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Pollen Morphological Study on the Genus *Cassia* L. *sensu stricto* from Sudan

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ABSTRACT

The significance of pollen morphology in the delimitation of various taxa and its importance to evaluate the systematic position of some species of the Leguminosae including the genus *Cassia sensu lato* have been provided by many authors. **Objective:** to revise the taxonomical relationship of members of the genus *Cassia* L. *sensu stricto* in Sudan, using pollen morphology as a primary character. **Results:** the genus *Cassia sensu stricto* can be identified at the generic level by the presence of tricolporate apertures, rugulate or psilate-microrugulate sculpture, perforate tecta and acute apices of ectoapertures. On the other hand, this genus exhibited notable interspecific variation with respect to characters such as: the presence of equatorial constrictions and ektexinous elements on the endexine membranes of the apertures. The species *Cassia sieberiana* encountered to exhibit intraspecific variation within the same species. **Conclusion:** Pollen morphological characters, being microscopic, in conjunction with attributes from other disciplines, may provide useful characters which will help in taxonomic treatments of this genus.

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INTRODUCTION

The genus *Cassia* L. *sensu lato*, a member of the Legume family (Family: Fabaceae/ Leguminosae, subfamily: Caesalpinioideae (APG, 2003)), has been segregated by Irwin and Barneby (1981, 1982) in their revision of subtribe Cassiinae in the New World, into three allied genera, namely *Cassia* L. *sensu stricto*, *Chamaecrista* Moench and *Senna* Miller. This treatment has been followed by many workers such as Lock (1988, 1989), Randell (1988, 1989, 1990) and Larsen and Hou (1996) in their works on the revision of subtribe Cassiinae Irwin & Barneby from Africa, Australia and Malaysia, respectively.

Several species of *Cassia* L. *sensu stricto* possess medicinal properties (Boulos, 1983; El Ghazali, 1986; AOAD, 1988; El Ghazali *et al*., 1998), hence they gained economical importance. They are included in many International Pharmacopoeias, for example British Herbal Pharmacopoeia (BHMA, 1983), Potter's New Cyclopaedia of Botanical Drugs and Preparations (Wren, 1988) and Indian Herbal Pharmacopoeia (IDMA, 2002) and were used for centuries ago.

Pollen morphological characters are often overlooked by many taxonomists due to the fact that they are so small (ca. 0.3 µm in diameter) for their identification (Faegri *et al*., 1989), and hence microscopic (both light and electron) techniques are prerequisite tools. The significance of pollen morphology in the delimitation of various taxa and its importance to evaluate the systematic position of some species of the Leguminosae including the genus *Cassia sensu lato* have been provided by many authors such as Erdtman (1952), Nair and Sharma (1962) and El Ghazali (1989). Perveen and Qaiser (1998), studying the pollen grains of the subfamily Caesalpinioideae in Pakistan, concluded that the pollen morphology of the subfamily is significantly useful at the generic and specific levels. They recognized three pollen types on the bases of apocolpium, mesocolpium and tectum features. Singh (2001) in a monograph on Indian subtribe Cassiinae, pointed out that, variations in ectine surface may be used as a base for delimiting certain related taxa.

In Sudan (including South Sudan), the genus *Cassia* L. *sensu stricto* is represented by seven species (Table 1). The present study is an attempt to elucidate the taxonomical relation between these species, using pollen morphology as a reference diagnostic character.

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MATERIALS AND METHODS

Collection of Pollen Material:

The study is based on the pollen morphology of six species out of the seven *Cassia sensu stricto* species present in Sudan (*Cassia manii* specimens examined do not contain pollen grains). Samples of pollen grains (taken from mature flowers/ buds) of each species were collected from living material or herbarium specimens. The permanent slides prepared and the voucher specimens for each of the species studied were deposited at the Herbarium of the Medicinal and Aromatic Plants Research Institute, National Centre for Research, Khartoum.

Preparation of Material For Light Microscopy (LM):

For preparation of permanent slides, the standard acetolysis method of Erdtman (1960) was used, which was summarized by El Ghazali (1989). The slides were examined under light microscope (OLYMPUS BX51) and photographed using digital camera (OLYMPUS DP20) attached to the same microscope. The dimensions of the polar axes (P) and the equatorial diameters (E) of all the pollen grains investigated were measured using a micrometer. The mean value (X) of 10 counts and the range for each parameter were calculated. The shape classes were deduced directly from the relationship between (P) and (E) (i.e P/E ratio) according to Erdtman (1952).

Preparation of Material For Scanning Electron Microscopy (SEM):

Samples of pollen for SEM of each species were collected from living material left to dry or from herbarium specimens. Material for SEM was prepared by mounting untreated dry pollen grains directly onto clean SEM stubs covered with double-sided cellotape. All stubs were coated with gold using a JEOL JFC 1100 E ion sputtering device. The pollen grains were then examined in a JEOL JSM 5400LV Scanning Electron Microscope (SEM), operated at an accelerating voltage of 15 kV and photographed using digital camera attached to the microscope.

Pollen Description And Terminology:

Pollen grains for each species were then described briefly (in a form of table of diagnostic characters) and an identification key for the studied species was constructed. Ten characters were described, namely: Polar axes (P), equatorial diameter (E) and Shape class; Type of apertures; Sculpturing; Shape in polar view; Shape of mesocolpi; Presence/ absence of ektexinous elements; Shape of apices of ectoapertures; Presence/ absence of costae colpi; Presence/ absence of equatorial constriction in ectoapertures; and finally the feature of endoapertures. The descriptive terminology of Faergri *et al.* (1989) was followed, more or less, throughout this study.

Results:

The list of the species representing the genus *Cassia sensu stricto* in Sudan, is summarized in the following table:

Table 1: List of species representing the genus *Cassia sensu stricto* in Sudan.

	Species name	Reference(s)	Note
1-	*Cassia arereh* Del.	Broun and Massey, 1929; Andrews, 1952; El Amin, 1990	Natural
2-	*C. fistula* L.	El Amin, 1990	Exotic but naturalized
3-	*C. mannii* Oliv.	Andrews, 1952; El Amin, 1990	Natural
4-	*C. javanica* ssp. *nodosa* (Roxb.) K. & S. Larsen	El Amin, 1990	Exotic but naturalized
5-	*C. thyrsoidea* Brenan	Herbarium specimens, Botany Department, Faculty of Science, University of Khartoum	Exotic but naturalized
6-	*C. grandis* L.f.	El Amin, 1981	Exotic but naturalized
7-	*C. sieberiana* DC.	Broun and Massey, 1929; Andrews, 1952; Wickens, 1976; El Amin, 1990	Natural

The morphology of the seven species representing the genus *Cassia sensu stricto* in Sudan, is summarized by the following key:

A.	Trees up to 8 m. high; branches glabrous; leaflets glabrous, apex acuminate, base cuneate; petioles 6-8 mm. long; racemes up to 10 cm long; bracts linear-lanceolate; pods brown, dehiscent …….. ***Cassia arereh***		
AA.	Trees 10 to 20 m. high; branches pubescent; leaflets pubescent, apex rounded or attenuate; petioles up to 5 cm. long; racemes more than 10 cm. long; bracts ovate or ovate-lanceolate; pods black, indehiscent:		
	B.	Leaflets 4-5 pairs:	
		C.	Trees up to 18 m. high; petals yellow; pods up to 60 cm. long ….. ***C. fistula***
		CC.	Trees up to 25 m. high; petals pink or white; pods up to 90 cm long …… ***C. mannii***
	BB.	Leaflets 6-14 pairs:	

		D.	Petioles 1-1.5 cm. long; inflorescence terminal leafy corymbs …….. ***C. javanica* ssp. *nodosa***		
		DD.	Petioles more than 1.5 cm. long; inflorescence solitary racemes or erect panicles:		
			E.	Leaflets sparsely pubescent beneath; inflorescence erect panicles …….. ***C. thyrsoidea***	
			EE.	Leaflets densely pubescent beneath; inflorescence solitary racemes:	
				F.	Leaflets oblong or elliptic-oblong; racemes erect, up to 15 cm. long; petals red or pink, up to 1.5 cm long …….. ***C. grandis***
				FF.	Leaflets elliptic or ovate-elliptic; racemes pendulous, up to 40 cm long; petals yellow, more than 1.5 cm long …….. ***C. sieberiana***

Pollen Morphological Characters examined:

A total number of 14 pollen morphological characters (quantitative and qualitative) were examined (table 2) for the species under consideration.

Polar axes (P), Equatorial diameters (E), P/E ratios and Shape Classes:

The smallest pollen grains (both in Polar axes and Equatorial diameters) was found to be that of *Cassia sieberiana* (P = 30.3 µm, E = 30.0 µm) (Fig. 5), while the largest Pollen grain (again in both Polar axes and Equatorial diameters) was found to be that of *C. grandis* (P = 49.2 µm, E = 35.0 µm) (Fig. 3). The shape classes which are deduced directly from the relationship between (P) and (E) (i.e P/E ratio) vary from prolate (P/E = 1.4) as in *C. grandis* (Fig. 3) to subprolate or prolate-spheroidal (P/E = 1.0 to 1.3) as for the other species.

Type of Apertures:

All the pollen grains of the various species examined are tricolporate, with three distinct ectoapertures (Colpi) and three distinct or indistinct endoapertures (Pores).

Sculpturing:

The sculpturing of the species examined are restricted to only two types. Psilate-microrugulate sculpturing type occurs in three species (*Cassia arereh* (Fig. 1), *C. grandis* (Fig. 3) and *C. javanica* subsp. *nodosa* (Fig. 4)), whereas rugulate type occurs in two species (*C. fistula* (Fig. 2) and *C. thyrsoidea* (Fig. 5)). *C. sieberiana* (Fig. 5), on the other hand, undergo intraspecific variations with respect to their sculpturing and the above mentioned two types can be identified in the same species.

Shape in Polar view:

Three shapes of pollen grains at the polar view are noticed, the subtriangular shape is dominant in four species (*Cassia arereh* (Fig. 1), *C. fistula* (Fig. 2), *C. grandis* (Fig. 3) and *C. thyrsoidea* (Fig. 5), triangular shape is noticed in one species (*C. javanica* subsp. *nodosa* (Fig. 4)), while one species (*C. sieberiana* (Fig. 5)) again undergo intraspecific variation between two shapes: subcircular-triangular.

Shape of Mesocolpi:

The shape of the mesocolpi in polar views may be convex, slightly convex or straight. Slightly convex shape of mesocolpi is the most dominant (four species: *C. arereh* (Fig. 1), *C. fistula* (Fig. 2), *C. javanica* subsp. *nodosa* (Fig. 4), *C. thyrsoidea* (Fig. 5), whereas convex shape is seen in one species (*C. grandis* (Fig. 3)) and straight/convex shapes also seen in one species (*C. sieberiana* (Fig. 5)).

Presence/ absence of ektexinous elements:

Ektexinous elements on the endexine membranes of the apertures are characteristically present in only one species (*C. javanica* subsp. *nodosa* (Fig. 4)), while it is absent in the other five species.

Shape of Apices of Ectoapertures:

The apices of the ectoapertures is predominantly acute in the six examined species.

Presence/ absence of costae colpi:

The presence of costae colpi (thickened edges of ectoapertures) occur in only two species (*C. fistula* (Fig. 2) and *C. grandis* (Fig. 3)), while it is totally absent from the other four species examined.

Presence/ absence of equatorial constriction in ectoapertures:

The presence of equatorial constrictions in the ectoapertures is a distinctive feature in two species (*C. arereh* (Fig. 1) and *C. thyrsoidea* (Fig. 5)), while it is totally absent from the other four species examined.

Feature of Endoapertures:

The endoapertures of the species examined are in the form of pores. These pores may be with or without protrusions, distinct or indistinct. Pores are distinct and protruding in one species (*C. arereh* (Fig. 1)), indistinct and protruding in two species (*C. grandis* (Fig. 3) and *C. thyrsoidea* (Fig. 5)), or indistinct and not protruding in three species (*C. fistula* (Fig. 2), *C. javanica* subsp. *nodosa* (Fig. 4) and *C. sieberiana* (Fig. 5)).

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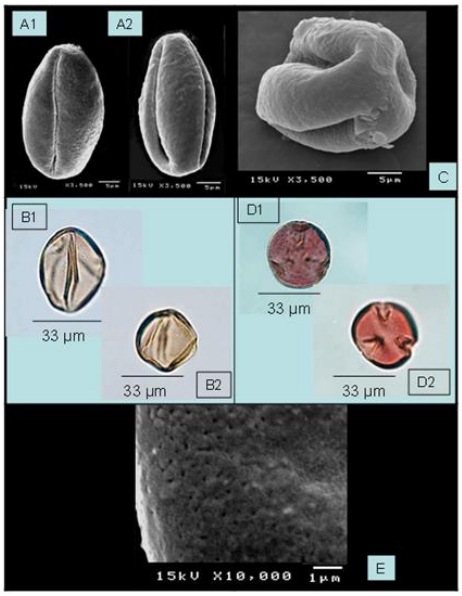

Fig. 1: Photomicrographs of *Cassia arereh* pollen grains; (A & B) equatorial view, (A) using SEM x3500, (B) using Light microscope; (C & D) polar view, (C) using SEM x3500, (D) using Light microscope; and (E) psilate sculpture, using SEM x10000.

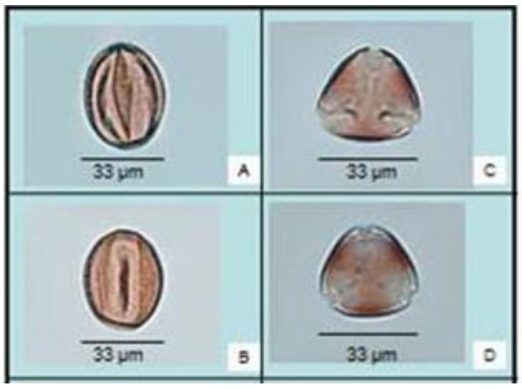

Fig. 2: Photomicrographs of *Cassia fistula* pollen grains using Light microscope. (A & B) equatorial view, (C & D) polar view.

An indented key to Pollen morphology of *Cassia sensu stricto* species examined:

A.	Pollen grains with rugulate sculpturing:			
	B.	Pollen with costae colpi ….		***Cassia fistula***
	BB.	Pollen without costae colpi:		
		C.	Ectoapertures (colpi) with equatorial constrictions ……	***C. thyrsoidea***
		CC.	Ectoapertures (colpi) without equatorial constrictions	***C. sieberiana***
AA.	Pollen grains with psilate-microrugulate sculpturing:			
	D.	Pollen with costae colpi …….		***C. grandis***
	DD.	Pollen without costae colpi:		
		E.	Endoapertures distinct ……....	***C. arereh***
		EE.	Endoapertures indistinct ……..…….....…	***C. javanica* subsp. *nodosa***

Table 2: Pollen morphological data of *Cassia sensu stricto* species examined.

	Species	P (µm)	E (µm)	P/E	Shape Class
1-	*Cassia arereh*	39.3 (33.3-43.3)	32.6 (30.3-33.3)	1.2	subprolate
2-	*C. fistula*	43.3 (40.0-46.7)	33.3 (30.0-36.7)	1.3	subprolate
3-	*C. grandis*	49.2 (43.3-50.0)	35.0 (30.0-40.0)	1.4	prolate
4-	*C. javanica* subsp. *nodosa*	36.0 (33.3-40.0)	33.3 (36.0-36.7)	1.1	prolate-spheroidal
5-	*C. sieberiana*	30.3 (23.3-33.3)	30.0 (26.7-33.3)	1.0	prolate-spheroidal
6-	*C. thyrsoidea*	36.3 (33.3-43.3)	33.3 (30.0-36.7)	1.1	prolate-spheroidal

Continue Table 2: Pollen morphological data of *Cassia sensu stricto* species examined.

	Species	Type of apertures	Sculp-ture	Shape in polar view	Shape of mesocolpi	Presence (+)/ absence (-) of endexine elements
1	*Cassia arereh*	Tricolporate	PM	SubT	Slightly convex	-
2	*C. fistula*	Tricolporate	R	SubT	Slightly convex	-
3	*C. grandis*	Tricolporate	PM	SubT	Convex	-
4	*C. javanica* subsp. *nodosa*	Tricolporate	PM	T	Slightly convex	+
5	*C. sieberiana*	Tricolporate	R/PM	SubC/ T	Straight/ convex	-
6	*C. thyrsoidea*	Tricolporate	R	SubT	Slightly convex	-

Continue Table 2: Pollen morphological data of *Cassia sensu stricto* species examined.

	Species	Ectoapertures			Endoapertures	
		Apices	Costae	Presence (+)/ absence (-) of equatorial constrictions	Pores with(+)/ without (-) protrusions	Pores distinct(+)/ indistinct(-)
1	*Cassia arereh*	Acute	Uniform	+	+	+
2	*C. fistula*	Acute	Thicker at the equator	-	-	-
3	*C. grandis*	Acute	Thicker at the equator	-	+	-
4	*C. javanica* subsp. *nodosa*	Acute	Uniform	-	-	-
5	*C. sieberiana*	Acute	Uniform	-	-	-
6	*C. thyrsoidea*	Acute	Uniform	+	+	-

Legend:
PM ≡ Psilate microrugulate sculpture; R ≡ Rugulate sculpture;
C ≡ Circular; SubC ≡ Subcircular; T ≡ Triangular; SubT ≡ Subtriangular shapes in polar view.

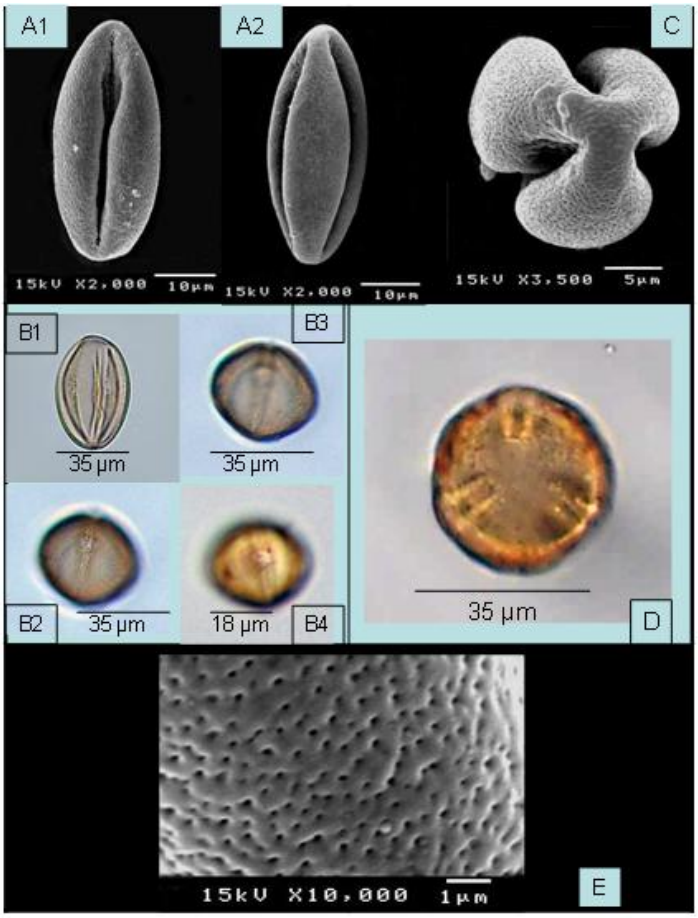

Fig. 3: Photomicrographs of *Cassia grandis* pollen grains: (A&B) equatorial view, (A) using SEM X2000 (B) using light microscope. See ectoaperture constricted at the equator in A1 and in B1; see granulated colpus in B2; see endoaperture thickened in B3&B4; (C&D) polar view, (C) using SEM X3500, (D) using light microscope and (E) sculpture, using SEM X10000.

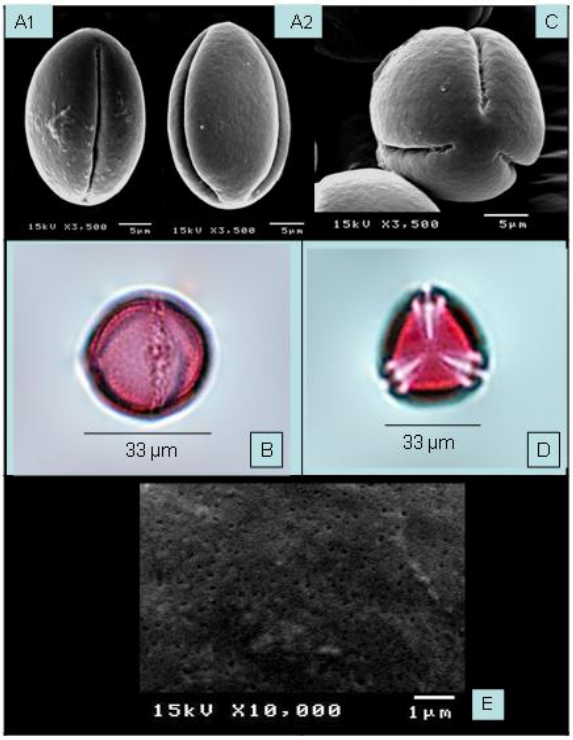

Fig. 4: Photomicrographs of *Cassia javanica* sub sp. *Nodosa* pollen grains: (A1&2; B) equatorial view, (A1&2) using SEM X3500 (B) using light microscope: (C&D) polar view, (C) using SEM X3500, (D) using light microscope and (E) sculpture, using SEM X10000.

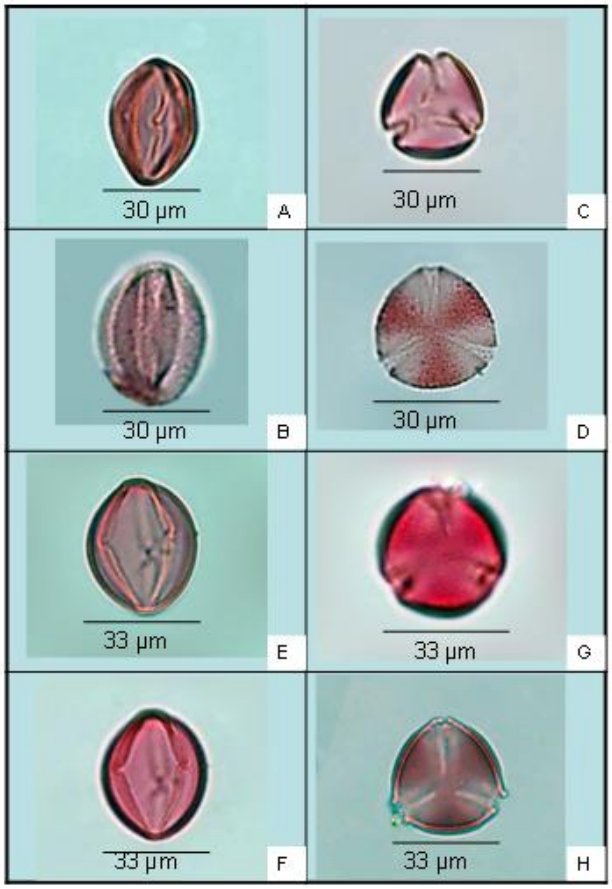

Fig. 5: Photomicrographs of *Cassia sieberiana* pollen grains using light microscope: (A&B) equatorial view, (C&D) polar view and *C. thyrsoidea*: (E&F) equatorial view, (G&H) polar view.

Discussion:

The pollen morphology of six species belonging to the genus *Cassia sensu stricto* present in the Sudan was investigated with Light microscope (LM) and Scanning Electron Microscope (SEM) to provide more microscopic attributes to contribute to the taxonomy of this taxon.

A total number of 14 characters (both quantitative and qualitative) were examined to highlight the most diagnostic attribute (s) to be used in the taxonomic treatments of the species examined.

All of the species studied have tricolporate pollen grains with rugulate or psilate- microrugulate sculpturing, perforate tecta and acute apices of ectoapertures. Other pollen morphological characteristics exhibited considerable variations, in spite of the existence of certain diagnostic features such as the presence of the equatorial constrictions and ektexinous elements on the endexine membranes of the apertures, the latter characters makes this genus interspecifically variable.

Pollen morphological descriptions of various species of the genus *Cassia sensu lato* were provided by a number of authors e.g. Vishnu-Mittre and Sharma (1962), Barth and Bouzada (1964), Smith (1964), Maley (1970), Fredoux (1977), etc. Most of these previous studies were done with Light microscopic (LM) techniques only; as a result, some discrepancies in their description from the present study were noticed. The use of Scanning Electron Microscopic (SEM) techniques in the present study have enormously extended the range of magnifications at which the pollen grains may be studied, and consequently the images obtained are more readily interpreted, so that micro-structures can be accurately elucidated. In addition, the use of different terminology is another factor which leads to differences in pollen morphological description between the present and previous studies. These differences may be due to different use of the same term or the same use of different terms, as previously noticed by El Ghazali (1993).

As revealed from the present study, the genus *Cassia sensu stricto* exhibited a notable intraspecific variability in three of its members with respect to its sculpturing namely; *Cassia fistula*, *C. sieberiana* and *C. thyrosoidea*. In addition, *Cassia sieberiana* showed intraspecific variation not only in sculpture but also in other characters such as shape in polar view and shape of mesocolpi. This phenomenon of intraspecific polymorphism was previously elucidated in the genus *Cassia* L. by Miege and Darrasse (1959), Nair and Sharma (1962), Melhem and Labouriau (1963), Barth and Bouzada (1964), Labouriau *et al*. (1965), and El Ghazali (1993), however, it is beyond the scope of this study to reinvestigate it, because different types of data need to be collected.

Conclusion:

1- The genus *Cassia sensu stricto* can be identified at the generic level by the presence of tricolporate apertures, rugulate or psilate-microrugulate sculpture, the perforate tecta and acute apices of ectoapertures.
2- The genus *Cassia sensu stricto* exhibited, on the other hand, notable interspecific variation with respect to characters such as: the presence of the equatorial constrictions and ektexinous elements on the endexine membranes of the apertures.
3- The species *Cassia sieberiana* encountered to exhibit intraspecific variation within the same species.
4- Pollen morphological characters, being microscopic, in conjunction with attributes from other disciplines, may provide useful characters which will help in taxonomic treatments of the genus.

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