

## TAXONOMIC IMPORTANCE OF THE PALYNOLOGICAL FEATURES OF SOME MEMBERS OF FAMILY LEGUMINOSAE- CAESALPINIOIDEAE

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### ABSTRACT

Acetolysed pollen grains of eight species in the Leguminosae- Caesalpinioideae were studied with the aim of identifying palynological characters of taxonomic importance among the taxa and determining their probable evolutionary relationships. The Acetolysis procedure and pollen type description were carried out following standard methods. The eight species include two species from genus *Cassia* Linn. – *Cassia sieberiana* DC and *Cassia fistula* Linn and six species from genus *Senna* Mill. - *Senna occidentalis* (Linn.) Link, *Senna siamea* (Lam.) Irwin and Barneby, *Senna podocarpa* (Guill. & Perr.) Lock, *Senna hirsuta* (Linn.) Irwin and Barneby, *Senna obtusifolia* (L.) Irwin and Barneby and *Senna sophera* (L.) Roxb. The pollens of all the species were shed as monads and they all had *perforate* sculpturing pattern on their exine. The pollen size of all the species falls into the group media (25 – 50 µm). All the species had ovate pollen shapes except for some additional circular, elliptic and club-shaped types observed in some of the species. All the species had monocolpate and tricolpate pollen grain types. Tetracolpate, pentacolpate and hexacolpate pollen grains, which are advanced pollen types were observed only in the species of *Senna* while the primitive pollen types, acolpate and monocolpate, were observed more in the members of the genus *Cassia*, thus, the evolutionary advancement of *Senna* over *Cassia* was discussed. Other pollen types observed in some of the species were bicolpate, trilete, diporate, tricolpate, and pentacolpate. Their importance in the identification and classification of the *Cassia* and *Senna* species was also discussed.

**Keywords:** Pollen grains, *Cassia*, *Senna*, Aperturate, Monocolpate, Tricolpate.

### INTRODUCTION

The Leguminosae family is one of the most successful flowering plants and the third largest family among the Angiosperms (LPWG, 2013) It is of much economic importance as food crops, ornamentals and weeds (Lewis *et al.*, 2005; Yahara *et al.*, 2013). Members of the family exhibit diverse habits and are found growing in different ecological zones of the world (Schrire *et al.*, 2005). About 770 genera and over 19,500 species have been reported for the family (Lewis *et al.*, 2005, 2013; LPWG, 2013). One of the best known economic importance of the family is the ability of its members to fix atmospheric nitrogen, thereby enriching the soil where they grow.

The family Leguminosae is classified into three sub-families, Caesalpinioideae DC., Mimosoideae DC., and Papilionoideae DC. (Lewis *et al.*, 2005; Cullen *et al.*, 2011). However, the classification was observed to be insufficient as a result of the diverse floral pattern in the family and especially the paraphyletic nature of sub-family

Caesalpinioideae (Doyle *et al.*, 1997; Bruneau *et al.*, 2008; LPWG, 2013). Based on this, the earlier classification into the three sub-families was pointed out as obsolete and a revision of the family was carried out using morphological and molecular data. This resulted into further division of the family into six subfamilies, Cercidoideae Detarioideae, Duparquetioideae, Dialioideae, recircumscribed Caesalpinioideae and Papilionoideae (LPWG, 2017).

Subfamily Caesalpinioideae has been reported to contain about 148 genera and ca. 4400 species which include trees, shrubs, lianas and suffruticose with some of its members armed with prickles or spines. The detailed morphological description of this subfamily is as presented in the writings of LPWG (2017). The subfamily has also been recognised as paraphyletic in which there is a need to clarify some taxonomic identity problems among its members (LPWG, 2017). The species worked on in this study are members of genera *Cassia* Linn. and *Senna* Mill. which both belong to the subfamily

Caesalpinioideae.

Genus *Cassia* L. *sensu lato* was raised to a subtribe level as subtribe Cassiinae from which three distinct genera were named: *Chamaecrista* Moench., *Senna* P. Mill. and *Cassia* L. *sensu stricto* (Irwin and Barneby, 1981 and 1982). This subdivision of *Cassia* L. *sensu lato* into three distinct genera has been ascertained by different authors around the world who employed data from various fields of evidence. Such authors include Marazzi *et al.* (2006); Acharya, *et al.* (2011) and Abdalla, *et al.* (2016).

Palynological evidences have proven useful at all taxonomic levels because the structure of the pollen wall which is usually being employed in the study is not affected by environmental factors Nnamani and Onu (2014). Palynological characters have therefore been variously employed in the taxonomy of species of plants, forensic sciences, crime site detection, archaeology, paleoecology, delimitation of closely related or sympatric species, valuable in phylogenetic inferences (Nyananyo, 1992; Akinwusi and Illoh, 1996; Edeoga *et al.*, 1998; Adedeji, 2005; Arogundade and Adedeji, 2009; Simpson, 2010, Arogundade and Lawal, 2018). According to Acharya *et al.* (2011), there are still a lot of taxonomic work to be done at the genera and specific levels of the subfamily Caesalpinioideae. This work therefore intends to use palynological evidence as a tool in the ongoing review of the paraphyletic subfamily Caesalpinioideae by identifying the different types of pollen grains and pollen characters of taxonomic importance among some species of *Cassia* and *Senna*.

## MATERIALS AND METHODS

The acetolytic method described by Erdtman (1960) and as modified by Arogundade and Lawal (2018) was employed in acetolysing the pollen grains collected from fresh anthers of eight members of the Caesalpinioideae. The members include two species from genus *Cassia* Linn. – *Cassia sieberiana* DC and *Cassia fistula* Linn. and six species from genus *Senna* Mill. – *Senna occidentalis* (Linn.) Link, *Senna siamea* (Lam.) Irwin and Barneby, *Senna podocarpa* (Guill. &

Perr.) Lock, *Senna hirsuta* (Linn.) Irwin and Barneby, *Senna obtusifolia* (L.) Irwin and Barneby and *Senna sophera* (L.) Roxb. The pollens were collected at different locations within Obafemi Awolowo University, Ile-Ife campus, Osun State 7.5165 °N, 4.5286°E and Saki, Oyo State, 8.6726°N, 3.3943°E, all in South Western part of Nigeria. They were stored in absolute ethanol prior to Acetolysis.

The acetolytic method includes series of centrifugation at 350 rpm for 20 minutes at each stage. The first stage involved the maceration of anthers in absolute ethanol. The supernatant was poured off and glacial acetic acid added for the next centrifugation. This solvent too was poured off after the centrifugation and acetolysis mixture (sulphuric acid and acetic anhydride in ratio 1:6) was added. The tubes containing these mixtures were then placed in a water bath and heated from a temperature of 70 °C to boiling point with intermittent stirring. Centrifugation was done again and the acetolysis mixture was poured off. Glacial acetic acid was added next, centrifuged and it was poured off. Finally, distilled water was added to the sediment and was followed by another centrifugation. The water was later decanted and the acetolysed pollen grains were mounted in dilute glycerine solution.

The acetolysed pollen grains were observed under Olympus XSZ-107BN binocular biological microscope (Zenith Laboratories, California). For each of the species, 150 pollens were chosen at random and studied. Attributes of the pollens which include the pollen shape, positions of the colpi and pores and pollen types based on the number of colpi and pores were described following the guidelines provided by Moore and Webb (1978) and Putt *et al.* (2007). Measurements of pollen diameter, depth of colpi, diameter of pore, distance between colpi and pollen wall thickness were taken with the aid of an ocular micrometer inserted into the eyepiece of the microscope. The various ocular readings were then converted into micrometer (µm) by multiplying each reading with the ocular constant which corresponds to the ocular magnification under which they were taken. The photomicrographs of the pollens were taken using an AmScope camera attached to the

microscope.

The data (quantitative and qualitative) generated from this study were subjected to univariate and multivariate statistical analysis. The mean values of each of the quantitative characters were calculated with the standard error. The data were coded and subjected to Single Linkage Cluster Analysis (SLCA) and Principal Components Analysis (PCA) using Paleontological Statistics Software Package (PAST).

## RESULTS

The pollen of all the species studied were shed as monads and the sculpturing pattern on their outer wall, the exine, is *perforate* sculpturing.

### Shape of Pollens

Four types of pollen shape were encountered in the species of *Cassia* and *Senna* studied (Figures 1 and 2; Table 1). They are ovate, circular, elliptic and club-shaped pollens. Ovate shape was observed as the main pollen shape in all the species. Elliptic pollen shape was observed in all the species as well but not in *S. podocarpa*. The frequency of the elliptic pollen shape was not as high as that of the ovate shape in the species where it was observed. Circular pollen shape, though sparsely encountered too, was observed in all the species except in *C. sieberiana* where only the ovate and elliptic pollen shapes were noted. The fourth pollen shape which is club-shape was observed only in *C. fistula* and not in any of the other species in this study.

### Position of Pores and Colpi

Pores were sparsely encountered among the species studied. When present they were observed in the association of one or two colpi at equatorial region and they are basically one, that is, the pollens with pores are monocolporate. The only exceptions to this were observed in *C. fistula* where some of its pollens were observed as having two pores, Diporate, in the equatorial region and in *S. podocarpa* where tetraporate pollen grains, with four pores were observed in the polar region. The colpi observed in the species ranges from one to six and they are situated at both the polar and equatorial regions of the pollens (Figures 1 and 2; Table 1).

### Pollen Types

The types of pollens observed among the species based on the number of colpi and pores on them are Acolpate (inaperturate), Monocolpate, Monocolporate, Bicolpate, Diporate, Tricolpate, Tricolporate, Trilete, Tetracolpate, Tetraporate, Pentacolpate, Pentacolporate and Hexacolpate.

All the species in this study had monocolporate and tricolpate pollen types. Pollens with no colpi at all, Acolpate types, were encountered in the two species of *Cassia* studied and *S. sophera*. Monocolpate pollen grain type was observed in *S. occidentalis*, *S. podocarpa*, *S. sophera* and *C. fistula*. Bicolpate types were observed in *S. occidentalis*, *S. siamea*, *S. obtusifolia*, *S. hirsuta*, *S. sophera*, *C. sieberiana* and *C. fistula*. Diporate pollen types with two pores were observed only in *C. fistula* while Tetraporate pollen grain types, pollens with four pores were observed only in *S. podocarpa*. Tricolporate types were observed in *S. occidentalis*, *S. siamea*, *S. obtusifolia*, *S. sophera* and *C. sieberiana*. Trilete pollen type was observed in all the *Senna* species except in *S. obtusifolia* and the two *Cassia* species. Tetracolpate pollens were encountered in *S. obtusifolia* and *S. hirsuta*. Pentacolpate pollens were encountered in *S. occidentalis* and *S. sophera*. Pentacolporate pollen types were observed only in *S. obtusifolia*. Hexacolpate pollen types were observed in *S. occidentalis*, *S. siamea* and *S. sophera* (Table 1; Figures 1 and 2).

Table 2 shows the mean values and the standard errors of the quantitative data measured on the pollens. Figure 3 shows the dendrogram of the Single Linkage Cluster Analysis which divided the species into two groups, in the first clustering, according to their genera. In the second grouping, *S. podocarpa* was distinctly separated from the other members of the genus *Senna*. Furthermore, *S. obtusifolia* and *S. hirsuta* were clustered together and separated from *S. siamea*, *S. sophera* and *S. occidentalis* with *S. sophera* and *S. occidentalis* being grouped together at a higher similarity level.

The result of the Principal Components Analysis corroborates the result of the Single Linkage

Cluster Analysis (SCLA) of the *Cassia* and *Senna* species. The first five components of ordination were responsible for 93.87% of the total variation observed among the species (Table 3). The graph

of the Principal Components Analysis of the species based on components 1 and 2 is as shown in figure 4 while that of components 1 and 3 is as shown in figure 5.

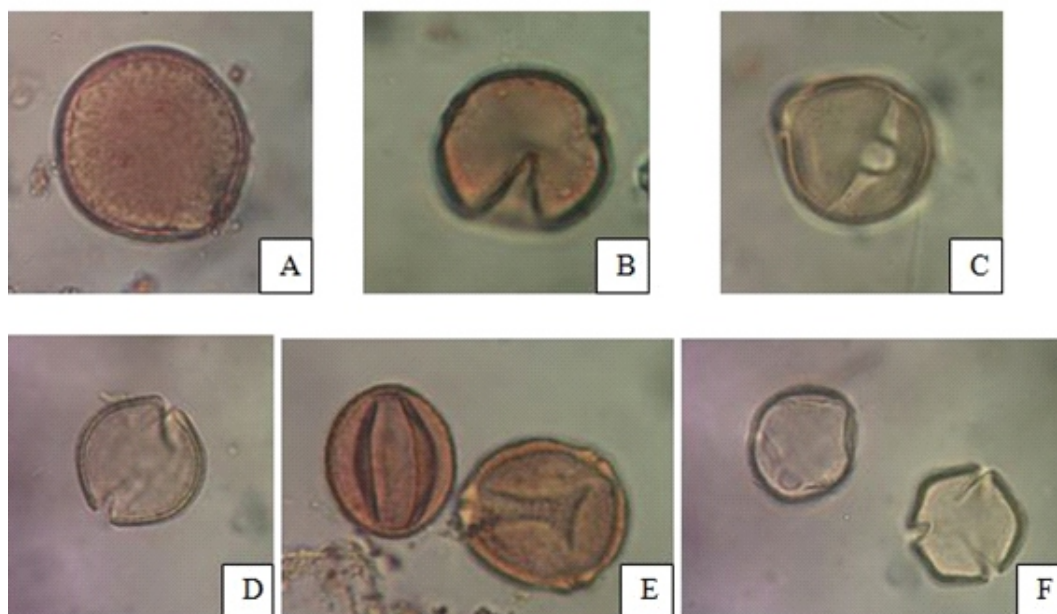
**Table 1:** Summary of the Qualitative Pollen Grain Characters of the Species of *Cassia* and *Senna* Studied

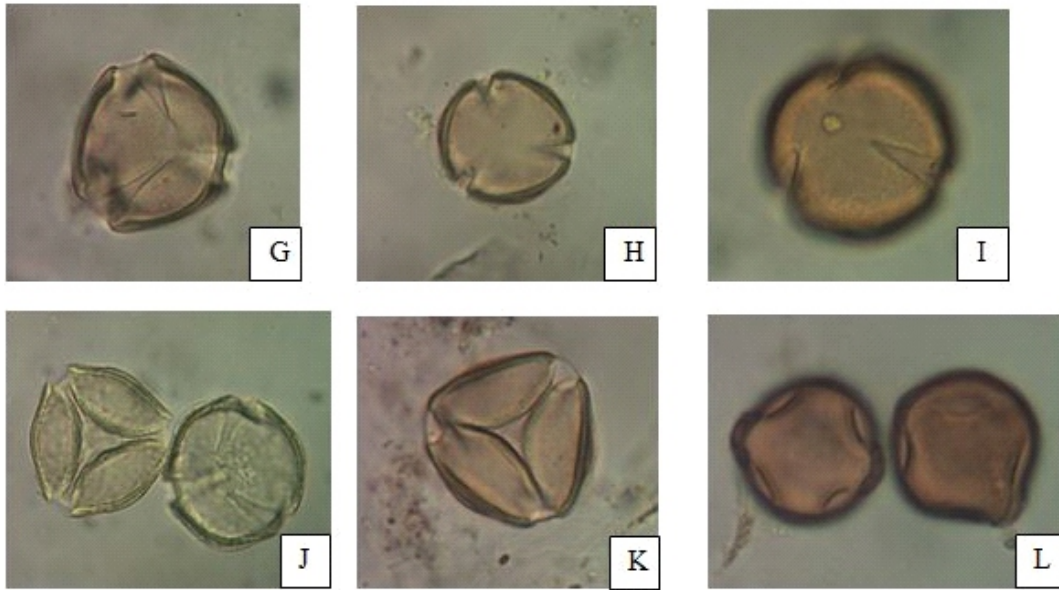
Species	Important Palynological Characters				Pollen types
	Shape of Pollens	Position of Colpi	Position of Pores		
<i>S. occidentalis</i>	Generally ovate, occasionally elliptic or circular	Polar, equatorial	Equatorial, associated with colpi		Monocolpate, Monocolporate, Bicolpate, Tricolpate, Tricolporate, Trilete, Pentacolpate, Hexacolpate,
<i>S. podocarpa</i>	Generally ovate, occasionally circular	Polar, equatorial	Polar and Equatorial. Associated or not-associated with colpi at the equatorial region		Monocolpate, Monocolporate, Tricolpate, Trilete, Tetraporate,
<i>S. siamea</i>	Generally ovate, occasionally circular or elliptic	Polar, equatorial	Equatorial, associated with colpi		Monocolpate, Bicolpate Tricolpate, Tricolporate, Trilete, Hexacolpate,
<i>S. obtusifolia</i>	Ovate, Circular, Elliptic	Polar, equatorial	Equatorial, associated with colpi		Monocolpate, Bicolpate, Tricolpate, Tricolporate, Tetracolpate, Pentacolporate
<i>S. hirsuta</i>	Ovate, Circular, Elliptic	Polar, equatorial	Equatorial, associated with colpi		Monocolpate, Bicolpate, Tricolpate, Trilete, Tetracolpate
<i>S. sophora</i>	Ovate, Circular, Elliptic	Polar, equatorial	Equatorial, associated with colpi		Acolpate, Monocolpate, Monocolporate, Bicolpate, Tricolpate, Tricolporate, Trilete, Pentacolpate, Hexacolpate
<i>C. sieberiana</i>	Generally ovate, occasionally elliptic	Polar region	Equatorial, associated with colpi		Acolpate, Bicolpate, Monocolporate, Tricolpate, Tricolporate
<i>C. fistula</i>	Circular, Ovate, Elliptic, Club-shape	Polar, equatorial	Equatorial, associated with colpi		Acolpate, Monocolpate, Monocolporate, Bicolpate, Diporate, Tricolpate

**Table 2:** Summary of the Quantitative Pollen Grain Characters of the Species of *Cassia* and *Senna* Studied

Species	Quantitative Palynological Characters				
	Pollen Diameter ( $\mu\text{m}$ )	Colpi depth ( $\mu\text{m}$ )	Pore Diameter ( $\mu\text{m}$ )	Pollen Wall Thickness ( $\mu\text{m}$ )	Distance between Colpi ( $\mu\text{m}$ )
<i>S. sophera</i>	32.88 $\pm$ 0.33	18.24 $\pm$ 0.39	0.27 $\pm$ 0.10	3.39 $\pm$ 0.01	21.26 $\pm$ 0.42
<i>S. obtusifolia</i>	35.84 $\pm$ 0.23	21.37 $\pm$ 0.49	5.44 $\pm$ 0.17	2.71 $\pm$ 0.05	21.19 $\pm$ 0.49
<i>S. hirsuta</i>	33.70 $\pm$ 0.20	18.85 $\pm$ 0.50	4.49 $\pm$ 0.14	2.64 $\pm$ 0.04	23.33 $\pm$ 0.32
<i>S. siamea</i>	34.73 $\pm$ 0.19	13.18 $\pm$ 0.39	7.18 $\pm$ 0.20	5.00 $\pm$ 0.05	29.42 $\pm$ 0.36
<i>S. occidentalis</i>	32.25 $\pm$ 0.35	10.85 $\pm$ 0.48	6.79 $\pm$ 0.22	4.33 $\pm$ 0.07	28.42 $\pm$ 0.32
<i>S. podocarpa</i>	31.95 $\pm$ 0.33	8.18 $\pm$ 0.25	8.35 $\pm$ 0.25	5.19 $\pm$ 0.06	26.07 $\pm$ 0.35
<i>C. fistula</i>	26.85 $\pm$ 0.29	14.20 $\pm$ 0.61	3.35 $\pm$ 0.13	2.58 $\pm$ 0.03	16.45 $\pm$ 0.39
<i>C. sieberiana</i>	25.02 $\pm$ 0.26	10.78 $\pm$ 0.30	4.53 $\pm$ 0.11	4.01 $\pm$ 0.06	19.98 $\pm$ 0.32

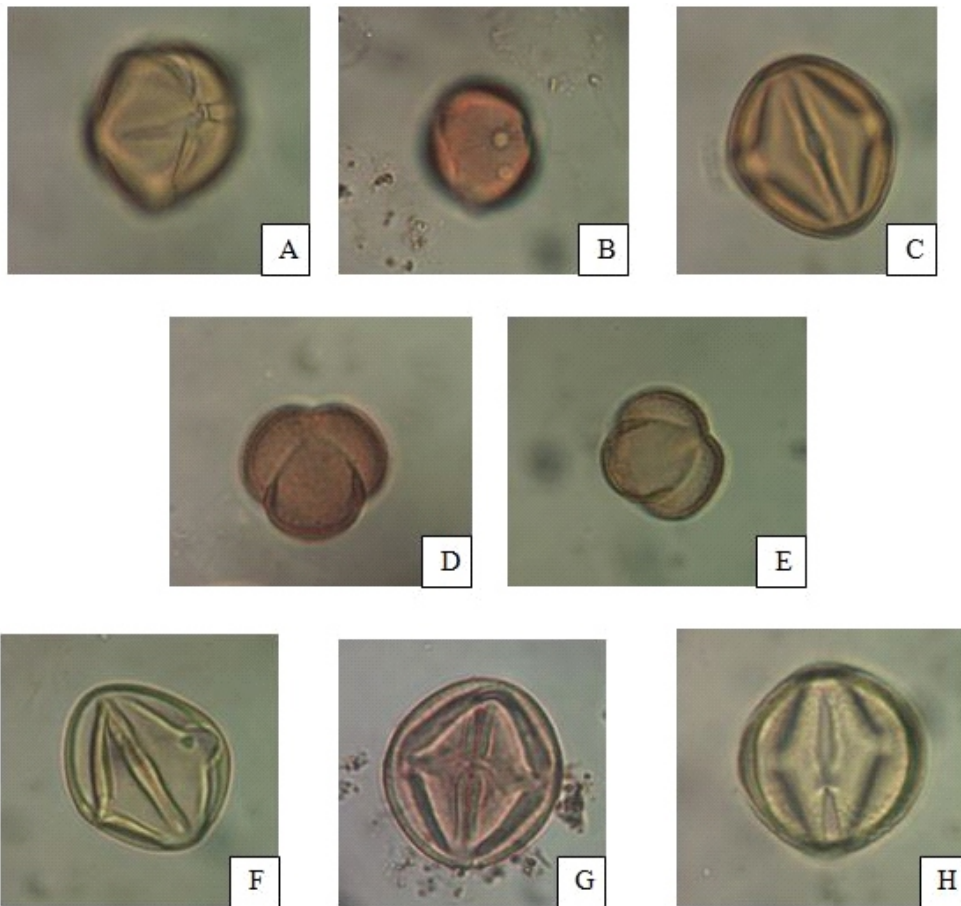
NB: values are means  $\pm$  Standard Error, n =150





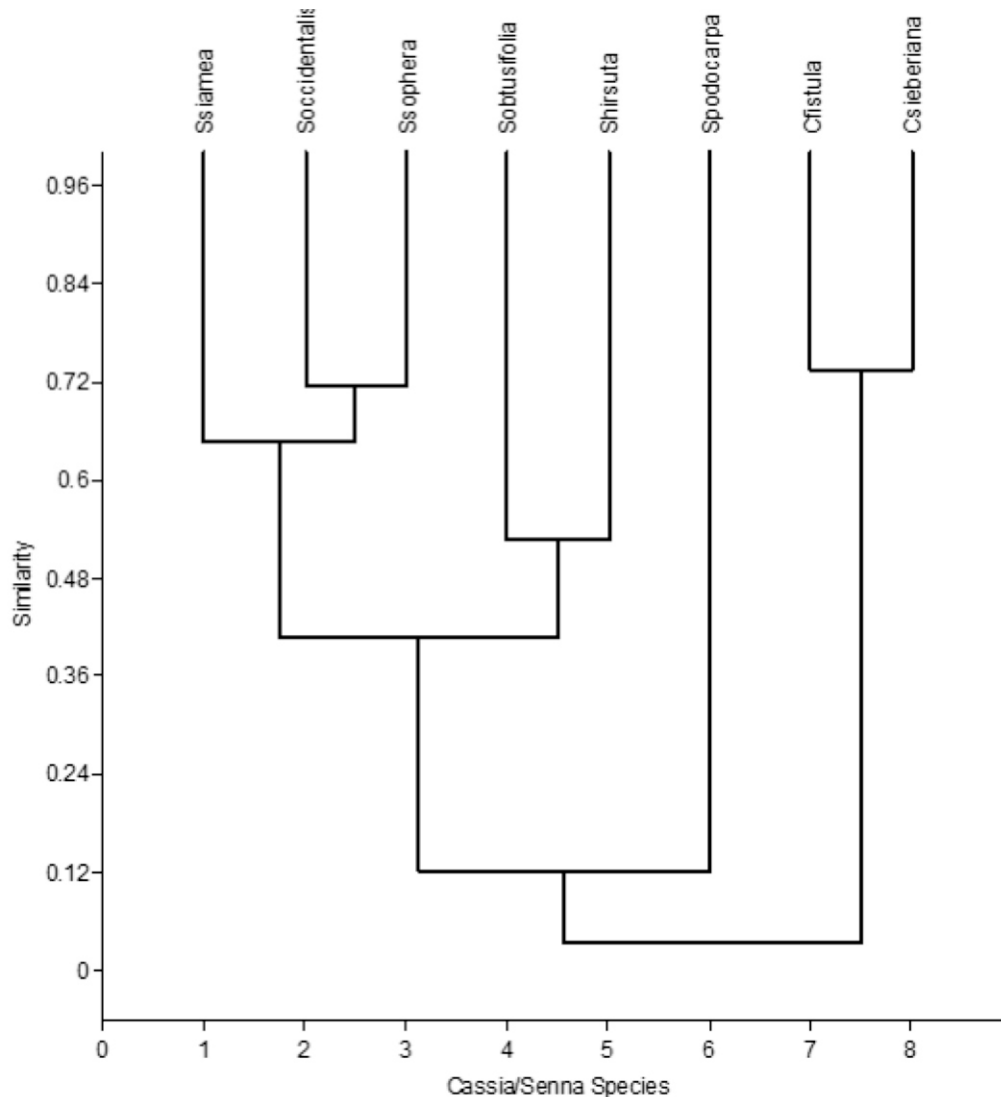
**Figure 1:** Pollen Grain Types in Species of *Cassia* and *Senna* Studied

A- Acolpate, B- Monocolpate, C- Monocolporate, D- Bicolpate, E- Bicolpate and Monocolpate, F- Monocolporate and Tricolpate, G and H- Tricolpate, I- Tricolporate, J- Trilete and Tricolpate, K- Trilete, L- Tetraporate. (Magnification x 400)



**Figure 2:** Pollen grain Types in Species of *Cassia* and *Senna* Studied

A- Tetracolpate, B- Diporate, C- Pentacolporate, D and E- Club-shaped (as found in *C. -fistula*), F- Pentacolpate, G and H- Hexacolpate. (Magnification x400)



**Figure 3:** Dendrogram of *Cassia* and *Senna* Species Based on their Qualitative and Quantitative Pollen Characters

## DISCUSSION

The members of the genera *Cassia* and *Senna* involved in this study had some pollen characters in common which affirms their sub-familial classification and close relationship as reported by Marazzi *et al.* (2006). All the species studied had aperturate pollen grains with either pores or colpi or both in diverse forms. Another important factor worthy of note is that all the pollens are dispersed as monads, that is, as single or individual units and the only sculpturing pattern observed on the exine of all the species is *perforate*.

However, a few pollen characters can still be employed in their delimitation both at the generic and specific levels. A character of interest in this

study is the shape of the pollens. Ovate shaped pollen grains were observed in all the species studied, yet other types of shapes encountered in some of the species can be employed in their delimitation. Elliptic pollen shape was observed in all the species both from the polar and equatorial views but not in *S. podocarpa*. In the same vein, pollen grains with circular shapes were observed in all the species except *C. sieberiana*. Another unique pollen shape described as club-shaped clearly demarcates *C. fistula* from *C. sieberiana* and the members of genus *Senna* in this study. Pollen shapes have been found useful in the delimitation of groups of plants (Moore and Web, 1978; Arogundade and Adedeji, 2009).

The pollen apertures (pores and colpi) observed in

this study also provided a good classification character among the species. Pores were not so commonly observed. The few ones encountered are circular in shape and were mostly found in association with colpi which are fissure-like openings. Varying number of colpi observed in the species offers a good classification character which is of high taxonomic significance. Nnamani and Onu (2014) reported only tricolporate pollens in the species of *Senna* studied. In this study, tricolporate pollens were observed in five of the species which cut across the two genera where additional pollen types were also observed. The only pollen types recorded to be common to all the species studied are monocolporate and tricolporate pollens.

Pollens with higher number of colpi were also reported in this study. The presence of higher number of colpi in any group of plants has been attributed to recent evolutionary advancement in such group of plants (Walker, 1976; Adedeji, 2005; Adedeji and Akinniyi, 2015; Arogundade and Lawal, 2018). Based on this assertion, the advancement of *Senna* over *Cassia* is established and this is as a result of the presence of more advanced pollen types- tetracolpate, pentacolpate and hexacolpate pollens, observed in the members of genus *Senna* and not in any of the members of *Cassia*. The primitive pollens types - acolpate and monocolpate pollen types observed in the species of *Cassia* also confirms the evolutionary advancement of *Senna* over *Cassia*. The presence of these primitive pollen grain types can thus be employed in the separation of the members of the genus *Cassia* from the members of the genus *Senna*. The uniqueness of diporate pollen types to *C. fistula*, tetraporate to *S. podocarpa* and pentacolporate types to *S. obtusifolia* provided a good basis for the identification of the species.

Polyploidization, which implies the presence of more than two chromosome sets per somatic cell, has been reported in the genus *Senna* (De Resende *et al.*, 2013; Cordeiro and Leonardo, 2018). This condition has also been ascertained to cause increase of cell size in plants where it occurs, pollen grains inclusive (Marinho, *et al.*, 2013). The pollen diameter of the *Senna* species in this study is relatively higher than that of the members of

the genus *Cassia* and this also supports the advancement of *Senna* over *Cassia* (Adedeji, 2005). Pollen grain sizes have been classified into various groups (Erdtman, 1952) and the classification has been adopted by many authors (Akinwusi and Illoh, 1996; Adedeji, 2005; Arogundade and Lawal, 2018). Based on this classification, the pollen size of all the species of *Cassia* and *Senna* in this study falls to the group *media*, with dimension between 25  $\mu\text{m}$  and 50  $\mu\text{m}$ .

The grouping of the species on the dendrogram resulting from the SCLA is in consonant with their respective genera, that is, members of genus *Cassia* were clearly separated from members of genus *Senna* in the first main grouping. The separation of *S. podocarpa* from the other *Senna* species in the second grouping was most likely due to the presence of tetraporate pollens in the species as this is the only species where tetraporate pollens were encountered. *S. podocarpa* is also the only species where bicolpate pollen grains were not observed. The closeness of *S. obtusifolia* and *S. hirsuta* was established in the third grouping and this is most likely due to the presence of tetracolpate pollen grains observed only in the two species. The fourth stage of grouping revealed *S. occidentalis* and *S. sophera* as close relatives at a higher similarity level which is due to the presence of pentacolpate and hexacolpate pollen types in the two. This similarity is not in consonant with the findings of Soladoye *et al.* (2010) who reported a distant relationship between *S. occidentalis* and *S. sophera* while conducting morphometric studies on some *Senna* species. There is therefore the need to carry out more research work especially on the members of genus *Senna* so as to clear any confusion that may arise as regards their relationships.

The outcome of the Principal Components Analysis shed more light on the identification and classification of the *Cassia* and *Senna* species. On the first component axis, the pollen diameter, pore diameter, distance between any two colpi, acolpate, bicolpate, diporate and trilete pollen grains are responsible for the separation. On the second axis component, the length of colpi, tetracolpate and pentacolpate pollen grain types



are responsible for the variation while component three recorded the pentacolpate pollen grain type as the character responsible for the variation observed. All these characters had high component loading and are helpful in the identification of the species.

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