute of Agricultural Research and Training (RIART) farm in the Rivers State University, Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.

Collection of Male Flowers and the Process of Acetolysis and Photography

At anthesis, male flowers were collected at 7.00am. Pollen grains were dislodged from the stamen with forceps, spread on a microscope slide and stained with 1% acetocarmine glycerol jelly (Marks, 1954). Pollen counts were made per microscopic field (10) from 2 random samples under a Leitz Diaplan binocular light microscope (x125 magnification). Only completely rounded and deeply stained grains were considered as fertile pollen. Pollen grains from flower buds of these species were analyzed for fertility within 1 hour of collection. Percentage Pollen fertility was calculated with these values. The diameters of fertile grains were measured with the aid of a graduated eyepiece. Giant pollen grains having diameters greater than or equal to 130µm were classified as 2n pollen, since 2n pollen normally have 1.25 to 1.50 times the diameter of haploid or n pollen (Darlinton, 1937).

Pollen **s**amples were acetolysed according to Erdtman's method with modifications (Erdtman, 1952; Bocianowski *et al*., 2016). The acetolysing mixture was made up of nine (9ml) of acetic acid anhydride and one (1ml) concentrated sulphuric acid in a 50 ml graduated cylinder. Anther of each of the species was added to each mixture and left for one hour. Thereafter, the mixture of acetic anhydride and sulfuric acid was decanted. The sample was poured in a test tube, containing 6ml of glacial acetic acid, stirred and centrifuged at 3000rpm for 5min. After which, it was decanted and washed 3 times. Silicone oil was then added and the process of acetolysis lasted for 5 minutes. After 5min, it was decanted and washed three (3) times using distilled water. Silicone oil was then added to the sample and slides were prepared with acetocarmine stain.

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Photography

Photomicrographs of well stained acetolyzed pollen grains were made with a Trinucular photographic research microscope (T340B) fitted with AmScope digital camera, to ascertain their qualitative features.

Data Analysis

Data collected were subjected to analysis of variance to estimate the differences among the species.

Results

The pollen sizes vary greatly among the different species: it ranges from 108.4 to 169.64 µm with a standard deviation of 11.05 and mean of 151.01 for *C moschata*. For *T. occidentalis* it ranges from 48.4 to 79.64µm with a standard deviation of 6.27 and mean of 63.23.

While *C. mannii* pollen sizes ranges from 42.44 to 80.32µm with a standard deviation of 8.73 and mean of 66.11 (Table 1). The result on Table 1 shows that the pollen size of *C. moschata* has the highest statistical values for mean/average as 151.01, followed by *C. mannii* (66.11) and *T. occidentalis* (63.23). Likewise, the Standard deviation (Table 1, Fig. 1) of the pollen diameter for *C. moschata* is highest (11.05) followed by that of *C. mannii* (8.73) and *T. occidentalis* (6.27). The result also shows that pollen fertility of *C. moschata, C. mannii and T. occidentalis* are high with the percentages of 91.1% for *C.moschata*, 77.5% for *C.mannii*, and 90.7% for *T. occidentalis* as shown in Table 1.

The frequency distribution graph depicts that all three species show variation in their pollen sizes. That of *C. moschata* is bimodal, while those of *T. occidentalis* and *C. mannii* are each unimodal (Fig. 1).

Table 1: Pollen diameter (µm) of the Cucurbitaceae species

Cucurbitaceae species	Maximum pollen size (µm)	Minimum pollen size (µm)	Mean pollen size (µm)	Percent pollen fertility	2n Pollen frequency (%)
Cucurmeropsis mannii	80.3	42.4	66.1±8.7	91.5%	4%
Cucurbita moschata	169.6	108.4	151.0±11.1	77.5%	0%
Telfairia occidentalis	79.6	48.4	63.2±6.3	90.1%	2%

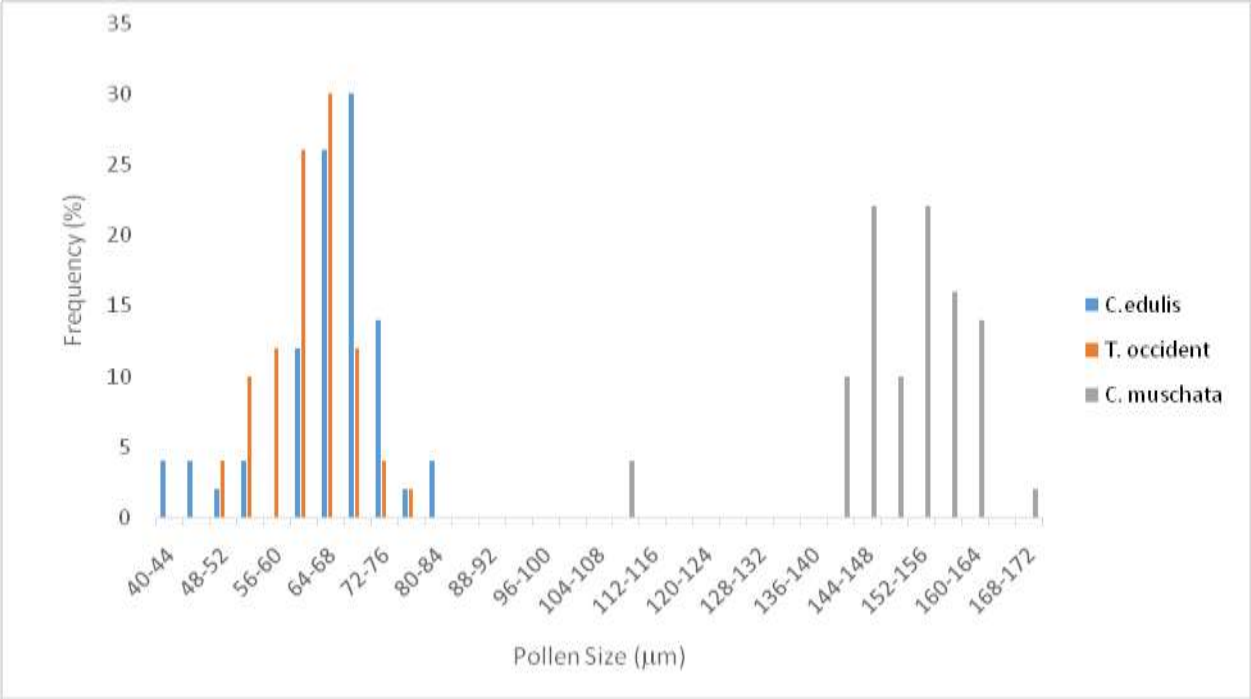

Fig. 1: Frequency Distribution of the Pollen Sizes of three Species of the Family Cucurbitaceae

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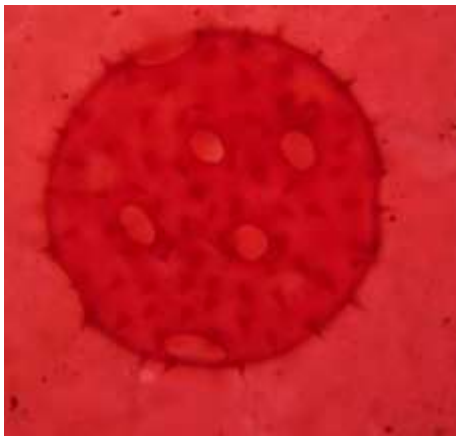
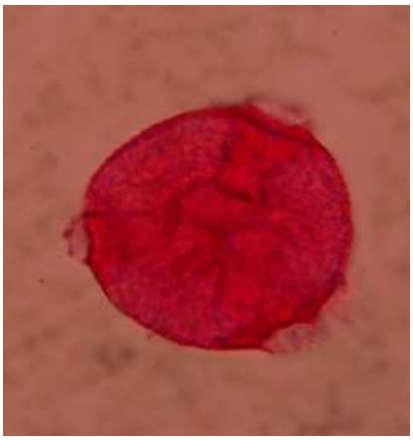
The photomicrographs of stained pollen structures of *C. moschata*, *C. mannii*, and *T. occidentalis* as observed under the microscope are presented in Plate 1. Pollens of *C. mannii* and *T. occidentalis* are triporate and similar, while that of *C. moschata* is spheroidal with spikes (Plate 1).



***C. moschata* (spheroidal) ×40**



***C. mannii* (triporate) ×100**

***T. occidentalis* (triporate) ×100**

Plate 1: Photomicrographs of Pollen structures of *C. moschata, C. mannii,* and *T. occidentalis*

Discussion

This study has revealed the size distribution, morphology and pollen types and pollen viability of the species of Cucurbits studied. The pollen sizes varies greatly among the different species, it ranges from 108.4 - 169.64 µm with a standard deviation of 11.05 and mean 151.01 for *C. moschata*. For *T. occidentalis* it ranges from 48.4- 79.64µm with a standard deviation of 6.27 and mean 63.23. While *C. mannii* pollen sizes range from 42.44- 80.32µm with a standard deviation of 8.73 and mean 66.11 as it is in Table 1. These results are close to those of Velthius (1992) that says Cucurbita pollen grains are large and vary from 80 to 150µm in diameter. Pollen size has been described as useful taxonomic tool at tribal level (El Naggar, 2003).

Furthermore, pollen grain size, according to Chin (1946) with his work on the cytology of polyploid Sorghum, has been said to be proportional to chromosome number. In addition, Pillay and Tenkouano (2011) were of the opinion that pollen diameter is the function of genome size since DNA content and cytoplasm increases as ploidy level increases. Bisognin (2002) reported that among the cucurbit genera, *Cucurbita moschata* has a higher chromosome number (2n=40). Uguru and Onovo (2011) reported a diploid chromosome number of 2n=22 in *T. occidentalis* and a diploid chromosome number of 2n=24 in *C. mannii* by Osuji *et al* (2006).

In this study, *C. moschata* with the highest chromosome number of 2n=40 has the highest value in pollen size (Table 1), followed by *C. mannii* with 24, and closely followed by *T.occidentalis* with 22. So there is some correlation in the pollen size with chromosome number of these three species, thus confirming the findings of Chin (1946) and Pillay and Tenkouano (2011).

The bimodal nature of the pollen grain sizes of *C. moschata* on the frequency distribution graph (Fig. 1) is also worthy of note in contrast to the unimodal of those of *T. occidentalis* and *C. mannii*. Early cytogenetic and isozyme studies as well as synteny analysis suggest possible whole-genome duplication (polyploidy) in this genus (Singh, 1990; Esteras *et al*., 2012).

In addition, the wide variation in pollen sizes within each of these species (Table 1; Fig. 1) points to the fact that there is some measure of irregular meiosis taking place, thus resulting in aneuploids and polyploids.

This has been confirmed by the work of Uguru and Onovo (2011) on *T. occidentalis* which show evidence of polploidy as follows: 2n=22+1 (4%); triploid (1.2%); tetraploid (1.2%); and normal 2n=22 (84%). Most of the landraces are diploid with polyploids as rare exceptions (Uguru and Onovo, 2011).

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The work of Bibi and Okoli (2014) on the cytology of *Lagenaria breviflora*, a cucurbit, which showed meiotic irregularities of univalents and bivalents at metaphase followed by lagging chromosomes at late anaphase I, further confirms aneuploids in this Family.

The varying sizes of pollen grains in Cucurbitaceae therefore, are a sign of aneuploids in this family, since pollen grain size is proportional to chromosome number (Chin, 1946). The pollen grains having 1.25–1.5 times the linear dimensions of haploid or n pollen grains are classified here as 2n (unreduced) pollen grains as has been suggested earlier (Darlington, 1937, Ortiz 1997; Oselebe *et al*., 2010; Xue *et al*., 2011). In this study, the pollen sizes occurring most frequently were considered the n pollen sizes, and those sizes that were up to at least x1.25 of the n pollen sizes were considered the 2n pollen. Hence the 2n pollen frequency for *T. occidentalis* is 2% while that for *C. mannii* is 4%. *C. moschata* on the other hand had 0% 2n pollen frequency (Table 1), probably due to the bimodal pollen size which suggests possible whole genome duplication (polyploidy) already. As the 2n pollen grains are apparently fertile, there is every possibility that such 2n (unreduced) gametes can produce intraspecific polyploids as has been advocated earlier by many researchers in different plants (Falistocco *et al*. 1995, Ortiz 1997, Kim *et al*. 2009, Kumar and Singhal 2011a, 2011b, 2012; Singhal *et al*. 2011). Such 2n (unreduced) pollen grains with somatic chromosome numbers are considered to be the main driving forces for the natural polyploidization of plants.

Pollen sizes that fall below the modal pollen size frequency therefore can be said to be n-1, n-2, e.t.c. aneuploids, while those just above the modal pollen size frequency would be n+1, n+2, up till the 2n pollen sizes (x1.25 of the n pollen). Aneuploidy has however been known to be associated with sterility. Meiotic irregularities resulting in the production of sterile and imbalanced gametes have been reported in several flowering plants (Riley and Law 1965, Sjödin 1970, Gottschalk and Kaul 1980, Bennetzen 2002, Bione *et al*. 2002, Pandit and Babu 2003, Gaut *et al*. 2007).

Alexander (2020) on pollen germination and viability of *Hydrangea macrophylla*, provides insight into a potential pre-zygotic barrier relating to pollen tube growth. Tubes of pollen from triploid parents grew more slowly than tubes of pollen from diploid parents, regardless of the ploidy level of the female parent. This may be because tubes from aneuploid pollen grains grow more slowly than tubes from haploid (n) gametes or diploid (2n) gametes, and all of the pollen produced by triploids in his study appeared to be aneuploid based on progeny genome sizes.

Viability assessment of pollen represents an important tool to estimate the physical, biochemical, and biological status and capacity of pollen to generate tubes to penetrate into the stigma, and elongate inside the style until two male gametes are released within female gametophyte (Daniel *et al*., 2002).

From this study it has been confirmed that pollen fertility is generally high (Table 1) among the three species, *C.moschata* (91.5%), *C.mannii* (77.5%), and *T.occidentalis* (90.1%). About the same value of 90% was obtained for *C. moschata* by Agbagwa *et al*. (2007) and he described it as highly viable. However he said this value is got when the flowers are newly opened, but decreases to about 62% on closure, and crashes to as low as 8% after one day. Dafni *et al*. (2005) reported that viability may be dependent on the conditions to which the pollen grains were subjected to prior to the test, and the pollen age. All these and more could explain the poor fruit set in this Family, compared to the number of flowers produced.

Pollen fertility has been said not to be synonymous with pollen viability. Pollen staining is widely used to assess male fertility and pollen viability; however, pollen staining only assesses fertilization potential because not all stainable grains may actually be viable. Alexander (2019) showed that aniline-blue staining overestimated pollen viability by an average of 2.3x for *Hydrangea macrophylla* when compared to *in vitro* germination. Results of pollen germination using growth medium are considered more accurate, but still fail to account for pollen-pistil interactions. Using fluorescent microscopy to monitor pollen germination and growth of the pollen tube most closely simulates *in vivo* conditions (Kho and Baër, 1968; Atlagić *et al*., 2012).

The photomicrographs of pollen structures of *C. moschata*, *C. mannii*, and *T. occidentalis* as presented in Plate 1 shows that he shape of the pollen of *T. occidentalis* and *C. mannii* are triporate with pores; there are similarities between the pollen grains of these two species. The shape of *C.moschata* on the other hand is spheroidal with pores.

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Of the three species studied, it is only *C.moschata* that was observed to have spines. The number, height and position of these spines vary in the different plant families where they occur, and constitute some of the most significant characters for identification purpose (Pope, 1925). It is obvious from the pollen shapes that *T. occidentalis* and *C. mannii* are more closely related than *C. moschata*.

In conclusion, the current study confirms that *C. moschata* is significantly different from *T. occidentalis* and *C. manni* in pollen size distribution and pollen structure, though they belong to the same Family. The variation in pollen size in the three species studied points to irregular meiosis and presence of aneuploids in this Family. The viability of the pollen grains is in question as aneuploids are known to be sterile.

Pollen staining is widely used to assess male fertility and pollen viability, however pollen staining assesses only fertilization potential, and is not commensurate with viability. There is very low percentage of 2n pollen in *T. occidentalis* and *C. manni*, but none at all in *C. moschata* which alone shows bimodal pollen size frequency, a pointer to whole genome duplication (polyploidy) and speciation.

The poor fruit set challenge among the species of this Family, despite its prolific production of especially male flowers, may be attributed more now to pollen viability due to the variation in pollen sizes that indicates aneuploidy. Other reasons being environment, pollinators and floral morphology.

It is recommended to further confirm what is actually going on in the meiosis of the pollen mother cells of these Cucurbitaceae species, and to carry out proper viability tests involving pollen germination of the aneuploids. Secondly, the bimodal pollen frequency sizes of *C. moschata* is worthy of note, and further investigation should be carried out at the molecular level to determine its speciation and relationship with *T. occidentalis* and *C. mannii*.

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