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Comparative Pollen Morphological Studies of Forest Plant Species of Leguminosae

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ABSTRACT

Pollen morphological studies of different forest plant species representing three families Fabaceae, Caesalpiniaceae and Mimosaceae of Leguminosae were undertaken. Morphological characters were studied using microscopic study and the pollen key based upon differentiating characters was prepared. The diversified characters of the sporomorphs provide an important basis for generic and specific delimitations. Among the various categories of pollen morphological characters, the aperture is considered as primary and most conservative, exine ornamentation as secondary and other characters as tertiary in order of their importance. The four plant species belonging to Fabaceae i.e. *Butea monosperma, Dalbergia sisoo, Gliricidia maculata* and *Pongamia pinnata* showed similarities in aperture pattern but variations in shapes and sizes. The five plant species *Bauhinia variegata, Cassia siamea, Delonix regia, Peltophorum pterocarpum* and *Tamarindus indica* belonging to family Caesalpiniaceae showed the variations in exine pattern. The pollen types belonging to Mimosaceae i.e. *Acacia arabica, Acacia nilotica, Albizzia lebbeck* and *Pithecellobium dulce* are polyado-polyad excluding *Prosopis juliflora* i.e. monad. An attempt has been made to see how far palynology helps in taxonomy and throws light on the evolutionary trends. Pollen morphology is widely used in taxonomic treatments particularly with differences in exine structure and aperture forms. These investigations revealed that the palyno-taxanomic characters help to arrange the pollen genera and families bio-systematically. The data generated through the work will be helpful to taxonomist and modern palynologist to predict the relation between these three families of Leguminosae.

Keywords: Pollen morphology, Fabaceae, Caesalpiniaceae, Mimosaceae, Leguminosae.

INTRODUCTION

Pollen morphology has wide applications in diverse fields of applied Palynology. The study of pollen morphology is applicable in various fields such as Palyno-taxonomy, Mellitopalynology, Aeropalynology, Palaeopalynology and Forensic Palynology. It helps to determine the phylogenetic relationship amongst different plant species and

subsequently helps to solve many stratigraphical and taxonomical problems (Sharma, 1970). Palynology used in morpho-taxonomy has attained a significant role in biosystematic investigations (Sudhakar and Rao, 1987). Microscopic studies provide information about pollen morphological characters such as shape, size, symmetry, AMB, pollen wall stratification, sculpture pattern, ornamentation and aperture type.

Palynology is the study of pollen and spores. Palynological studies are not only helpful in solving many taxonomic problems but also useful to know the incidence of pollen which causes pollenosis and to know the bee forage plants by means of melittopalynological studies. So, research on pollen morphology is undertaken to know the importance of pollen characters in plant taxonomic and phylogenetic relationship.

As circumscribed by Bentham and Hooker (1962), the order Rosales includes nine families including Leguminosae. Hutchinson (1959) included Leguminosae under a separate order Leguminales and treated its three sub-families i.e. Papilionoideae, Caesalpinioideae and Mimosoideae. According to recent treatment these three sub-families are considered as separate families i.e. Fabaceae (Papilionaceae), Caesalpiniaceae and Mimosaceae. So the interest have been come forward to collect the pollen morphological relationship in between these three families.

Earlier investigations on pollen morphology and palynotaxonomy revealed that pollen morphological characters are interrelated within genus and family and are phylogenically important. Such reports were produced in some species belonging to Bombacaceae (Sharma, 1970), Compositae (Perveen, 1999) Tiliaceae and Sterculiaceae (El-Husseini, 2006) and Moringaceae (Perveen and Quiser, 2009). Nayar (1990) studied *Dalbergia volubilis* and *Pongamia pinnata* belonging to Fabaceae and observed some morphological similarities in between these two species. Zuraw (2007) studied some *Allium* species, Thangaraja and Ganesan (2008) studied *Terminalia paniculata* and Dematteis and Pire (2008) studied some species of *Vernonia* and also reported some inter-generic and inter-specific similarities in pollen morphological features. Study of pollen diversity in Osmanabad district was carried out by Devarkar (2011). Rajurkar, Tidke and Patil (2011) conducted studies on pollen morphology of *Ipomoea* species. Rajurkar *et al.* (2013) described Palynomorphological studies on family Mimosaceae. The pollen morphological studies of Anacardiaceae from Chandrapur and Gadchiroli districts of Vidarbha region of Maharashtra state is conducted by Athavale (2014). Rajurkar and Tidke (2017) conducted study on family Solanaceae. The study of pollen morphology in some dicotyledonous families including Malvaceae, Asteraceae, Convolvulaceae and Verbenaceae was carried by Nasare (2022) from Nagpur, Maharashtra.

The study of morphology of pollen grains is necessity for Palynology in the recognization and identification of pollen grains (Arora and Modi, 2008). The pollen morphology of 10 endemic legumes of India belonging to 10 different genera was studied by Balan *et al.* (2016). In the recent years the data from pollen morphology has been significantly used for taxonomic purposes. Therefore, the present research was undertaken to study the pollen morphology of some selected members of Fabaceae, Caesalpiniaceae and Mimosaceae.

MATERIALS AND METHODS

Pollen morphological studies of different forest plant species representing three families Fabaceae, Caesalpiniaceae and Mimosaceae has been undertaken during 2020 to 2023 from the collected pollen grains from the forest of Marathwada and Vidarbha region of Maharashtra State in India. Particularly visits are made in forest areas of Amravati, Akola, Washim, Nanded, Parbhani, Jalna and Hingoli Districts.

The plant species selected from Fabaceae were *Butea monosperma, Dalbergia sisoo, Gliricidia maculata* and *Pongamia pinnata.* The plant species selected from Caesalpiniaceae were *Bauhinia variegata, Cassia siamea, Delonix regia, Peltophorum pterocarpum* and *Tamarindus indica.* The pollen types selected from Mimosaceae were *Acacia arabica, Acacia nilotica, Albizzia lebbeck, Pithecellobium dulce* and *Prosopis juliflora.*

Pollen samples were collected in bulk before anther dehiscence. Intact anthers were crushed and pollen sediment was obtained in 70% ethanol. The sediment was mounted with glycerin jelly on microscopic slide. Same samples were also carried for pollen preparation by acetolysis to note the exine characters. Various

pollen morphological characters were recorded after microscopic studies. The pollen measurements were taken using occular and stage micrometer. Key based upon differentiating characters was prepared.

The acetolysis of pollen sample was carried to remove protoplast so that the exine characters would be made discernible to facilitate better identification of pollen. The pollen material was acetolysed as per the method suggested by Erdtman (1960) and modified by Nair (1960). Pollen sediment was mounted with glycerin jelly on slides for microscopic observations. The pollen grains were observed for various morphological characters under Light Microscope. The reference slides were prepared for further studies.

OBSERVATIONS AND RESULTS

The pollen types of Mimosaceae i.e. *Albizzia lebbeck* and *Pithecellobium dulce* show similar pollen morphological characters as polyads, 16 celled, individual pollen triporate and AMB squarish. The pollen types *Acacia arabica* and *Acacia nilotica* also showed similar characters as rest of the species of Mimosaceae but differing in AMB shape i.e. squarish to irregular. The pollen types belonging to Fabaceae and Caesalpiniaceae were found to be tricolporate differing in AMB shape i.e. spheroidal, spheroidal to rounded, rounded, rounded- triangular and triangular.

All studied pollen types belonging to Fabaceae and Caesalpiniaceae showed similarities in exine surface i.e. reticulate excluding *Butea monosperma, Gliricidia maculata, Tamarindus indica* and *Cassia siamea* which observed with psilate to granular surface. The four plant species belonging to Fabaceae i.e. *Butea monosperma, Dalbergia sisoo, Gliricidia maculata* and *Pongamia pinnata* showed similarities in aperture pattern but variations in shapes and sizes. The five plant species belonging to family Caesalpiniaceae i.e. *Bauhinia variegata, Cassia siamea, Delonix regia, Peltophorum pterocarpum* and *Tamarindus indica* showed the variations in exine pattern. The pollen types belonging to Mimosaceae i.e. *Acacia arabica, Acacia nilotica, Albizzia lebbeck, Pithecellobium dulce* and *Prosopis juliflora* are found to be polyado-polyad excluding *Prosopis juliflora* i.e. monad. Pollen grains of Fabaceae are found to be small in size, isopolar, radially symmetric, prolate spheroidal, tricolporate mostly. Where as in Caesalpiniaceae they are generally symmetrical, isopolar, oblate to prolate-spheroidal, rarely sub-prolate, and tricolporate. In Mimosaceae, pollen grains may be monads or polyads or both (Table No. 1).

Table No. 1 Pollen morphological characterization.

Sr. No.	Pollen Type	Family	Shape, Size and Symmetry	Apertural Pattern (NPC Parameter)	Pollen Wall Structure and Sculpture
1.	*Butea monosperma*	Fabaceae	Oblate spheroidal, AMB – rounded, medium-sized, 35-41 µm, P = 35-37 µm, E = 38-41 µm, P/E = 0.90-0.92, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters -colporate, NPC: $N_3 P_4 C_5$	Exine thick, tectate, surface psilate to granular
2.	*Dalbergia sissoo*	Fabaceae	Prolate spheroidal, AMB - triangular, medium sized, 20-23 µm, P = 18-20 µm, E = 20-23 µm, P/E = 0.86-0.90, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters - colporate, NPC: $N_3 P_4 C_5$	Exine thick, surface faintly reticulate
3.	*Gliricidia maculata*	Fabaceae	Spheroidal, AMB - rounded triangular, medium sized, 26-30 µm, P = 28-30 µm, E = 26-28 µm, P/E = 1.06-1.07, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters - colporate, colpi long with tapering ends, ora lalongate, NPC: $N_3 P_4 C_5$	Exine thick, tectate, surface psilate to faintly granular
4.	*Pongamia pinnata*	Fabaceae	Subprolate, AMB - triangular, medium	Tricolporate, number - 03 (tritreme),	Exine thick, subtectate, surface

Sr. No.	Pollen Type	Family	Shape, Size and Symmetry	Apertural Pattern (NPC Parameter)	Pollen Wall Structure and Sculpture
			sized, 28-32 µm, P = 28-30 µm, E = 28-32 µm, P/E = 0.93-1.0, radially symmetrical	position - zonotreme, characters - colporate, colpi linear, acute tips, NPC: $N_3 P_4 C_5$	reticulate
5.	*Bauhinia variegata*	Caesalpiniaceae	Subprolate to prolate, AMB – rounded triangular, medium-sized, 30-37 µm, P = 30-32 µm, E = 34-37 µm, P/E = 0.86-0.88, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters -colporate, NPC: $N_3 P_4 C_5$	Exine thick, seriate reticulate pattern
6.	*Cassia siamea*	Caesalpiniaceae	Subprolate to prolate, AMB – rounded triangular, medium-sized, 32-35 µm, P = 32-34 µm, E = 34-35 µm, P/E = 0.94-0.97, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters -colporate, colpi linear, long, ora circular NPC: $N_3 P_4 C_5$	Exine thick, tectate, surface psilate to granular
7.	*Delonix regia*	Caesalpiniaceae	Oblate to suboblate, AMB - spheroidal, large sized, 50-63 µm, P = 48-53 µm, E = 57-63 µm, P/E = 0.84, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters - colporate, colpi long, ora faint and rounded, NPC: $N_3 P_4 C_5$	Exine thick, subtectate, surface reticulate, heterobrochate, reticular meshes smaller near aperture
8.	*Peltophorum pterocarpum*	Caesalpiniaceae	Oblate spheroidal, AMB - spheroidal to rounded, large sized, 52-57 µm, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters - colporate, colpi long, NPC: $N_3 P_4 C_5$	Exine thick, subtectate, surface reticulate, heterobrochate
9.	*Tamarindus indica*	Caesalpiniaceae	Spheroidal, AMB - rounded triangular, medium sized, 38-45 µm, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters - colporate, colpi long, acute tips, ora lolongate, NPC: $N_3 P_4 C_5$	Exine thick, surface striate to radially granular
10.	*Acacia arabica*	Mimosaceae	Polyad, 16-celled, spheroidal, large-sized, 50-54 µm, each cell 16-18 µm, AMB – squarish to irregular, radially symmetrical	Individual pollen triporate, number - 03 (tritreme), position - zonotreme, characters- porate, NPC: $N_3P_4C_4$	Exine - thick, tectate, surface psilate
11.	*Acacia nilotica*	Mimosaceae	Polyad, 16-celled, spheroidal, large-sized, 44-48 µm, each cell 13-15 µm, AMB – squarish to irregular, radially symmetrical	Individual pollen triporate, number - 03 (tritreme), position - zonotreme, characters- porate, NPC: $N_3P_4C_4$	Exine - thick, tectate, surface granular
12.	*Albizzia lebbeck*	Mimosaceae	Polyad, 16-celled, spheroidal, large-sized, 72-74 µm, each cell 17-19 µm,	Individual pollen triporate, number - 03 (tritreme),	Exine - thick, tectate, surface psilate to faintly

Sr. No.	Pollen Type	Family	Shape, Size and Symmetry	Apertural Pattern (NPC Parameter)	Pollen Wall Structure and Sculpture
			AMB – squarish, radially symmetrical	position - zonotreme, characters- porate, NPC: $N_3P_4C_4$	granular
13.	*Pithecellobium dulce*	Mimosaceae	Polyad, 16-celled, spheroidal, large-sized, 58-64 µm, each cell 15-18 µm, AMB – squarish, radially symmetrical	Individual pollen triporate, number - 03 (tritreme), position - zonotreme, characters- porate, NPC: $N_3P_4C_4$	Exine - thick, tectate, surface psilate to granular
14.	*Prosopis juliflora*	Mimosaceae	Prolate to subprolate, AMB - rounded triangular, small-sized, 21-23 µm, P = 13-15 µm, E = 21-23 µm, P/E = 0.61-0.65, radially symmetrical	Tricolporate, number - 03 (tritreme), position - zonotreme, characters - colporate, colpi tapering towards poles, acute tip, ora lalongate NPC: $N_3P_4C_5$	Exine thick, subtectate, surface faintly reticulate

AMB – Ambitus, µm - Micrometer, P - Polar diameter, E - Equatorial diameter, P/E - Polar to equatorial diameter ratio

Key for identification of pollen grains:

A1) Pollen in polyads, 16-celled, individual pollen triporate

AA1) AMB – squarish

P1) 72-74 µm, each cell 17-19 µm, surface psilate to faintly granular

Albizzia lebbeck

P2) 58-64 µm, each cell 15-18 µm, surface psilate to granular

Pithecellobium dulce

AA2) AMB- squarish to irregular

P3) 50-54 µm, each cell 16-18 µm, surface psilate

Acacia arabica

P4) 44-48 µm, each cell 13-15 µm, surface granular

Acacia nilotica

A2) Pollen in monads, tricolporate

AA1) AMB - spheroidal

P5) Oblate to suboblate, colpi long, ora faint and rounded, 50-63 µm, subtectate, surface reticulate, heterobrochate, reticular meshes smaller near aperture

Delonix regia

AA2) AMB - spheroidal to rounded

P6) Oblate spheroidal, 52-57 µm, subtectate, surface reticulate, heterobrochate

Peltophorum pterocarpum

AA3) AMB - rounded

P7) Oblate spheroidal, 35-41 µm, tectate, surface psilate to granular

Butea monosperma

AA4) AMB - rounded triangular

P8) Spheroidal, colpi long with tapering ends, ora lalongate, 26-30 µm, tectate, surface psilate to faintly granular

Gliricidia maculata

P9) Spheroidal, colpi long, acute tips, ora lolongate, 38-45 µm, surface striate to radially granular

Tamarindus indica

P10) Subprolate to prolate, colpi linear, long, ora circular, 32-35 µm, tectate, surface psilate to granular

Cassia siamea

P11) Subprolate to prolate, 30-37 µm, seriate reticulate pattern

Bauhinia variegata

P12) Prolate to subprolate, colpi tapering towards poles, acute tip, ora lalongate, 21-23 µm, subtectate, surface faintly reticulate

Prosopis juliflora

AA5) AMB - triangular

P13) Prolate spheroidal, 20-23 µm, surface faintly reticulate

Dalbergia sissoo

P14) Subprolate, colpi linear, acute tips, 28-32 µm, subtectate, surface reticulate

Pongamia pinnata

DISCUSSION

Several pollen morphological characters viz; aperture, exine pattern, stratification, shape and size of the grains, have been considered as bearing evolutionary significance (Sharma, 1970). Among the various categories of pollen morphological characters, the aperture is considered as primary and most conservative, exine ornamentation as secondary and other characters as tertiary in order of their importance (Sharma, 1970 and Sudhakar and Rao, 1987).

The morphological characteristics of the pollen grains embodied in the exine are generally categorized into four broad groups namely (1) aperture, (2) exine ornamentation (3) exine strata and (4) grain shape and size, in the order of their importance in terms of conservation with regard to each category (Sudhakar and Rao, 1987).

Prosopis juliflora is found to be monad and rest of Mimosaceae pollen types are polyads. *Prosopis juliflora* though belongs to Mimosaceae, showed similarity in pollen morphological characters with Fabaceae pollen types particularly in reticular surface pattern. The pollen types belonging to Fabaceae and Caesalpiniaceae were found to be tricolporate differing in AMB shape. It is also concluded that all studied pollen types belonging to Fabaceae and Caesalpiniaceae showed similarities in exine surface i.e. reticulate.

Pollen grains of Fabaceae are small in size, isopolar, of radial symmetry, prolate spheroidal, amb subcircular, 3-colporate, longiaperturate, colpi with pointed apices, no central constriction, and without a covering exinous membrane, endoaperture lalongate, well delimited, sexine microreticulate with smaller brochi next to the apertural area, looking like a psilate margo, sexine slightly thicker than the nexine (Luz CF and *et.al.* 2013).

Caesalpiniaceae species show more variation in sporoderm stratification than apertural partten, polarity and symmetry. These taxa consist the dominance of tricolporate apertural partten.

Pollen grains of Caesalpiniaceae were generally symmetrical, isopolar, oblate to prolate-spheroidal, rarely sub-prolate, and tricolporate. The tended to be either thicker or thinner the nexine, rarely being the same thickness. The ornamentation is reticulate, rugulate, psilate, striate and rarely rugulate. Apertural type, shape and ornamentation are important characters in the palynotoxanamy to identification of taxa up to species level (Ganga Kailas and *et.al.* 2014).

Some peculiar characteristics as well as the great variation in the pollen morphology of the Mimosaceae have been long known. It was investigated that Mimosaceous pollen may be monads or polyads or both. However, the complete range of pollen variation was discovered as monads, tetrads, octads and different types of polyads (Pentti Sorsa, 1969).

Phyletic development of pollen grains is assumed to have led from colpate types through colporate to porate types, and, as regards the exine, from relatively thin and simple types. The exine, from relatively thin and simple types to thicker exines with distinctly differentiated layers and relief. Parallel to such an evolution an increase in the size of the grains has taken-place (Pentti Sorsa, 1969).

The species investigated here illustrate the pollen morphological variation in the Mimosoideae. The greatest variation occurs in pollen unit types which ranged from monads to polyads of grains (Jumah A., 1991-96).

A certain degree of intraspecific variation in pollen morphology was noted in some cases. This involved polyad types, shapes and sizes. It is, therefore, important that in pollen studies, attention is paid to probable intraspecific variation so that variants of one species are not inadvertently assigned to two or more different species (Jumah A., 1991-96).

Monads are considered as a simplest in the evolutionary line while polyads are most advanced in their characters (Panicker, 2004). With the development of bird and bat pollinated flowers, the evolution of pollen grains may take place which forms monads-tetrads-polyad in pollen grains. The present finding supports to the pollen - pollinator co-evolution. A considerable variation in pollen morphology especially in apertural characters and exine patterns was observed in the pollen grains of the legume species (Balan and *et.al.* 2016). Great variations observed in the apertural characters and exine ornamentations are found to be significantly helpful at

generic as well as species level delimitation. The pollen grains are generally 3-zonocolporate or rarely 3-zonocolpate with ora lalongate or lolongate or circular. The exine ornamentation shows highest variation amongst Leguminosae.

CONCLUSION

From the above study, pollen morphological character is found to be useful in identification and discrimination of taxonomically related genera and species as it's going to reveal by minute characters. The pollen identification key is given in present research work which has taxonomic importance.

It is concluded that pollen grains are an expression of the structural and functional evolution in plants. It has increased the potentiality of pollen for use as an experimental material in biological investigations. Similarly, greater knowledge on the morpho-taxonomical interlink of pollen in several other branches in biology. Pollen characters are typically considered in Paleobotany, Taxonomy and Evolutionary biology. The morphological studies are important as they are helpful to understand details of mechanism of pollination, in vivo-germination and for hybridization experiments. These studies are also helpful in plant taxonomy, embryology, Biochemistry and modern biological sciences.

Conflict of Interest: The authors declare no conflict of interest in relation to this research.

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