

MICROGRAPHIC ANALYSIS OF MICROSPORES OF SOME MEMBERS OF THE FAMILY FABACEAE, LAHORE, PAKISTAN.

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Abstract

Some selected plant species of the Family Fabaceae were collected from Lahore, Pakistan for palynological studies. Ten plant (10) species belonging to seven (07) genera of the family Fabaceae were collected in different seasons and palynologically examined. The represented pollen types were tricolporate and tetracolporate types. Palynomorphographic features were measured carefully. Pollen diameter in polar and equatorial view was measured. Largest pollen was found in *Cassia glauca* (60µm) and smallest in *Millettia ovalifolia* (18µm). Colpi length in colporate pollen ranged between 48 µm (*Cassia glauca*) to 10µm (*Albizia julibrissin*) while colpus width from 15µm (*Cassia glauca*) to 02 µm (*Albizia julibrissin*). Largest pores 20µm were measured in *Cassia glauca* and smallest (2µm) in *Albizia Julibrissin* respectively. Pollen exine thickness in various plant species varied from 1µm (*Caesalpinia pulcherima*) to 5µm (*Bauhinia purpurea*). Shape of pollen in different species was prolate, spheroidal, oblate spheroidal or semi rounded. Exine ornamentation was found to be microreticulate in seven species while four (04) species (*Acacia modesta*, *Bauhinia purpurea*, *Caesalpinia pulcherima* and *Millettia ovalifolia*) showed coarsely reticulate to intra reticulate. All of these palynological characteristics were used to develop a dichotomous key.

Keywords: Pollen morphology, Microscopy, Fabaceae, exine ornamentation

Introduction

H.A. Hyde and D.A. Williams were the scientists who first proposed the term palynology in 1994 and 1st investigation on pollen was done by Paul Sears. Palynology is further divided into two branches paleopalynology and neopalynology. Pollen morphology is illustrated with the help of palynogram. Fabaceae contains seven hundred twenty seven genera and more than nineteen thousand species (Lewis *et al.*, 2005). With reference to economic importance it is 2nd largest family. Usually it is called legume family because fruit of species is legume. Members of

this family shows huge diversity in physical & morphological structures (Lewis *et al.*, 2003). Plants are perennial & annual trees, vine, shrubs & herbs. They have worldwide distribution. Members of family Fabaceae are present in almost all kinds of region. Stem is herbaceous. Leaves are petiolate, pinnate, alternate, compound & venation is parallel. Roots are Fibrous tap root. Flowers are zygomorphic, hermaphrodite, pedicellate & complete. Calyx & corolla are five or four in no & these are united, monocarpellary, superior ovary is present. Seeds may or may not contain endosperm. Marginal placentation.

Pollen grains have variety in shape, size, ornamentation, structure and sculpture. Spores and pollen have two to three cells and all these cells are within the wall. This wall has two different regions e.g., intine and exine. Outer wall is made up of sporopollenin and inner wall is of cellulose. Pollens are spheroidal, prolate, reticulate to intra-reticulate and infra-granulate in structure (Chaudhary, 1983). Pollen grains are mostly uniform, isopolar, radially symmetrical, prolate & tricolporate. Exine Ornamentation is perforate or reticulate. (Al-Zoghet *et al.*, 1995). In fabaceae family pollen grains are 3-colporate, 3-6 colporate, monads, tetrads, polyads, regulate, pistillate and faveolate (Erdtman, 1952).

Basic & applied palynology are two different classes of palynology & Applied palynology have many other groups e.g. pharmacology, Meliso-palynology, Palynotaxonomy, Aero-palynology, Forensic palynology, Paleo-palynology, Copro-palynology, Entomo-palynology & Latro-palynology. (Mildenhall *et al.*, 2006)

Palynology used in coal, oil gas and petroleum and also helpful in forestry, forensic, agriculture and other sciences. It is used to find out pollination mechanism, foraging sources, insect and pollinator zones. Family Fabaceae is categorized into 3 sub families Caesalpinoideae, Papilionoideae and Mimosaceae (Chaudhary, 1983). Mimosaceae includes 3,100 species and 58 genera in the world (Mabberley, 1997). Mimosaceae contains 19 genera which are distributed worldwide, including tropical, subtropical, arid and semiarid zones. This subfamily consist of three tribes Acacieae, Ingeae and Mimoseae (Elias, 1981). *Acacia* Mill. is an

important genus for preparing medicines for humans and animals (Springuel & Mekki, 2000).

The subfamily Caesalpinoideae is a paraphyletic grade of 171 genera and approximately 2250 species from which derived the monophyletic sub-families Mimosoideae and Papilionoideae. (Lewis *et al.*, 2005). Distribution of Caesalpinoideae includes tropical, subtropical and temperate regions.

Family Fabaceae is an economically important family. Plants related to this family are source of fuel and also used as fodder, green manure, herbal medicines and lightened fiber. The main purpose of this research work is to explore the data of plants present in Pakistan. Identification of different Species of Pakistan can be done on the basis of their pollen analysis. Through collected data we can define pollen shape, size and other morphological features which provides help to explore the flora of Pakistan (Lashin, 2006).

Methodology

Field survey

During field survey fresh floral buds were plucked from the trees, shrubs and herbs and their pictures were captured. Then these flowers were placed in zip lock bags on which their names were mentioned. Both common and scientific names were noted. In Pakistan, different sites of Lahore, Punjab were surveyed from March to September for the collection of different flowers (Table 1.1). *Acacia farnesiana*, *Cassia fistula*, *Milletia ovalifolia* and *Sophora secundifolia* were collected from PU Botanical garden, *Caesalpinia pulcherima* and *Acacia modesta* from GCU Botanic Garden, *Albizia julibrissin*, *Bauhinia purpurea* and *Cassia glauca* were collected from Jinnah Garde, *Cassia alata* and *Caesalpinia*

pulcherima were collected from Jallo park, Lahore.

Sampling & Anther isolation

Flowers of selected plants species were collected at bud stage for the isolation of pollen. Placed the sample in polythene zipper bag and the name of plant was mentioned on it. Anthers were exposed by removing the petals with the help of forceps. These anthers were separated to the flower. A clean test tube was taken and labeled with the name of peculiar plant. Anthers were crushed with the help of blunt ended needle and then transferred into the label test tube.

Maceration

Preparation of fixative

In order to preserve the histological sections of sample, a chemical known as fixative was used:

Sr. No	Chemicals used	Volume used
1	Glacial acetic acid	15 mL
2	Formalin	5 mL
3	Tertiary butyl alcohol	80 mL

Fixative was prepared by adding 15 ml glacial acetic acid and 5 ml formalin in 80 ml of tertiary butyl alcohol. Floral buds were taken and anthers were extracted from the buds and crumpled them gently and then put these crushed anthers in the test tube and labelled it with the specimen name and date. 20 ml fixative was added in test tube and covered it tightly. Placed the test tube in test tube stand and kept it for 3 days.

Neutralization

Each sample was neutralized after three days with water by performing 5 to 6 decantation. In this process test tubes were vortex and filled with distilled water and placed them in centrifuge machine for 20 minutes. After twenty minutes water was removed carefully with the help of dropper. In order to neutralize the sample, dropper was pressed, drenched and released. Again, water was added in the test tube and repeated the process at least times for complete neutralization.

KOH Treatment

After neutralization, specimens were treated with 2 percent KOH and heated the specimens on water bath at 80 c for 5 minutes. Then specimens were kept to lower the temperature. After alkali treatment, again 5-6 decantation were performed in order to neutralize the sample.

Sample preservation

Samples were preserved by adding 1-2 drops of 1% thiomersal solution in it.

Staining

Each specimen was stained with one drop of safranin.

Mounting stages

Glycerin jelly or Canada balsam were used as mounting medium and following steps were involved.

Preparation of Glycerine jelly

Glycerine jelly was prepared by adding 10 g gelatin powder in 100ml distilled water and kept it over-night. This solution was heated on a hot plate and 60ml glycerin and 2-3 drops of 1% thiomersal were added in it. Then solution was refined by using silk cloth and It was stored at below 20°c in refrigerator.

Slide preparation

Glycerin jelly was melted by putting it in the hot water. One drop of glycerin jelly was taken on a glass slide and sample was added on it. Glass slide is covered with cover slip with the help of needles in order to avoid bubbles between cover slip and glass slide carefully. Then cover slip was sealed with nail enamel. Prepared slides were stored in refrigerator below 20°C.

Microphotography

Pollen morphological characters were observed by placing the slides in Meiji trinocular compound microscope at different powers like 20x, 40x and 60x. Photographes were taken with the help of luminera digital camera and positive color prints were obtained.

Results

Genus *Acacia* Mill.

A. farnesiana : Pollen shape is semi-rounded, colpus is present; colporate. Pollen is polyad 32 no of monads are present. Surface of tectum is micro-reticulate. P/E ratio is 0.89 μ m. Colpus width is 2 μ m and length is 5 μ m. Exine ornamentation is microreticulate and thickness is 5 μ m. Polar & equatorial diameter is 30 μ m & 29 μ m.

A. modesta : The shape of pollen grain is prolate spheroidal while pollen type is tricolpate. Polar diameter is 18 μ m while equatorial diameter is 20 μ m. pollen grain is prolate in shape. P/E ratio is about 0.91 μ m. Colpus length is about 10 μ m and width is 4 μ m. Exine ornamentation is reticulate and thickness is about 4 μ m. Pore diameter is 4.5 μ m. Polar & equatorial diameter is 47 μ m & 42 μ m.

Genus *Albizia* Durazz.

A. julibrissin.: pollen grains are Tetracolpate. Pollen shape is polyad. Pollen is

prolate to sub prolate in polar view and prolate in equatorial view respectively. Polar diameter is 52 μ m, while equatorial diameter is 42 μ m. Colpi is 10 μ m long while 2 μ m wide. Spines are absent. Diameter of pore is 2 μ m. Exine ornamentation is granulate to micro-reticulate and thickness is about 3 μ m. Pore diameter is 2 μ m. Polar & equatorial diameter is 52 μ m & 42 μ m.

Genus *Bauhinia* L.

B. purpurea : Pollen shape is prolate, amb triangular to circular, aperture is tricolporate. Shape of pollen is prolate. P/E ratio is 1.2 μ m. Pollen grain size is 58.42 μ m. Colpus length is 40.95 μ m and width is 4.68 μ m. Ora length 8.96 μ m and width 1.64 μ m. Exine ornamentation is intra to infra reticulate and thickness is about 5 μ m. Polar & equatorial diameter is 35 μ m & 30 μ m.

Genus *Cassia* L.

C. fistula.: Pollen grains are isopolar. Symmetry is radial, syncolpate and 3- colporate. Colpi narrow with conspicuous margins. Pollen pore areas are faintly demarcated. P/E ratio is 1.39 μ m, prolate; amb circular. Equatorial outline is rhomboidal to elliptical. Exine is about 2.0 μ m; sexine and nexine of equal thickness; sexine granular. Exine ornamentation is microreticulate and thickness is about 3.5 μ m. Pore diameter is 20 μ m. Polar & equatorial diameter is 47 μ m & 42 μ m.

C. glauca: pollen grains are oblate and tricolpate. Symmetry is radial. Outline is circular and spheroidal in polar and equatorial view respectively. Polar diameter is 60 μ m, while equatorial diameter is 70 μ m. Colpi is 48 μ m long while 15 μ m wide. P/E ratio is 0.93 μ m. Exine ornamentation is microreticulate and thickness is about 1.81- 2.58 μ m. Spines absent. Pore diameter

is 20µm. Polar & equatorial diameter is 60 µm & 70 µm.

C. alata : pollen shape is oblate. Pollen type is tricolporate. Exine ornamentation is granulate to micro-reticulate and thickness is

about 2-3µm. P/E ratio is about 0.98µm. Outline is sub-circular in polar view and circular in equatorial view. Colpus length is 30µm and width is 7µm. Pore diameter is 5 µm. Polar & equatorial diameter is 27 µm & 25 µm.

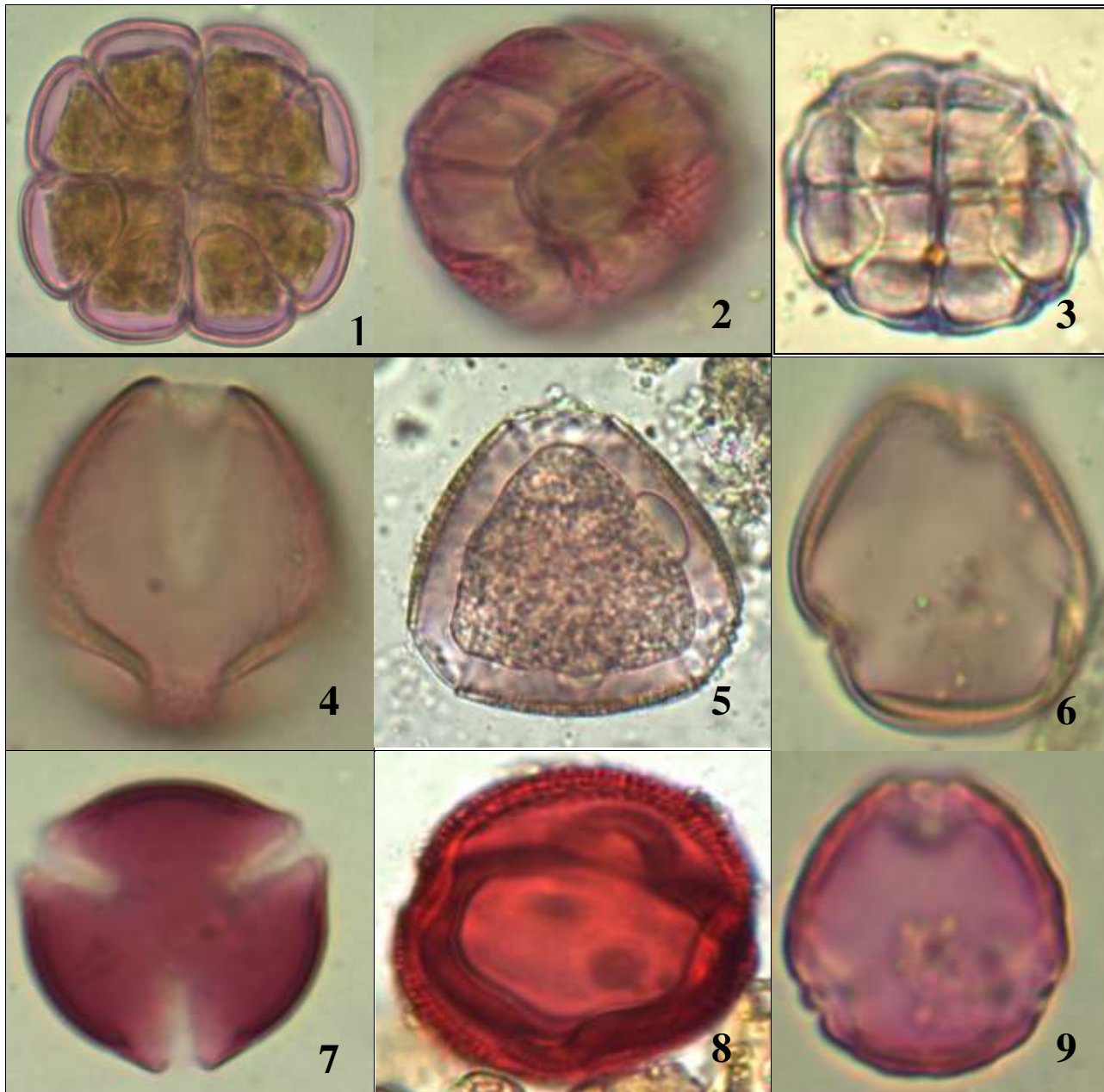


Fig.1-9. LM photographs of pollen Morphology of family Fabaceae. 1) *Acacia modesta* polyad pollen 2) *Acacia farnesiana* prolate spheroidal pollen. 3) *Albizzia julibrissin* (4) *Cassia glauca* tricolporate pollen. 5) *Bauhinia purpurea*. 6) *Cassia fistula*. 7) *Cassia alata*. 8) *Ceaselpinia pulcherima*. 9) *Millelita ovalifolia*.

Genus *Caesalpinia* L.

C. pulcherima: Pollen grains are isopolar. Colpus is 3-colporate and syncolpate. Symmetry is radial. Colpi are very broad and colpi membranes are granular. Ora is longitudinally elongated. P/E ratio is 0.93 μ m. Pollen shape is oblate spheroidal; amb circular to triangular. Equatorial outline is elliptical. Exine ornamentation is coarsely reticulate and thickness is about 6.5 μ m; sexine is thicker than nexine; sexine is reticulate. Size measurements are: P78.08 \pm 2.70 μ m (range 73.6 - 80.0 μ m). E83.84 \pm 2.52 μ m (range 80.0 - 86.4 μ m). Polar & equatorial diameter is 28 μ m & 24 μ m.

Genus *Millettia* L.

M. ovalifolia: colpi in pollens are tricolpate. Pollens are circular in shape. Polar diameter is 20 μ m, while equatorial diameter is 18 μ m. Colpi is 10 μ m long while 3 μ m wide. Spines are absent. Diameter of pore is 3 μ m. P/E ratio is 0.3 μ m. exine ornamentation pattern is reticulate and thickness is 2.5 μ m. Polar & equatorial diameter is 20 μ m & 18 μ m.

Genus *Sophora* L.

S. secundiflora L.: pollen grains are triporate. Pore diameter is 5 μ m. Pollen grains are circular in shape. Polar diameter is 30 μ m, while equatorial diameter is 28 μ m. Colpi and spines are absent. Diameter of pore is 7 μ m. Exine ornamentation is micro-reticulate and thickness is 3.5 μ m. Polar & equatorial diameter is 30 μ m & 28 μ m.

Discussion

Ten plant species were collected for pollen extraction. Palyno-morphological features of these ten species were studied with the help of microscope and in this investigation different pollen structures were observed. All of the

collected species were cultivated. Pollen grains of collected samples were observed under high power \times 100 of Meiji Trinocular Compound Microscope. *Albizia julibrissin* is the only species which presented tetracolporate pollen type while all other remaining species showed tricolporate pollen type. Tectum surface was micro-reticulate and spines were absent in all samples. Al- Watban (2013) revealed pollens of 7 sp. of *Acacia*. Among these species tectum surface of *A. farnesiana* & *A. nilotica* was microreticulate.

Different pollen shapes were studied i.e., prolate, oblate spheroidal, spheroidal or semi rounded. Dekui *et al.*, (2005) studied pollen grains of *Sophora* that were prolate spheroidal with 3-colporate as shown in the results. Karachai and Chantranothai (2009) examined twelve taxa of *Cassia*. and concluded that pollen was monad, tricolporate, radially symmetrical and small to medium sized. Pollen grains of *Cassia glauca* and *C. alata* were also monad & tricolporate. All species had monad pollen type except *A. farnesiana*, *A. modesta* and *A. julibrissin* (polyad).

Fitri and Des (2018) revealed that pollens of peacock flower were monad, isopolar and radially symmetrical. Antonio-Domigues *et al.*, (2018) demonstrated the palynological characteristics of 16 species of family Fabaceae. Results revealed that most of the species under study shows pollen shape which was polyad and tetrad. Khan *et al.*, (2019) investigated 6 species of *Acacia* through LM and SEM. In *Acacia modesta* pollen shape was oblate spheroidal and in *A. farnesiana* prolate shape was observed. Al-Timen *et al.*, (2013) reported the pollen morphology of eleven species. This study includes ornamentation and size of pollen grain. Among these species, *Cassia fistula* pollens were

tricolporate and exine ornamentation was reticulate. Jamwal (2021) examined the 6 polleniferous and nectariferous species of family Fabaceae under LM and SEM. In *Cassia fistula* exine ornamentation was regulate- reticulate and oblate spheroidal shape was reported. All the pollens under study were isopolar and radially symmetrical.

Liao *et al.*, (2022) Studied the palynological features of 4 species of *Sophora* by using SEM and results revealed that pollens were tricolporate and shape was oblate spheroidal or prolate. 11 species of *Bauhinia* and *Phanera* were examined by Francisco (2012). Results was similar with this paper pollen type was colporate and exine of *bauhina* was reticulate.

Heteromorphism in the number of apertures was observed in species of both genera.

In order to measure the pollen diameter, equatorial and polar diameter readings were noted. In polar view, pollen diameter was observed to be lowest in *M. ovalifolia* and highest in *C. glauca* while in equatorial view, pollen diameter was lowest in *M. ovalifolia* and highest in *C. glauca*. P/E ratio was measured. This ratio was ranged between 0.3µm (*S. secundifolia*) to 1.39 µm (*C. glauca*). In almost all members of collected species colpi were observed and their length and width were measured. Colpus width was found to be smallest in *A. julibrissin* and largest in *Cassia glauca*. Length of colpi were smaller in *A. julibrissin* and largest in *Cassia glauca* species.

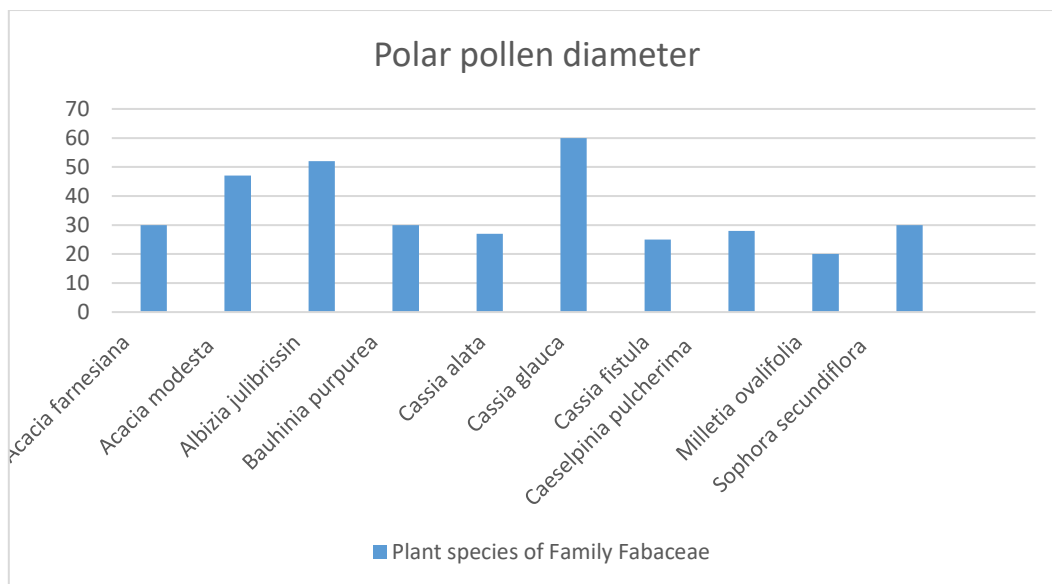


Fig.10: Polar pollen diameter.

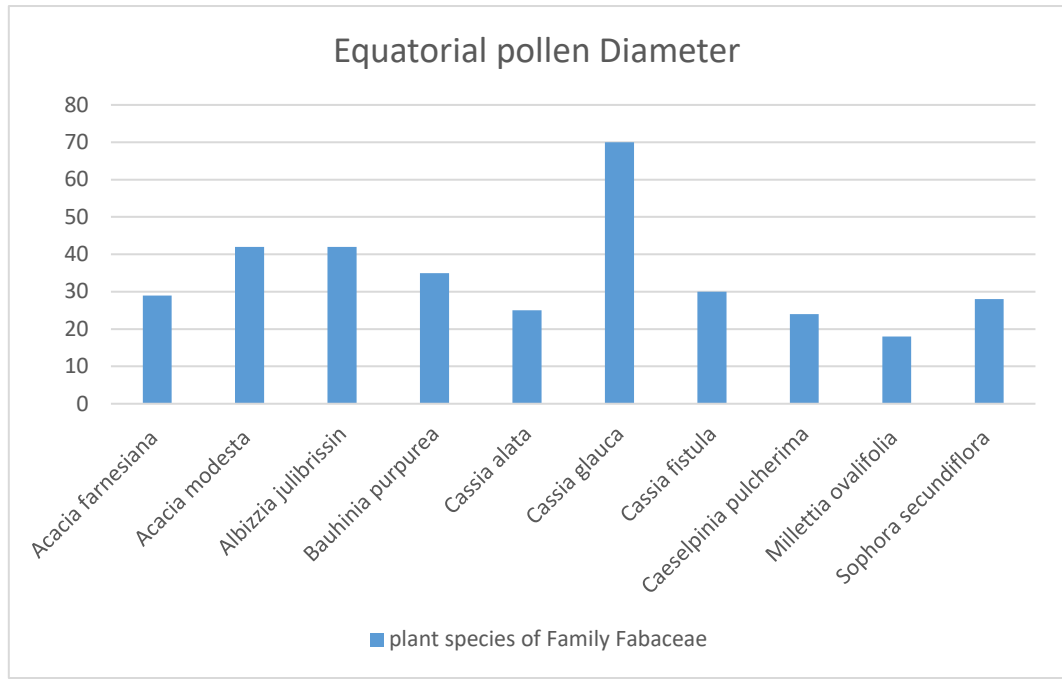


Fig.11: Equatorial pollen diameter

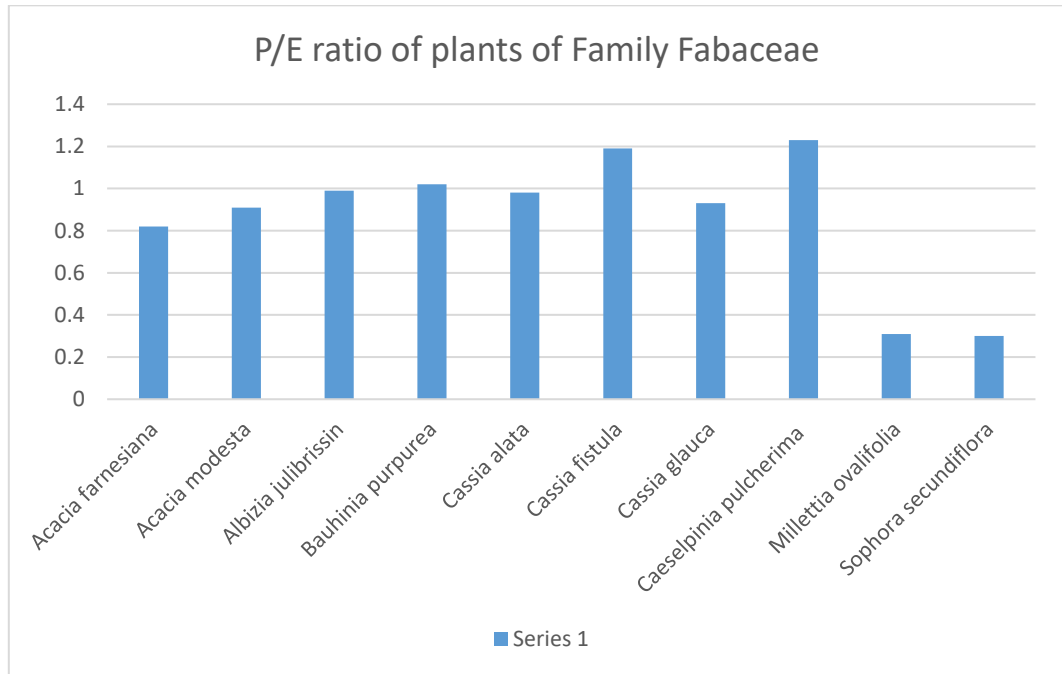


Fig. 12: P/E Ratio of collected plant species

Table 1: Flowering Season, Habit, Habitat & Distribution of the Studied Species.

Species	Local Name used in Pakistan	Color of the Flower	Flowerng Season	Habit	Habitat	Distribution in Pakistan
<i>Acacia modesta</i>	Phulahi, Palausa, Phulau or Amritsar gum	White	March - May	Smalldecidious tree	Dry or even arid areas and sandy soils.	India, Afghanistan & W. Pakistan
<i>Acacia farnesiana.</i>	Sweet Acacia, Needle bush, Fragrant Acacia, Durgandh khair, Vilayati babul or Guh babul,	Small bright yellow Puff like flowers	Nov- march	Decidious Small tree or shrub	Dry areas, on loamy and sandy soils, occurs in coastlands, deserts and riparian regions	India, W.Pakistan, & native to America
<i>Albizia julibrissin</i>	Silk tree, Kurmura, Shirin and Pink siris	Pink flowers like pom poms	Flowering in summer	Small decidous tree	Open habitats, woodland and scrubland	United states, America and Asia
<i>Bauhinia purpurea</i>	Orchid tree, Purple kachnar or Dev Kanchan	Light Purple or Pink	Sep- Nov	Perennial tree	Mountain forests, dry forests and scrubland	All over the world and mostly in Tropical and sub-tropical regions
<i>Cassia fistula</i>	Amaltas, golden shower or purging cassia	Bright-yellow showy	April- June	Decidious tree	Shallow and dry areas	Burma, India, Ceylon and W.Pakistan
<i>Cassia glauca</i>	Scrambled egg bush or Pivla amaltas	Bright yellow flowers in cluster	April- June	Perennial small tree of dry season	Along roadside, garden weed, Meadows, Shrubland	Sri Lanka, India, Pakistan and Malaysia
<i>Caeselpinia pulcherima</i>	Peacock Flower, Dwarf poinciana or Guletura	orange red	July- August	Perennial or Annual Flowering plant	Grasslands, meadows, plains, valleys, montane	All Pakistan mainly Lahore, Islamabad, Peshawar, Karachi
<i>Milletia ovalifolia</i>	Moulmein rosewood or Mitoshika	light pink	Feb- march	Annual tree	plains, valleys	Sikkim, india, Afghanistan
<i>Sophora secundiflora</i>	Texas mountain laurel	Purple	Feb- April	Annual tree	Moist forests	Ceylon, China and Antarctica
<i>Cassia alata</i>	Candle bush, craw plant or ringworm plant	Bright yellow	Aug- Sep	Perennial shrub	Meadows and grasslands	Southern hemisphere and Asia

Table 2: Pollen morphological characters of Family Fabaceae

Species	Pollen size			Colpus size			Pollen type	Pollen shape	Exine ornamentation
	Equatorial diameter	Polar diameter	P/E ratio	Colpi length	Colpi width	Pore diameter			
<i>Acacia farnesiana</i>	29 μm	30 μm	0.89 μm	5 μm	2 μm	5 μm	Tricolporate	Prolate	Micro-reticulate
<i>Acacia modesta</i>	42 μm	47 μm	0.91 μm	10 μm	4 μm	5 μm	tricolporate	Oblate spheroidal	Reticulate
<i>Albizia julibrissin</i>	42 μm	52 μm	0.96 μm	10 μm	2 μm	2 μm	Tricolporate	Oblate spheroidal	Micro-reticulate
<i>Bauhinia purpurea</i>	30 μm	35 μm	1.2 μm	40 μm	4 μm	3 μm	Tricolporate	Prolate	Intra to infra reticulate
<i>Cassia fistula</i>	42 μm	47 μm	1.39 μm	20 μm	10 μm	5 μm	Tricolporate	Prolate	Micro-reticulate
<i>Cassia glauca</i>	70 μm	60 μm	0.93 μm	48 μm	15 μm	20 μm	Tricolporate	Spheroidal	Micro-reticulate
<i>Cassia alata</i>	25 μm	27 μm	0.98 μm	30 μm	7 μm	5 μm	Tricolporate	Oblate	Granulate to micro-reticulate
<i>Caesalpinia pulcherrima</i>	24 μm	28 μm	0.93 μm	20 μm	4 μm	3 μm	Tricolporate	Oblate spheroidal	Coarsly reticulate or intra-reticulate
<i>Milletia ovalifolia</i>	18 μm	20 μm	0.35 μm	10 μm	3 μm	3 μm	Tricolporate	Spheroidal	Reticulate
<i>Sophora secundiflora</i>	28 μm	30 μm	0.3 μm	12 μm	7 μm	7 μm	Tricolporate	spheroidal	Micror-reticulate

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