

POLLEN GRAIN MORPHOLOGY OF THREE SPECIES AND A VARIETY OF *OCIMUM* LINN. (LAMIACEAE) IN SOUTHWESTERN NIGERIA

O.O. Arogundade and O. Adedeji

Department of Botany,
Obafemi Awolowo University,
Ile-Ife, Nigeria.

ABSTRACT

Pollen grains from fresh anthers of three species and a variety of *Ocimum* occurring in Southwestern Nigeria were collected and acetolysed. This was in an attempt to use the pollen characteristics to delimit, classify and trace probable evolutionary relationships among the taxa. The structural morphology of the pollen grains carried out with a light microscope is reported. Characters measured on the pollen grains were pollen diameter, pollen wall thickness, depth of colpi, distance between colpi and pore diameter. This was done with the aid of an ocular micrometer. Number of pores per pollen was counted. Pollen grain sizes of the species and the variety of *Ocimum* studied fall into two groups, Media (diameter 25-50µm) and Magna (diameter 50-100µm). All the species and the variety had acolpate and hexacolpate pollen grains. Heptacolpate and octacolpate pollen grains which are advanced types of pollen grains were encountered in *O. canum* only. These advanced types of pollen grains are a mark of recent evolutionary development in the species.

Keywords: *Ocimum*, pollen grains, morphology, acetolysis, evolution

INTRODUCTION

The genus *Ocimum* Linn. belongs to the tribe Ocimeae, subfamily Nepetoidea, family Lamiaceae and the order Lamiales. About six species are currently recognized in West Africa and are generally distributed throughout the region as well as in parts of the world outside the African continent (Burkill, 1995). In Nigeria, five species of *Ocimum* have been reported which are distributed to both Southern and Northern Nigeria (Hutchinson and Dalziel, 1963). Three species and a variety of one of the species are found in Southwestern Nigeria.

These are: *O. canum* Sims, *O. basilicum* Linn., *O. gratissimum* Linn. and *O. basilicum* var. *purpurascens* Benth.

The genus *Ocimum* is one of the economically important groups of aromatic herbaceous plants extensively used in perfumery, flavouring and pharmaceutical products (Khosla, 1993). Many species of *Ocimum* have been grown by local people as medicinal plants, culinary herbs and insect controlling agents (Grayer *et al.*, 1996).

Pollen characteristics have been used considerably in the taxonomy of angiosperms and can be applied in tracing the history of plant groups

and species (Moore and Webb, 1978). Patel and Datta (1958) and Sowunmi (1973) are some of the researchers who have worked extensively on the morphology of pollen grains and have emphasized the significance of pollen architecture in phylogeny. The work of Akinwusi and Illoh (1996) on the pollen morphology of *Hibiscus* showed that the study of pollen grains (Palynology) provides useful data for the taxonomy of the genus. Adedeji (2005) traced the evolutionary relationships among three species of *Emilia* based on their pollen morphology. There is no reported work on the pollen morphology of the genus *Ocimum* in Nigeria. The aim of this study is therefore to use the characters of the pollen to delimit, classify and trace evolutionary relationships among the taxa of *Ocimum* in Southwestern Nigeria.

MATERIALS AND METHODS

Collection of Pollen grains

Pollen grains from fresh anthers of flowers of the four taxa of *Ocimum* found largely in the

Southwestern part of Nigeria, namely, *O. canum* Sims, *O. basilicum* Linn., *O. gratissimum* Linn. and *O. basilicum* var. *purpurascens* Benth were collected across the region (Table 1).

Acetolysis

The collected pollen grains were acetolysed following the procedure of Erdtman (1960). The fresh anthers were placed in a centrifuge tube and macerated in 70% ethyl alcohol with a stirring rod. This dehydrated the pollen grains. These were then centrifuged at the speed of 5,000 revolutions per minute for fifteen minutes. The alcohol was poured off, glacial acetic acid was added and the mixture was centrifuged again at the same speed. Acetolysis mixture (sulphuric acid and acetic anhydride) was added next and the tube containing the pollen grains and the mixture was kept in water bath and heated from 70°C to boiling point. The mixture was then stirred with a glass rod and centrifuged again at the same speed as above,

after which the and glacial ac then centrifug followed by a was then dec grains were m

Microscopy

Acetolysed po ined under the of the diamete depth of colpi pore diameter pollen grains the aid of an eyepiece of t ments were la stant with res they were tak tolysed pollen

Data Analysis

The means of were calculat Analysis of \ Multiple Ran differences an A table illust characters me gram of the ve

RESULTS

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Table 1: Provenances of the specimens studied

Species	Area/Place of collection
<i>O. canum</i> Sims	In front of a house, Ajebandele area, Ile-Ife, Osun State.
<i>O. canum</i> Sims	In a garden, Line 3, Ooni Layout, Ajebandele, Ile-Ife, Osun State.
<i>O. canum</i> Sims	In a garden, Road 9, O.A.U. Staff Quarters, Ile-Ife, Osun State.
<i>O. canum</i> Sims	Along Road 7, Irèpodun Street, Ile-Ife, Osun State.
<i>O. canum</i> Sims	Seramo guest house junction, Eleyele, Ile-Ife, Osun State.
<i>O. canum</i> Sims	Along the road, Agoare, Oyo State.
<i>O. basilicum</i> Linn.	In a garden, Road 9, O.A.U. Staff Quarters, Ile-Ife, Osun State.
<i>O. basilicum</i> Linn.	Isale Igbagbo street, Sepeteri, Oyo State.
<i>O. basilicum</i> Linn.	In a garden, Line 3, Ooni Layout, Ajebandele, Ile-Ife, Osun State.
<i>O. basilicum</i> Linn.	No 15, opposite Seramo guest house, Eleyele, Ile-Ife, Osun State.
<i>O. basilicum</i> Linn.	Along the road, Owo, Ondo State.
<i>O. basilicum</i> Linn.	In front of Flat 19, Omole Estate, Ile-Ife, Osun State.
<i>O. gratissimum</i> Linn.	Old buka, O.A.U. Campus, Ile-Ife, Osun State.
<i>O. gratissimum</i> Linn.	In a garden, Line 3, Ooni Layout, Ajebandele, Ile-Ife, Osun State.
<i>O. gratissimum</i> Linn.	In a garden, Road 9, O.A.U. Staff Quarters, Ile-Ife, Osun State.
<i>O. gratissimum</i> Linn.	Beside a house, Akinjagunla, Ondo, Ondo State.
<i>O. gratissimum</i> Linn.	Ibadan - Oyo road, Ibadan, Oyo State.
<i>O. gratissimum</i> Linn.	Erelu road, Oyo, Oyo State.
<i>O. gratissimum</i> Linn.	Along the road, Ijebu-Ode, Ogun State.
<i>O. gratissimum</i> Linn.	Along the road, Ipapo, Oyo State.
<i>O. gratissimum</i> Linn.	Along the road, Oye, Ekiti State.
<i>O. basilicum</i> var. <i>purpurascens</i>	By the roadside, Modakeke market, Ile-Ife, Osun State.
<i>O. basilicum</i> var. <i>purpurascens</i>	In a garden, Road 9, O.A.U. Staff Quarters, Ile-Ife, Osun State.
<i>O. basilicum</i> var. <i>purpurascens</i>	In a garden, Line 3, Ooni Layout, Ajebandele, Ile-Ife, Osun State.
<i>O. basilicum</i> var. <i>purpurascens</i>	Behind Botany Dept., O.A.U., Ile-Ife, Osun State.

Table 2: Qu stu sig

Param
Pollen diameter (μ)
Pollen wall thick
Depth of colpi (μ)
Distance between
Pore diameter (μ)
Number of pores

after which the acetolysis mixture was decanted and glacial acetic acid added. The mixture was then centrifuged again, distilled water added, followed by another centrifugation. The water was then decanted and the acetolysed pollen grains were mounted in dilute glycerin solution.

Microscopy

Acetolysed pollen grains were properly examined under the light microscope. Measurements of the diameter of pollen grain, wall thickness, depth of colpi, distance between apertures, and pore diameter as well as numbers of pores of 50 pollen grains were taken for each taxon with the aid of an ocular micrometer inserted in the eyepiece of the microscope. These measurements were later multiplied by the ocular constant with respect to the power under which they were taken. Photomicrographs of the acetolysed pollen grains were taken.

Data Analysis

The means of the data generated from the work were calculated. Statistical analyses include Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) for significant differences among the species and the variety. A table illustrating the means of the pollen characters measured was prepared. Cluster diagram of the values was also made.

RESULTS

Generally, the pollen grains of the four taxa of *Ocimum* studied were spherical to ellipsoidal in shape. The two types of aperture, the colpi and

the pores were present in the four taxa. Colpi were fissure-like or slit-like apertures, also known as furrows while pores were reticulate. All the species and the variety were polyporate. Furthermore, the pollens are reticulate, light yellow to yellowish in colour.

O. canum

The pollen types present were Acolpate (Plate 1 A), Monocolpate (Plate 1 G), Bicolpate (Plate 1 H), Pentacolpate (Plates 1 J, K & L), Hexacolpate (Plates 2 A, B, C), Hexazonocolpate (Plate 1 E), Heptacolpate (Plates 3 A & B), Octacolpate (Plates 3 C & D), Figure 8 shape (Plates 1 I & 3 F) and Ribbon-like shape (Plate 2 L). The mean diameter of the pollen grains was $79.01 \pm 1.09 \mu\text{m}$ Table 2. The apertures were situated in both the polar and equatorial regions of the pollen grains.

O. basilicum

The pollen types present were Acolpate (Plate 1 A), Monocolpate (Plate 1 G), Bicolpate (Plate 1 H), Hexacolpate and convex in shape (Plate 2 G). The mean diameter of the pollen grains was $65.30 \pm 0.94 \mu\text{m}$ Table 2). Colpi were situated in the polar region only while pores were situated both in the polar and equatorial regions of the pollen grains.

O. gratissimum

The pollen types present were Acolpate (Plate 1 C), Pentacolpate (Plate 2 D), Tetraxonocolpate (Plate 1 D), Hexazonocolpate and convex in shape (Plate 2 H & J), Ribbon-like shape (Plate

Table 2: Quantitative characteristics and Duncan grouping of the four taxa of *Ocimum* studied based on the pollen grain attributes (means with the same letter are not significantly different).

Parameters	<i>O. canum</i>	<i>O. basilicum</i>	<i>O. basilicum</i> var. <i>purpurascens</i>	<i>O. gratissimum</i>
Pollen diameter (μm)	$(79.01 \pm 1.09) \text{ A}$	$(65.30 \pm 0.94) \text{ B}$	$(44.80 \pm 0.73) \text{ C}$	$(49.45 \pm 0.49) \text{ D}$
Pollen wall thickness (μm)	$(5.55 \pm 0.15) \text{ A}$	$(7.63 \pm 0.13) \text{ B}$	$(4.38 \pm 0.12) \text{ C}$	$(3.95 \pm 0.11) \text{ D}$
Depth of colpi (μm)	$(26.10 \pm 0.43) \text{ A}$	$(20.58 \pm 0.52) \text{ B}$	$(14.28 \pm 0.40) \text{ C}$	$(13.04 \pm 0.38) \text{ D}$
Distance between colpi (μm)	$(26.49 \pm 0.32) \text{ A}$	$(24.78 \pm 0.18) \text{ B}$	$(13.15 \pm 0.13) \text{ C}$	$(18.08 \pm 0.19) \text{ D}$
Pore diameter (μm)	$(7.37 \pm 0.13) \text{ A}$	$(8.37 \pm 0.49) \text{ B}$	$(5.02 \pm 0.11) \text{ C}$	$(6.53 \pm 0.16) \text{ D}$
Number of pores	$(57.82 \pm 1.40) \text{ A}$	$(37.88 \pm 0.88) \text{ C}$	$(46.58 \pm 1.48) \text{ B}$	$(45.64 \pm 1.40) \text{ B}$

2 K). The mean diameter of the pollen grains was $49.45 \pm 0.49 \mu\text{m}$ Table 2. The apertures were situated both in the polar and equatorial regions of the pollen grains.

O. basilicum var. *purpurascens*

The pollen types present were Acolpate (Plate 1 B), Tetrazonocolpate (Plate 1 D), Hexacolpate and convex in shape (Plate 2 E, F & I). The mean diameter of the pollen grains was $44.80 \pm 0.73 \mu\text{m}$ Table 2. The apertures were situated in both the polar and equatorial regions of the pollen grains. The pollen diameter, the wall thickness, the depth of colpi, the distance between the colpi, the pore diameter, and the number of pores of the pollen grains are shown in Table 2.

The pollens quantitative characteristics of the species and the variety clustered into two main

groups. *O. basilicum* var. *purpurascens* and *O. gratissimum* clustered together in the first main cluster while *O. basilicum* and *O. canum* clustered together in the second main cluster (Fig. 1).

DISCUSSION

The pollen grains of the *Ocimum* species and the variety studied looked similar, though there were some differences especially in *O. canum* where more colpi were encountered. The pollen grains of all the species and the variety were spherical to ellipsoid in shape.

The acolpate pollen grains were encountered in all the species and the variety studied. Monocolpate and bicolpate pollen grains were encountered occasionally in *O. canum* and *O. basilicum*. Pentacolpate pollen grains were encountered in *O. canum* and *O. gratissimum*. All

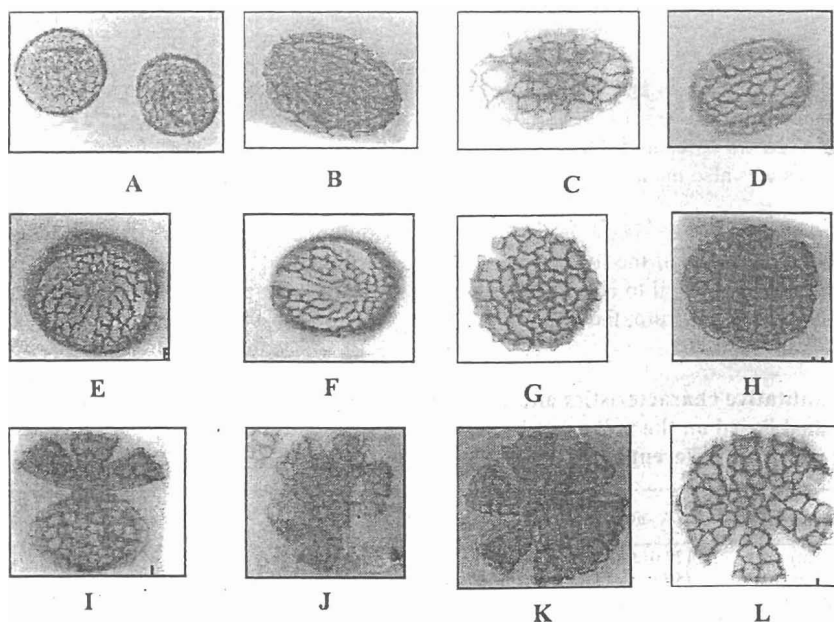


Plate 1: Pollen grains of *Ocimum* species

A – C: Acolpate, D: Tetrazonocolpate, E and F: Hexazolocolpate, G: Monocolpate, H: Bicolpate
I: Tetrazonocolpate (Figure 8 shape), J – L: Pentacolpate



A

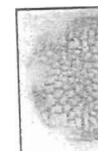
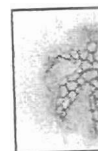


Plate 2: Pollen
A – C: Hexacolpate; I

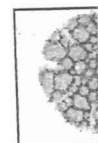


Plate 3: Poller
A and B: Heptacolpate

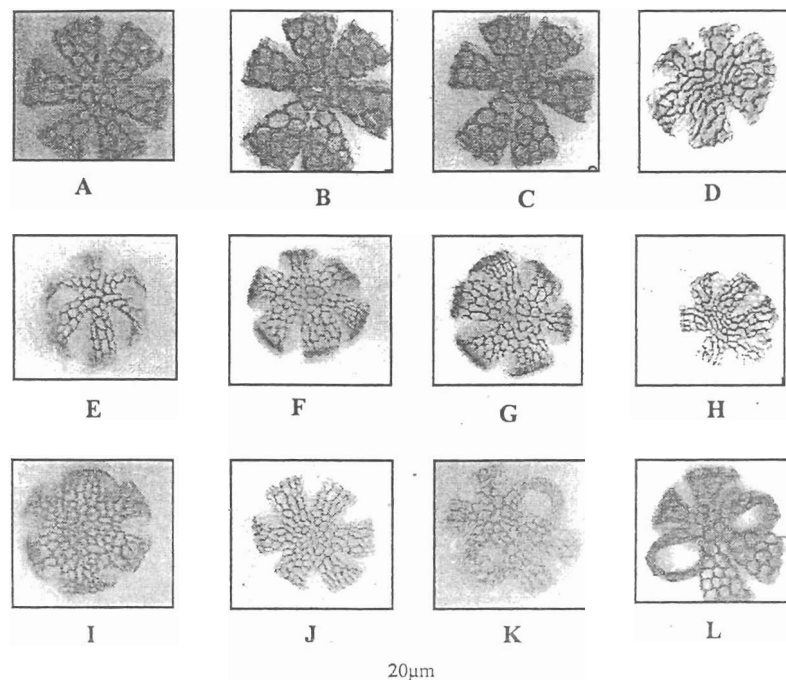


Plate 2: Pollen grains of *Ocimum* species

A - C: Hexacolpate; D: Pentacolpate, E-J: Hexacolpate (Concave in Shape), K and L: Hexacolpate (Ribbon-like shape)

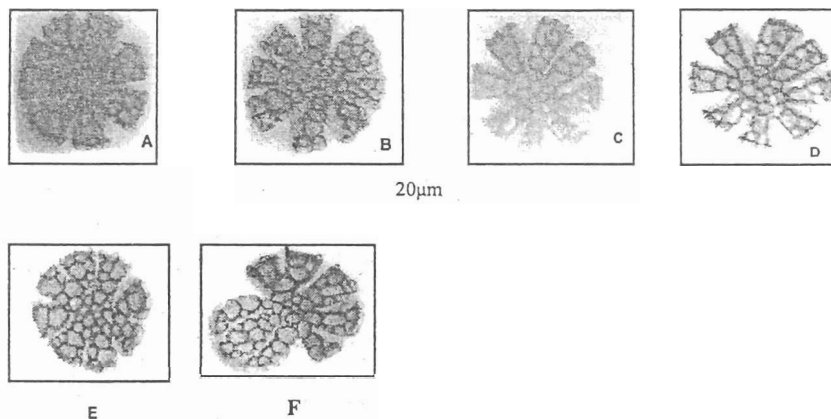


Plate 3: Pollen grains of *Ocimum* species

A and B: Heptacolpate, C - E: Octacolpate, F: Octacolpate (Figure 8 shape)

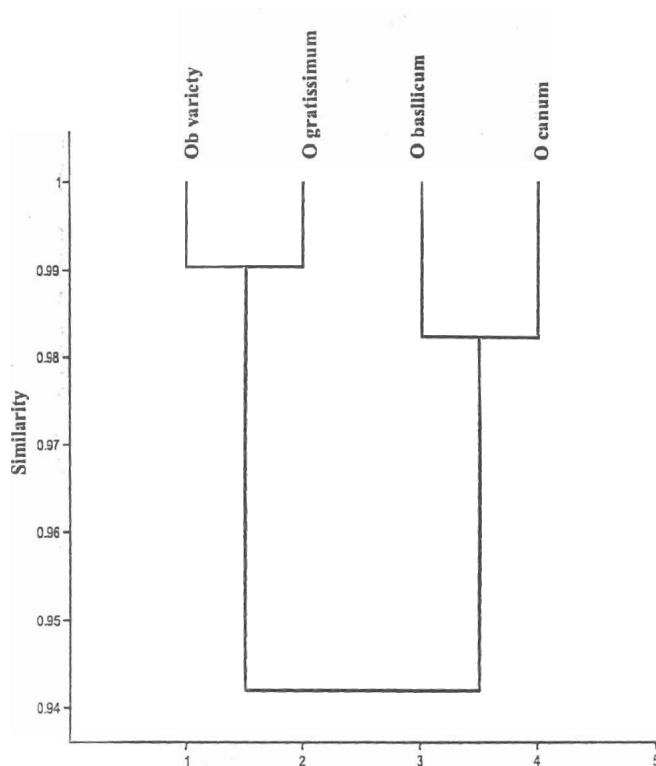


Fig. 1: Dendrogram of the pollen grain attributes of the four taxa of *Ocimum* studied

the species and the variety had hexacolpate pollen grain. Heptacolpate and octacolpate pollen grains were encountered only in *O. canum* which separated this species from the others while the morphologically different pollen grains like the concave in shape were encountered in *O. basilicum*, *O. gratissimum* and *O. basilicum* var. *purpurascens*, the ribbon-like were encountered in *O. canum*, *O. gratissimum* and *O. basilicum* var. *purpurascens* and the figure 8 types were encountered in *O. canum* only which again delimits *O. canum* from the other species and the cultivar.

Tetrazonocolpate pollen grains with four colpi arranged in an equatorial zone were encountered in *O. gratissimum* and *O. basilicum* var. *purpurascens* while hexazonocolpate pollen grains with six colpi in an equatorial zone were encountered in *O. canum* only according to the

descriptions of Moore and Webb (1978).

Heptacolpate and Octacolpate pollen grains, which are more advanced types of pollen, found to be occurring in *O. canum* were conspicuously absent in the other species and the variety. *O. canum* also has the largest size, highest colpi depth, highest number of pores and the largest distance between any pair of apertures. These further separate *O. canum* from the other species. The pollen grains of *O. basilicum* were distinct in having the thickest wall and the largest pore diameter size while *O. gratissimum* had the thinnest wall.

Analysis of Variance (ANOVA) reveals that there are significant differences within each attribute studied. Among the species and the variety, Duncan Multiple Range Test grouping of the *Ocimum* species and the variety based on

the pollen grain are significant grain attribute studied except *O. canum* and *O. gratissimum* which are not significantly different.

Pollen grains according to the classification of Perminuta (diameter 10-50µm), Magnata (diameter greater than 50µm), *Ocimum* could be classified into *O. canum* and *O. basilicum* var. *purpurascens* belong to the same group. In a similar study, pollen grains of *Hibiscus* in Nigeria were related here because they cluster at a similarity level of 0.94.

Number of colpi is a useful tool in identifying species among the species of dicotyledons, with either none at all (ac) (Asteraceae) (2005). It can be used to separate and identify only in *O. canum* and *O. basilicum* var. *purpurascens*.

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the pollen grain attributes also reveal that there are significant differences among the pollen grain attributes of the species and the variety studied except in *O. basilicum* var. *purpurascens* and *O. gratissimum* where there is no significant difference in the number of pores.

Pollen grains have been classified into groups according to their sizes by Erdtman (1952) as Perminuta (diameter less than 10µm), Minuta (diameter 10-25µm), Media (diameter 25-50µm), Magna (diameter 50-100µm), Permagna (diameter 100-200µm), Giganta (diameter greater than 200µm). Based on this classification, the pollen grains of the genus *Ocimum* could be described as media (diameter 25-50µm) and magna (diameter 50-100µm). *O. basilicum* var. *purpurascens* and *O. gratissimum* belong to the group media while *O. canum* and *O. basilicum* belong to magna. In a similar study, Akinwusi and Illoh (1996) used pollen grain sizes to group some species of *Hibiscus* in Nigeria. *O. basilicum* var. *purpurascens* and *O. gratissimum* are more closely related here based on pollen grain attributes, since they clustered together at a higher similarity level than that of *O. basilicum* and *O. canum*.

Number of colpi on pollen grains has been a useful tool in tracing evolutionary relationship among the species of a genus. The advanced dicotyledons have more colpi than the primitive ones, with either a colpus (monocolpate) or none at all (acolpate) (Walker, 1976; Adedeji, 2005). It can thus be affirmed that the heptacolpate and octacolpate pollen grains observed only in *O. canum* is a mark of recent evolutionary development in the species.

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Editors: Prof. S. E. Owusu
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