ISSN: 2287-688X

Annals of
Plant Sciences

Volume 11, Issue 01 (2022) pp. 4662-4669

Research Article

Diversity Assessment in Some *Cassia* Species Based on Pollen Morphological Characters from Vidarbha Region of Maharashtra, India

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Abstract

The inert nature of pollen characters towards strong selective forces favored its utility as a taxonomic tool. The present work assessed the diversity among the selected species of *Cassia* using pollen morphological characters. Pollen morphology of ten *Cassia* species was studied by Scanning Electron Microscope and Light. A total of 23 pollen morphological characters were observed. All the species showed distinct variations in terms of shape, size, aperture, and exine ornamentation characters. In all ten *Cassia* species, a major similarity in aperture type was observed having tricolporate to trizonocoplorate. Size ranges between 23-40 µm, while, shapes were oblate, spheroidal-prolate, spheroidal. Psilate or psilate perforate, reticulate, or microreticulate tectum was noted in SEM observation.

Keywords: *Cassia, Caesalpiniaceae, LM, Pollen morphology, SEM.*

Introduction

The genus *Cassia* L. is widely distributed and consists of about 500 to 600 species (Airy-Shaw 1973; Singh 2001). It is probably the largest of the leguminous genera in the sub-family Caesalpinioideae. The genus *Cassia* is categorized among the 25 largest genera of the dicotyledonous plants (Irwin &Turner, 1960). *Cassia* L. has been recognized as a heterogeneous group. The taxonomy and nomenclature of *Cassia* L. species are quite complex and intriguing. Bentham (1871) divided the genus *Cassia* into three genera and nine sections. Britton & Rose (1930) split the genus *Cassia* into twenty-eight genera. Irwin and Baneby (1982) realizing diversity and complexity raised the genus *Cassia* L. to the levels of subtribe (Cassiinae) and raised the sub-genera *Senna* Mill. and *Chamaecrista Moench* to genetic level. Currently, this genus has followed several probing lines of classification, because of the extreme morphological resemblances and ambiguous boundaries between taxa (Soladoye *et al.*, 2010). This unstable taxonomic boundary is mainly due to difficulty in the taxonomic identification and interpretation of similar morphological features in the genus *Cassia*. Marazzi *et al.*, (2006) noted that the high degree of specialization typical of the buzz-pollinated *Cassia* flowers complicates the identification of characters that can be unambiguously used for their taxonomic identification and delineation.

The advances in micro-diagnostic techniques endorsed palynology as a significant field of study. Different features of pollen external surface such as apertural pattern, exine pattern, shape, and symmetry are very conventional for the taxonomic judgment of the plants (Perveen, 2006; Bera *et al.*, 2007; Keshavarzi *et al.*, 2012). The external surface features of pollen 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 electron and light) techniques are prerequisite tools for

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DOI: http://dx.doi.org/10.21746/aps.2022.11.01.14

discrimination among the plant species. The importance of pollen features in the discrimination of different taxon and the significance in evaluating the systematic position of some species of the Leguminosae including the genus *Cassia* has been provided by many authors such as Erdtman (1952); Nair and Sharma (1962); El Ghazali (1989); Jumah (1991-1996) & Aftab and Perveen (2006). Labouriau *et al.,* (1965) studied the pollen variation of polar axis and equatorial diameters in two species of *Cassia* viz., *C. cathartica* and *C. rugosa*. Wherein, magnitude of polar axis was reported to be more than the equatorial axis. Singh (2001) in a monograph on Indian sub-tribe Cassiinae, pinpointed the variations in ectine surface that may be used as a base for delimiting certain related taxa. Recently, Abdalla (2014) made an attempt to elucidate the taxonomical relation between seven *Cassia* sp. from Sudan using pollen surface features as a reference diagnostic character.

The high level of magnification for the evaluation of pollen features was achieved by the electron microscope. The present investigation assessed the diversity among available species of *Cassia* based on pollen morphology evaluation using Scanning Electron Microscopy (SEM) and Light Microscopy (LM).

Materials and Methods

The pollen grains from ten genera of *Cassia* (Table 1) was collected from the campus of Sant Gadge Baba Amravati University, Amravati (Maharashtra), and stored in 70% alcohol. The studied taxa were identified from Floras (Naik, 1998 and Yadav, 2005). The collected material was crushed with a glass rod in a plastic centrifuge tube and crushed material was filtered through fine meshes to isolate pollen grains. The methods described by Erdtman (1960) and Arora & Modi (2008) were utilized for the preparation of pollen and subsequent analysis by electron and light microscopy. Pollen grains mounted in glycerin jelly were taken for observations by Carl Zeiss's Trinocular Fluorescence Microscope (Axiostar HBO 50/AC). For Scanning Electron Microscopy (SEM) studies, pollen grains were suspended in a drop of ethanol and directly transpired with a fine pipette to a metallic stub using double-sided cello tape and coated with gold-palladium in a sputtering chamber (POLARON SPUTTER COATER). The SEM examination was carried out on a LEO electron microscope (LEO 430). The measurements are based on data from 10 pollen collected by an ocular micrometer. For the diversity assessment, pollen grain size, colpi size, pore size was measured (Table 1). The terminology used is in accordance with Faegri and Iverson (1964), Erdtman (1971), Bhattacharya *et al.,* (2006), Agashe (2006), and Punt *et al.,* (2007). A total of 23 features of *Cassia* sp. were observed separately for morphological analysis.

Results

Description of pollen morphological characters**:**

Cassia alata L.

Pollen grain features showed Polar Axis (PA) 31.30-32 µm, Equatorial Axis (EA) 28-30 µm, radially symmetrical, prolate spheroidal, polar outline triangular obtuse, equatorial outline elliptic, trizonocolporate, colpi 19.19 µm long and 4.5-5 µm wide, colpi tapering towards the end, separated into two by ori, mesocolpium 18.1-19.21 µm, apocolpium 5.51-6.66 µm, pori circular, 5.5-6.6 µm in diameter, exine 1.65-2.0 µm, tectum psilate [Fig. 1 (LM, Polar View (PV)), Fig. 2 (SEM, PV, Mag. 2.23 KX) Table 1].

Cassia auriculata L.

Pollen grain PA 25.2 (26.04) 27µm, EA 29.97(34.86) 36.52, radially asymmetrical, prolate spheroidal-sub-prolate, polar outline triangular-obtuse, equatorial outline rhombic acuminate obtuse, trizonocolporate, colpi 26.56 µm long and 3.99 µm wide at equator, colpi broad, sides parallel with rounded ends, pori 2.66-3.16 µm in diameter, mesocolpi 23.56-26.09 µm, exine 1.76-2.40 µm thick, tectum fossulate-rugulate or rarely coarsely reticulate [Fig. 3-4 (LM, PV and Equatorial View (EV)), Fig. 5 (SEM, EV, Mag. 4.31 KX) Table 1].

***Cassia bicapsularis* L.**
Pollen grain PA 23.97 µm, EA 27.66 µm, radially symmetrical, suboblate-oblate spheroidal, polar outline triangular, equatorial outline elliptic, trizonocolporate, colpi 21.20 µm long and 3.22-3.68 µm wide, mesocolpium 18.90 µm, apocolpium 8.75 µm, pori circular, 2.30 µm wide, colpi linear to narrowly elliptic, tips obtuse, exine 1.93-2.44 µm thick, tectum microreticulate perforate, N3P4C5 [Fig. 6-7 (LM, PV and EV), Fig. 8-9 (SEM, PV and EV, Mag. 4.03 KX and 5.40 KX) Table 1].

***Cassia biflora* L.**
Pollen grains, PA 23.43-25 µm, EA 26.98-32.66 µm, radially symmetrical, sub-oblate, Polar Outline triangular, equatorial outline elliptic, trizonocolporate, colpi 22.72 µm long and 2.84-3.55 µm wide, colpi narrowly elliptic, mesocolpium 15.2 µm, apocolpium 6-6.8 µm, pori circular, 3.55µm in diameter, exine 1.47-2.31 µm thick, tectum psilate to perforate N3P4C5 [Fig. 10(LM, EV), Fig. 11 (SEM, EV, Mag. 1.38 KX) Table 1].

***Cassia fistula* L.**
Pollen grain PA 34.86-36.92 µm, EA 32.69-33.43 µm, radially symmetrical, prolate spheroidal, polar outline triangular, equatorial outline elliptic, tricolporate, colpi linear to narrowly elliptic, tips acute, colpi faint, 22.5-24.57 µm long and 5.6-5.76 µm wide, pori 3.22-3.72 µm wide, mesocolpi 26.8-28.55 µm, apocolpi 15.7-18.3 µm, exine 1.86-2.40 µm thick, punctitegillate or tectum microreticulate perforate, N3P4C5 [Fig. 12(LM, PV), Fig.13 (SEM, PV, Mag. 5.46 KX) Table 1].

***Cassia javanica* L.**
Pollen grain PA 24.32- 26.86 µm, EA 36.8-37.2 µm, radially symmetrical, oblate-sub-oblate, polar outline triangular, equatorial outline elliptic, tricolporate, colpi narrowly elliptic, 30-32 µm long and 5.28-5.77 µm wide, pori circular, mesocolpi 19.96-21.83 µm, apocolpi 12.76-13.69 µm, exine 2.24-3.17 µm in thickness, tectum psilate- microreticulate perforate, N3P4C5 [Fig. 14-15 (LM, PV and EV), Fig. 16 (SEM, EV, Mag. 3.58 KX) Table 1].

***Cassia occidentalis* L.**
Pollen grains, PA 32.92 µm, EA 37.63 µm, radially symmetrical, sub-oblate-oblate spheroidal, polar outline triangular, equatorial outline elliptic, trizonocolporate, colpi 29.52-38 µm long and 3.52-4.70 µm wide, colpi narrowly elliptic, tips acute, mesocolpium 27.04 µm, apocolpium 8.82-9.40 µm, pori circular, 4.70 µm in diameter, exine 1.77-2.62 µm in thickness, tectum finely reticulate, N3P4C5[Fig. 17-18(LM, PV and EV), Fig. 19-20 (SEM, PV and EV, Mag. 1.14 KX and Mag. 1.14 KX) Table 1].

***Cassia obtusifolia* L.**
Pollen grain PA 36.45-38.80 µm, EA 41.16-42.84 µm, radially symmetrical, oblate spheroidal, polar outline triangular obtuse, equatorial outline elliptic, trizonocolporate, colpi 34.10 µm long and 3.52-4.70 µm wide, colpi narrowly elliptic, separated into two by a bridge formed over ori, tips acute, ori circular-elliptic, 5.88 µm wide, mesocolpium 31.75-32.92 µm, apocolpium 13.52-15.87 µm, exine 2.08-2.31 µm thick, tectum microreticulate perforate,N3P4C5 [Fig. 21-22(LM, PV and EV), Fig. 23-24 (SEM, PV and EV, Mag. 1.21 KX and Mag. 1.21 KX) Table 1].

***Cassia siamea* Lam.**
Pollen grains, PA 33.32-34.15 µm, EA 35.11-37.48 µm, radially symmetrical, oblate-spheroidal, polar outline triangular obtuse, equatorial outline elliptic, trizonocolporate, colpi 28.99-30.82 µm long and 6.8 µm wide, colpi tapering towards the end, separated into two by pori, mesocolpium 26.4-28.4 µm, apocolpium 6-7.2 µm, pori circular, 5.8-6.4µm in diameter, exine 2.73-3.30 µm in thickness, tectum psilate to perforate, N3P4C5 [Fig. 25-26 (LM, PV and EV), Fig. 27-28 (SEM, PV and EV, Mag. 3.27 KX and Mag. 3.27 KX) Table 1].

***Cassia tora* L.**
Pollen grain PA 29-30 µm, EA 34-36 µm, radially symmetrical, prolate spheroidal-Sub-prolate, polar outline triangular obtuse, equatorial outline elliptic, trizonocolporate, Colpi linear to narrowly elliptic, colpi 27-29 µm long and 4.59-5.35 µm wide, mesocolpium 25.24 µm, apocolpium 8.41-9.18 µm, pori

circular, 3-5 µm wide, exine 1.44-2.04 µm thick , tectum psilate- verrucate, heterobrochate, N3P4C5 [Fig. 29-30(LM, PV and EV), Fig. 31-32 (SEM, PV and EV, Mag. 1.32 KX and Mag. 1.32 KX) Table 1].

Table 1: Pollen grain characteristics of *Cassia* species.

Sr. No.	Name of taxon	Pollen grain size (µm) P×E	Pollen shape	Aperture pattern	Colpi/pori size (µm)	Exine ornamentation
1	*Cassia alata*	31.30 × 28	Prolate Spheroidal	Trizonocolporate	19.19 × 4.5	Psilate
2	*Cassia auriculata*	26.04 × 34.86	Prolate spheroidal-Sub-prolate	Trizonocolporate	26.56 × 3.99	fossulate-rugulate or rarely coarsely reticulate.
3	*Cassia bicapsularis*	23.97 × 27.66	Suboblate-oblate spheroidal	Trizonocolporate	21.20 × 3.68	Microreticulate perforate
4	*Cassia biflora*	23.43 × 26.98	Sub-oblate	Trizonocolporate	22.72 × 2.84	Psilate perforate
5	*Cassia fistula*	24.5 × 27.25	Oblate spheroidal	Tricolporate	9.81 × 2.18	Punctitegillate?
6	*Cassia javanica*	24.32 × 36.8	Oblate-sub-oblate	Tricolporate	30-32 × 1.81	Psilate-microreticulate perforate
7	*Cassia occidentalis*	32.92 × 37.63	Sub-oblate-oblate spheroidal	Trizonocolporate	28 × 3.52	Finely reticulate
8	*Cassia obtusifolia*	36.45 × 42.84	Oblate spheroidal	Trizonocolporate	34.10 × 4.70	Microreticulate perforate
9	*Cassia siamea*	33.32× 35.11	Oblate-spheroidal	Trizonocolporate	28.99× 6.8	Psilate perforate
10	*Cassia tora*	29 × 34	Prolate spheroidal-Sub-prolate	Trizonocolporate	4.59 × 5.35	Psilate

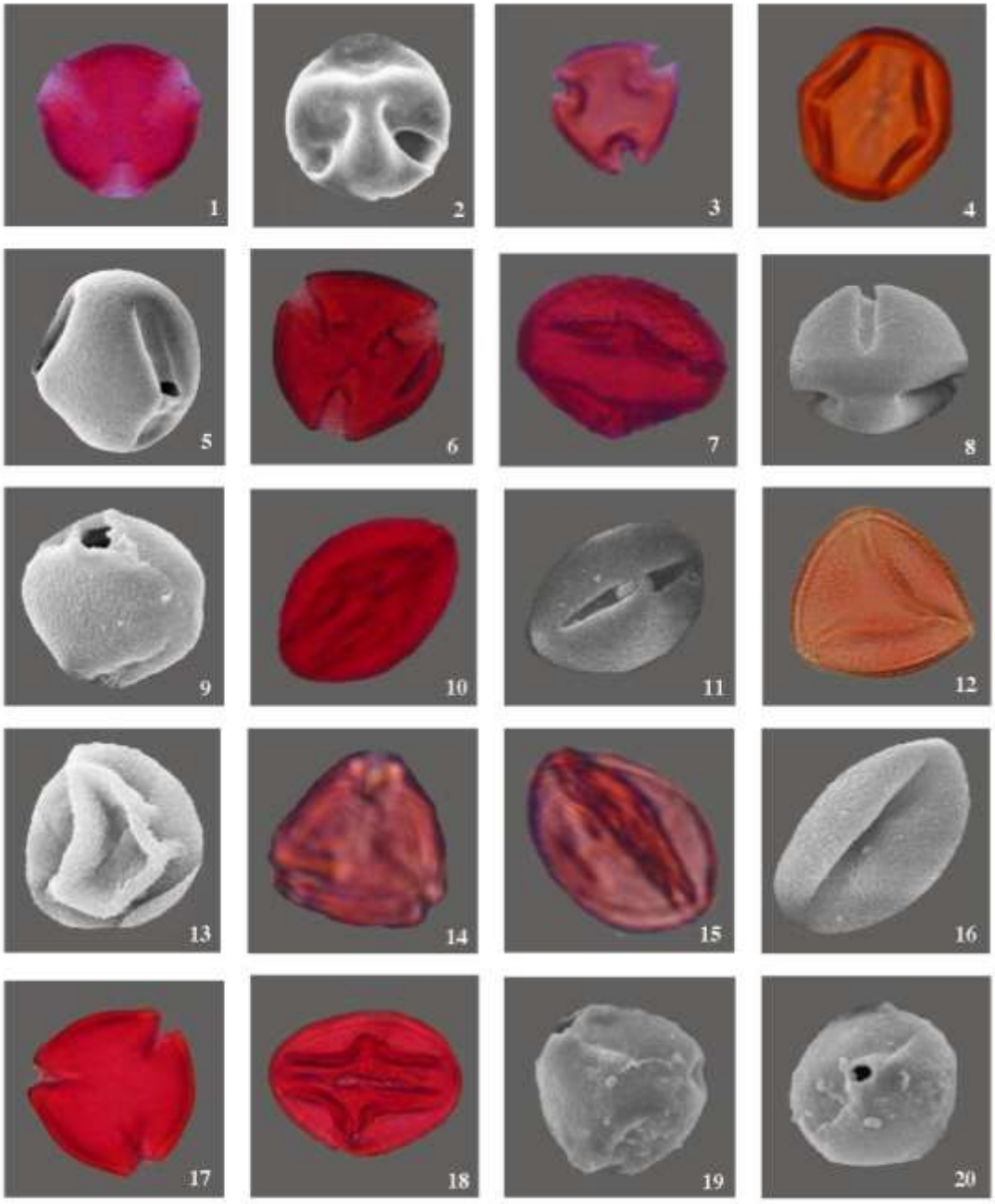

Fig. 1-20 Light and Scanning Electron Micrograph showin structure and exine sculpture of pollen grain: **Fig. 1-2** *Cassia alata* (PV), **Fig. 3-5** *Cassia auriculata* (PV and EV), **Fig. 6-9** *Cassia bicapsularies* (PV and EV), **Fig. 10-11** *Cassia biflora* (EV), **Fig. 12-13** *Cassia fistula* (PV), **Fig. 14-16** *Cassia javanica* (PV and EV), **Fig. 17-20** *Cassia occidentalis* (PV and EV). PV- Polar view, EV- Equatorial view.

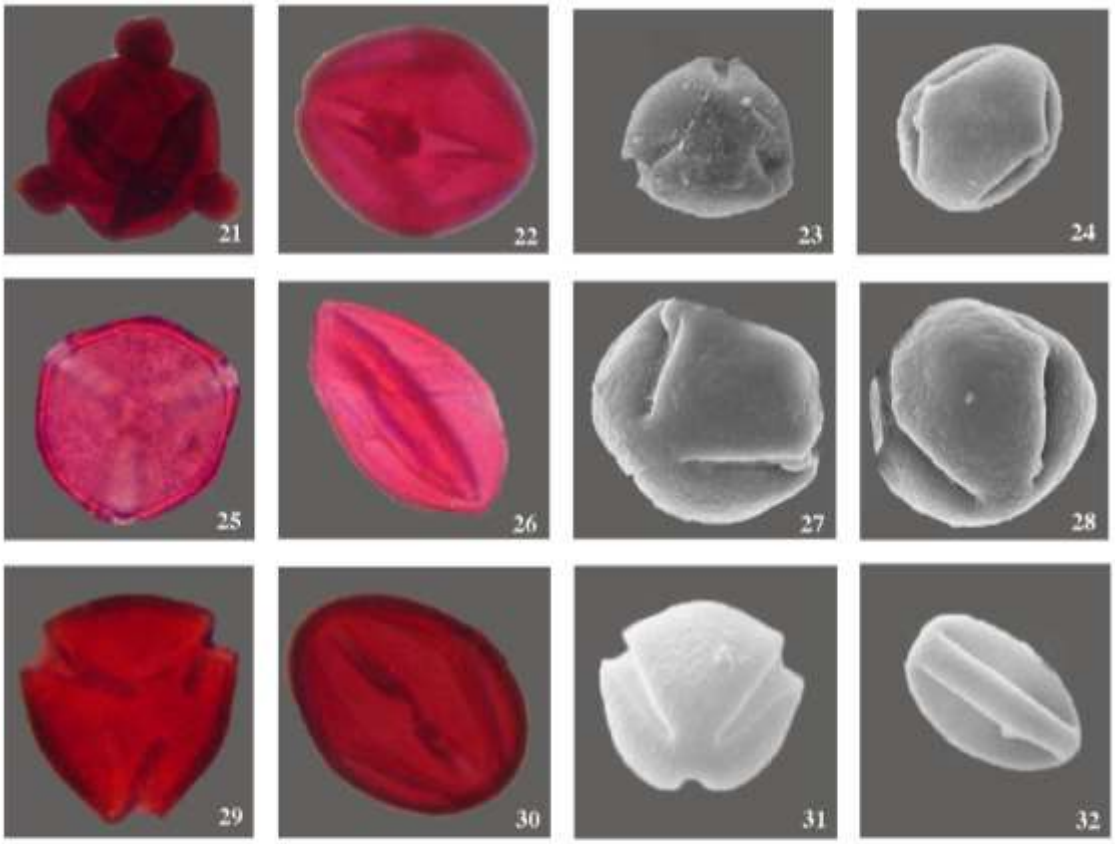

Fig. 21-32 Light and Scanning Electron Micrograph showin structure and exine sculpture of pollen grains: **Fig. 21-24** *Cassia obtusifolia* (PV and EV), **Fig. 25-28** *Cassia siamea* (PV and EV), **Fig. 29-32** *Cassia tora* (PV and EV). PV- Polar view, EV- Equatorial view.

Discussion

Pollen morphological characteristics study is a precise method of relating and differentiating one plant genus from another. The present investigation on pollen micro-morphological characters suggests that qualitative and quantitative features of the pollen could be used to discriminate species.

In all ten *Cassia* species, many similarities in aperture type were observed, i.e. having feature tricolporate to trizonocoplorate. Psilate or psilate perforate tectum is noted in *Cassia tora*, *Cassia alata*, *Cassia siamea*, *Cassia javanica*, *Cassia biflora*, whereas reticulate or microreticulate exine pattern occurred in *Cassia occidentalis*, *Cassia auriculata*, *Cassia bicapsularis*, *Cassia obtusifolia*. In contrast to the above, *Cassia fistula* showed punctitegillate exine ornamentation. Rao and Lee (1970) and Aftab and Perveen (2006) reported similar exine ornamentation i.e. reticulate-rugulate tectum within *Cassia fistula*, *Cassia javanica* and *Cassia siamea* based on LM. Whereas, in the present investigation, SEM observation reveals more clear reports showing different tectum namely punctitegillate or microreticulate perforate in *Cassia fistula*, psilate- microreticulate perforate in *Cassia javanica*, and psilate perforate in *Cassia siamea*. Besides, the other characters were found to be similar. Nayar (1990) studied pollen morphology of five genera of *Cassia* viz. *Cassia auriculata*, *Cassia fistula*, *Cassia mimosoides*, *Cassia occidentalis* and *Cassia tora*; and noted psilate tectum in all of them. While, in the present study, the same taxon of *Cassia* showed variable exine ornamentation, and the remaining characters showed homology.

Pollen morphological descriptions of various species of the genus *Cassia* L. were provided by a number of authors e.g. Vishnu-Mittre & Sharma (1962), Barth and Bouzada (1964),

Smith (1964), Maley (1970), Fredoux (1977). Most of these previous studies were based on LM techniques only. As a result, some discrepancies in their description from the present study were noticed. In addition, the use of different terminology is another factor that 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).

Conclusion

In the genus *Cassia* L., all ten species showed major similarity in aperture type i.e. tricolporate to trizonocoplorate. Pollen micro-morphological characters suggest diversity in the studied genus *Cassia*. Among all studied characters SEM based surface structure was found to be most significant for the identification of species. The qualitative and quantitative micro-morphological features considered in this study have the potential utility to discriminate the species.

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Source of support: Nil; **Conflict of interest:** Nil.

Cite this article as:

Rajurkar, A.V. and Vivek, R.N. "Diversity Assessment in Some *Cassia* Species Based on Pollen Morphological Characters in Vidarbha Region of Maharashtra, India." *Annals of Plant Sciences*.11.1 (2022): pp. 4662-4669.

DOI: http://dx.doi.org/10.21746/aps.2022.11.1.14