

Pollen morphological study of some selected Indian taxa of Rosaceae

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Abstract

In present study pollen morphology of 24 taxa from 12 genera of the family Rosaceae were investigated by using Scanning Electron Microscope (SEM) and Light Microscope (LM). Except the surface ornamentation the pollen data supports the general classification. Based on surface ornamentation three general types (A, B and C) were recorded, i.e. striae, perforate-reticulate and granulate-microscabrate. The striae ornamentations were again categorized into six subtypes on the basis of arrangement of muri and perforations along with structure of the muri. Type A includes Striae: I. Faint to striae-found in *Cotonester* sp., *Eriobotrya elliptica* etc.; II. Distinct striae found in *Fragaria daltoniana*; III. Striato-reticulate found in *Rosa* sp., *Fragaria* sp.; IV. Irregular striae-found in *Potentilla kleiniana*, *Potentilla fulgens*, *Potentilla atosanguinea*; V. Regular striae-found in *Pyraecantha crenulata*, *Rubus paniculatus* and VI. Y-shaped striae-recorded in *Rubus hypargyrus*, while type B represents Perforate-reticulate: as recorded in *Rubus acuminatus*, *Rubus ellipticus* and *Rubus lineatus*, and C type exhibits granulate-microscabrate features observed in *Rosa* sp., *Ribes* sp., *Pyrus* sp., and *Pyrus sikkimensis*.

With the advancement of Scanning Electron Microscopy (SEM) surface ornamentation of exine became more important along with apertural types as because these are more or less fixed or stable characters and generally do not vary much with the variation of the environmental parameter. Moreover scanning electron microscopy (SEM) used on the pollen morphology provides a useful tool for plant identification.

Palynological studies of the family Rosaceae L. have been conducted earlier which include Erdtman⁸, Szafer and Pawlowski²⁴, Gerstberger¹¹, Kurtto *et al.*¹⁵, Muntzing¹⁸, Asker and Frost⁵, Asker⁴, Ascherson and Graebner², Juzepczuk¹³, Sojak²³, Eide^{6,7}, Faegri and Iversen¹⁰, Kumar and Panigrahi¹⁹, Leht¹⁷, Asker and Jerling³, Pilarek and Boratynska²⁰, Kolodziejek and

Gabara¹⁴. Keys based on pollen grain morphology are included in: Reitsma²¹, Eide⁶. In the key prepared by Reitsma²¹, he had distinguished 13 types of pollen grains in Rosaceae. Classes of exine thickness were determined on the basis of its thickness in selected species of the family Rosaceae by Eide⁶. Reitsma²¹ had opined that shapes of the pollen grain of Rosaceae are oblate, Prolate, spheroidal; while Eide⁶ considered these as spheroidal, prolate and subprolate. However, the pollen morphology has been used to elucidate taxonomic relationships in many angiosperm taxa⁸, including Rosaceae^{6,21}. The principal aim of this study was to describe the morphology of pollen grains of the investigated species and to characterize the exine sculpturing with the help of LM and SEM analysis.

In the present study 24 taxa from 12 genera of the family Rosaceae have been studied. Fresh polliniferous samples were obtained from field collection. The investigated materials are shown in Table-1.

Fresh materials were acetolysed for L M investigation. The method used, as suggested by Erdtman^{8,9} after slight modification. In all cases measurement and other observations were based on acetolysed grains unless otherwise mentioned. The measurements quoted in the pollen descriptions are generally based on an average of 5/10/15 reading randomly chosen. In case of scanty occurrence, however, fewer grains were measured. For the determination of shape, a shape class of Erdtman⁸ was followed. To determine the shape the values of PA (Polar axis length) and ED (Equatorial diameter) were measured and the P/E ratio was calculated. Then the ratio of

P/E was multiplied with 100, from value, the shape of pollen grain was determined.

To determine the length of the colpus that is either small or moderate or long the colpus length (average) was divided by the 'length of polar axes (average) and then multiplied with 100, that is $\{(CA/PA) \times 100\}$ (it is a percentage of the length of polar axis covered by each colpus) and shown as discrete variables. A colpus is considered as small when the value is ≤ 50 or moderate when it is > 50 but < 70 or long when it is ≥ 70 . Diameter of pore, exine thickness, width of colpi was also measured.

For the Scanning Electron Microscopic analysis of exine sculpture, pollen grains were mounted on metal stubs, sputtered with technical gold, examined and photographed in a S530-Hitachi SEM.

The pollen morphology of the 24 studied taxa of Rosaceae showed diversity in size, shape, exine characters but not in aperture types (Table-2). The present study showed that the main characteristic features of pollen of Rosaceae are isopolar, radially symmetric, prolate/subprolate/ob-prolate in shape, a zona-colporate/zona-colpate aperture in all genera and species with fixed (three) number of apertures. The exine sculpturing pattern is striae mainly with few exceptions. These characters agree well with those reported earlier for Rosaceae^{12,25,14,16}.

The SEM observations within the family showed a variation (synapomorphic character) in the exine sculpture only (Plate 1; table 2). Other characters are more or less uniform in the family (symplesiomorphic

Table-1. List of specimens used in investigation

Sl.No.	Name of the species/taxa	Locality
1.	<i>Cotonester</i> sp. Linn.	Lloyd Botanic Garden; Darjeeling (W.B.)
2.	<i>Eriobotrya elliptica</i> Lindl.	Kucheri Compound, Darjeeling (W.B.).
3.	<i>Fragaria daltoniana</i> J. Gay.	Tiger Hill, Darjeeling (W.B.).
4.	<i>Fragaria</i> sp. Linn.	Tiger Hill, Darjeeling (W.B.).
5.	<i>Fragaria</i> sp. Linn.	Tiger Hill, Darjeeling (W.B.).
6.	<i>Malus baccata</i> (L.) Borkh.	Pankha Bari Road, Darjeeling (W.B.).
7.	<i>Neillia thyrsoflora</i> D. Don	North Point, Darjeeling (W.B.).
8.	<i>Photinia integrifolia</i> Lindl.	Lloyd Botanic Garden, Darjeeling (W.B.).
9.	<i>Potentilla atrosanguinea</i> G.Lodd. ex D. Don	Tiger Hill, Darjeeling (W.B.).
10.	<i>Potentilla fulgens</i> Wall. ex Sims	Tiger Hill, Lebong, Darjeeling (W.B.).
11.	<i>Potentilla kleiniana</i> Wight & Arn.	Tiger Hill, Darjeeling (W.B.)
12.	<i>Pyracantha crenulata</i> (Roxb.ex D. Don) Roem.	Lloyd Botanic Garden, Darjeeling (W.B.)
13.	<i>Pyrus sikkimensis</i> (Wenz.)Hook. f.	Darjeeling (W.B.)
14.	<i>Pyrus</i> sp. Linn.	Darjeeling (W.B.)
15.	<i>Ribes</i> sp. Linn.	Ghoom, Sonada, Darjeeling (W.B.)
16.	<i>Rosa sericea</i> Wall. ex Lindl.	Tiger Hill, Sandakpheu, Darjeeling (W.B.)
17.	<i>Rosa</i> sp. Linn.	Lloyd Botanic Garden, Darjeeling (W.B.)
18.	<i>Rosa</i> sp. Linn.	Lloyd Botanic Garden, Darjeeling (W.B.)
19.	<i>Rubus acuminatus</i> Sm.	Darjeeling, Ghoom (W.B.)
20.	<i>Rubus ellipticus</i> Sm.	Tiger Hill, Ghoom, Darjeeling (W.B.)
21.	<i>Rubus hypargyrus</i> Edgew.	Ghoom, Sonada, Darjeeling (W.B.)
22.	<i>Rubus lineatus</i> Reinw. ex Blume	Tiger Hill, Darjeeling (W.B.)
23.	<i>Rubus paniculatus</i> Sm.	Darjeeling (W.B.)
24.	<i>Rubus wardii</i> Merr.	Darjeeling (W.B.)

characters), so palynologically the family is heterobathmic, in nature (with both primitive and advanced characters in a single taxa). The overall observations revealed that pollen of Rosaceae are admixture of wide variety of morphological characters. The present description of the pollen grain under L M showed a significant difference found in grain

sizes. Two deferent types of pollen sizes have been identified. Some of the species with larger pollen grain (20.48 μm to 30.4 μm) e.g. are found in *Rosa* sp., *Rubus paniculatus*, *Rosa sericea* and *Rubus hypargyrus* and other species with comparatively smaller pollen (8 μm to 10.08 μm) are found in *Rosa* sp., *Pyracantha crenulata*, *Cotonester* sp. It is

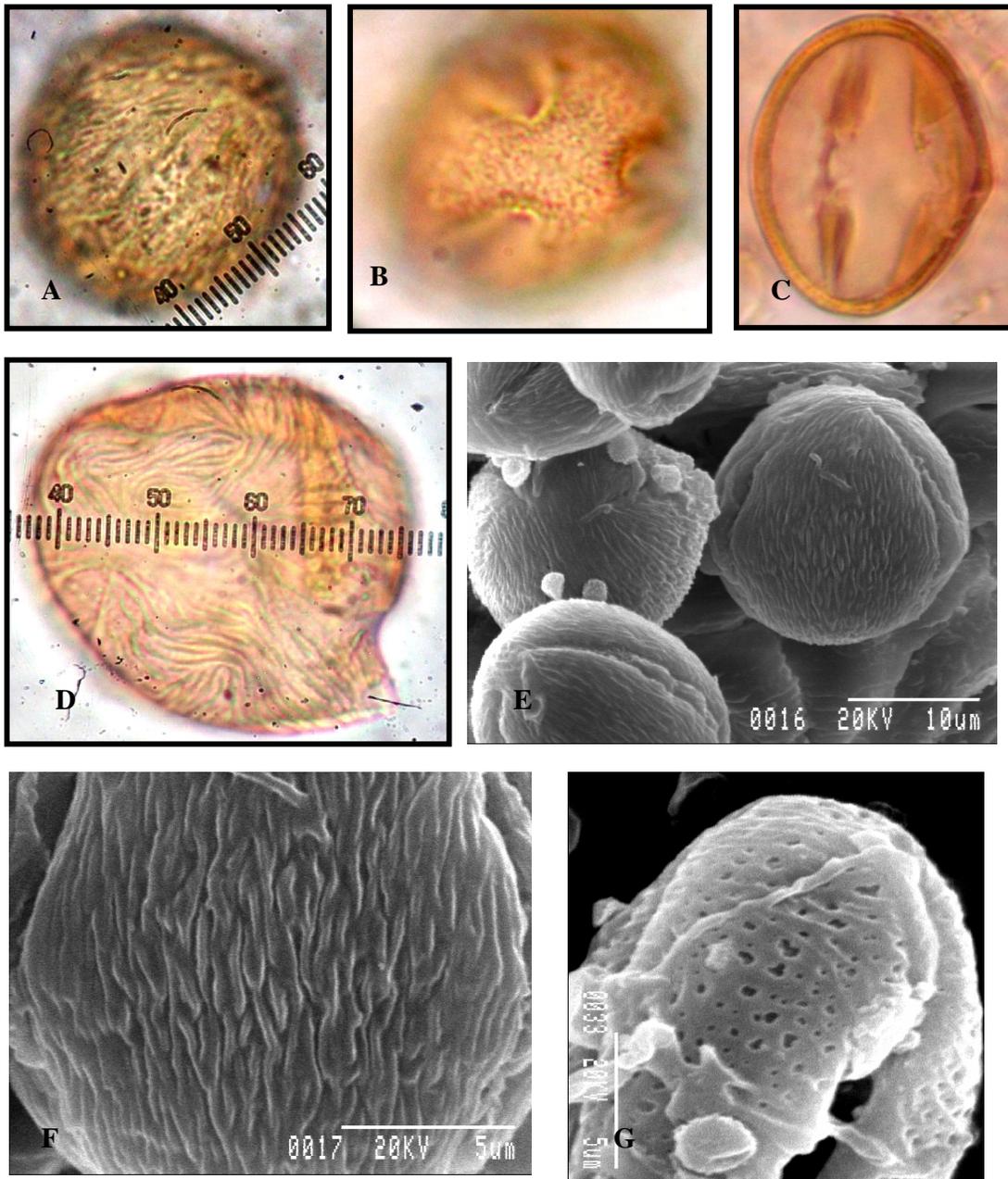


Plate 1 Microphotographs of pollen surface of different investigated taxa of Rosaceae
A. *Malus baccata* (X1000) ; B. and C. *Pyrus sikkimensis* (x1000); D. *Rubus hypargyrus*
(x1000); E. and F. *Rosa sericea*; G. *Rubus ellipticus*. (A-D-LM photographs; E-G-SEM
photographs) .

well known that pollen grain dimensions in many genera of Rosaceae are correlated with chromosome number (Muntzing¹⁸, Anchev and Deneva¹) but we have not studied the correlation between pollen sizes in the studied taxa of Rosaceae.

Apertures are more or less homogenous with tri-colporate, zona-perturate pollen grains (in 23 taxa) and tricolpate pollen grain is found in *Potentilla fulgens*, considering this apertural types there are colporate pollen in *Cotonester* sp., *Eriobotrya elliptica*, *Malus baccata*, *Photinia integrifolia*, *Pyrus sikkimensis*, *Rosa sericea* etc., colpate in *Potentilla fulgens*. The pollen of 23 taxa (out of 24 taxa) is with tri-colporate aperture. This complex aperture is considered to be as advanced character and is found in numerous advanced families of dicotyledonous, though these tri-colporate pollen grains are also found in some primitive families also.

The present result showed (Table 2) a considerable proportion of sub-prolate (*Malus baccata*, *Eriobotrya elliptica*, *Potentilla atrosanguinea*, *Potentilla fulgens*, *Pyracantha crenulata*, *Rubus paniculatus*, *Rubus hypargyrus*) and prolate (*Rubus lineatus*, *Rubus acuminatus*, *Rosa* sp., *Potentilla kleiniana*, *Cotonester* sp.) grains, with an average frequency of prolate-spheroidal (*Rubus wardii*, *Rosa sericea*, *Pyrus sikkimensis*, *Fragaria* sp. etc.) grains and very rarely spheroidal grains and agreed with the opinion of Reitsma²¹ and Edie⁶. The morphology of pollen is more or less circular in polar view in all the studied species.

On surface ornamentation three

general types were recorded (*i.e.* striae, perforate-reticulate and granulate-microscabrate). The striae ornamentation again categorised in to six subtypes on the basis of arrangement of muri and perforation along with structure of the muri. Types are as follows- A. Striae: i. Faintly striae- found in *Cotonester* sp., *Eriobotrya elliptica* etc.; ii. Distinct striae- found in *Fragaria daltoniana*; iii. Striato-reticulate- found in *Rosa* sp., *Fragaria* sp.; iv. Irregular striae- found in *Potentilla kleiniana*, *Potentilla fulgens*, *Potentilla atrosanguinea*; v. Regular striae- found in *Pyracantha crenulata*, *Rubus paniculatus*; vi. Y-Shaped striae- recorded in *Rubus hypargyrus*; B. Perforate-reticulate: Recorded in *Rubus acuminatus*, *Rubus ellipticus* and *Rubus lineatus*; C. Granulate-microscabrate: Observed in *Rosa* sp., *Ribes* sp., *Pyrus* sp., and *Pyrus sikkimensis*.

In SEM, apocarpial exine sculpture varies from granulate-reticulate to striae, from faintly striae to clearly striae. Exine sculpture along the colpi is similar to that appearing at distal pole, but the mesocolpal exine has a tendency to decrease in lateral extension and sometimes forming 'Y'-shaped striae (*Rubus hypargyrus*).

Most exine sculptures in the studied taxa of the family were classified into 6 subtypes within 3 types based on tendency from secondary sculpturing. The genus *Rubus hypargyrus* shows a peculiarity among the species. Exine is clearly, deeply perforate or striae (*Rubus ellipticus* to *Rubus paniculatus*). The exine sculpture sometime varies among the species and sometime within a species and sometimes form a unique 'Y'-shaped striae ornamentations (*Rubus hypargyrus*).

Table-2 Pollen morphology of the investigated taxa of Rosaceae

Sl. No	TAXA	P.A. (In μm)		E.D. (In μm)		P/E RA TIO	SHAPE	APERTURE	COLPUS (In μm)			CL/PAK 100	PORE (In μm)	EXINE (In μm)			SURFACE ORNAMENTATION		
		R	A	R	A				R	A	R			A	R	A			
i	ii	iii	iv	v	vi	vii	viii	ix	x	xi	xii	xiii	xv	xvi	xvii	xviii	xix	xx	xxi
1	<i>Cotoneaster</i> sp.	10.4-11.2	10.72	7.2-8.8	8	134	Prolate	Tricolporate, zonoaperturate	6.4-7.2	6.88	1.2-1.6	-	1.6	1.2-1.6	-	0.4-0.8	-	Faintly striate	
2	<i>Eriobotrya elliptica</i>	20-21.6	20.8	16-18.4	17.6	118.19	Subprolate	Tricolporate, zonoaperturate	16.8-19.2	17.6	-	1.6	1.6-2.4	-	1.2-2.4	-	-	Faintly striate	
3	<i>Fragaria daltoniana</i>	16-18.4	17.28	16-16.8	16	108	Prolate-spheroidal	Tricolporate, zonoaperturate	14.4-16	15.04	.8-1.6	-	2.4	1.6-2.4	-	.8-1.2	-	Distinctly striate	
4	<i>Fragaria</i> sp.	-	17.6	16-16.8	16.32	107.84	Prolate-spheroidal	Tricolporate, zonoaperturate	14.4-16	15.04	1.6-2	-	3.2-4	1.6-2.4	-	1.6-2.4	-	Striato-reticulate or striate	
5	<i>Fragaria</i> sp.	-	-	-	-	-	-	Tricolporate, zonoaperturate	-	-	-	-	-	1.6-2.8	-	-	-	Striae	
6	<i>Malus baccata</i>	-	14	-	12	116.67	Subprolate	Tricolporate, zonoaperturate	-	8.8	-	.4	1.6-2	.66-.8	-	.2-.4	-	Striae	
7	<i>Neillia thyrsiflora</i>	-	8.8	-	7.2	122.22	Subprolate	Tricolporate, zonoaperturate	6.4-6.8	6.6	1-1.2	-	-	.4-1	.66	.1-.3	.2	Scabulate	
8	<i>Photinia integrifolia</i>	14.4-16	15.52	12.8-14.4	13.18	114.12	Prolate-pheroidal / Subprolate	Tricolporate, zonoaperturate	12.8-14.4	13.28	.8-1.6	-	1.6-2.4	1.6-2.4	-	1.6-3.2	-	Striae	
9	<i>Potentilla atrosanguinea</i>	12.8-17.6	15.36	9.6-14.4	12	128	Subprolate	Tricolporate, zonoaperturate	1.2-11.76	12.8	1.2-2.4	-	1.6-3.2	1.2-1.6	-	.8-1.6	-	Irregularly striate	
10	<i>Potentilla fulgens</i>	16.8-18.4	17.6	14.4-16	15.2	115.78	Subprolate	Tricolpus	12.8-15.2	14.08	1.6-2.4	-	2.4-3.2	1.6-2.4	-	0.8-1.2	-	Irregular linear striae	
11	<i>Potentilla kleiniana</i>	16-17.6	16.64	11.2-12.8	12.16	136.84	Prolate	Tricolporate, zonoaperturate	12.8-14.4	13.6	1.2-1.6	-	1.6	1.2-1.6	-	.4-.8	-	Irregular linear striae	
12	<i>Pyracantha crenulata</i>	11.2-14.4	12.32	8-11.2	9.6	128.33	Subprolate	Tricolporate, zonoaperturate	8.9-6	8.64	.8-1.2	-	1.2	1.2-1.6	-	.4-.8	-	Regular linear striae	
13	<i>Pyrus sikkimensis</i>	22.4-24.8	23.52	19.2-22.4	20.8	113.07	Prolate-spheroidal	Tricolporate, zonoaperturate	18.4-20	19.2	.8-1.6	-	1.2-1.6	1.2-3.2	-	1.2-3.2	-	Slightly granulate to microscabrate	

14.	<i>Pyrus</i> sp.	12.8-13.6	12.8	-	11.2	114.29	Prolate-pheroidal / Subprolate	Tricolporate, zonoaperturate	9.6-11.2	10.24	-	1.6	80	1.2-1.6	-	1.6-3.2	Granulate
15.	<i>Ribes</i> sp.	17.6-19.2	18.24	14.4-16.8	16	114	Prolate-pheroidal / Subprolate	Tricolporate, zonoaperturate	14.4-16	15.52	1.2-1.6	-	85	-	1.2	.8-1.6	Striato-granulate or striato-reticulate
16.	<i>Rosa sericea</i>	25.6-28	16.56	24-26.4	12.8	107.10	Prolate-spheroidal	Tricolporate, zonoaperturate	21.6-24	22.4	1.2-1.6	-	84.34	2.4-4	-	1.2-2.4	Roughly striate
17.	<i>Rosa</i> sp.	24-25.6	24.48	16-17.6	16.64	147.12	Prolate	Tricolporate, zonoaperturate	19.2-20.8	20.32	.8-1.6	-	83	3.2-4	-	1.2-2.4	Striato-reticulate
18.	<i>Rosa</i> sp.							Tricolporate, zonoaperturate								1.6-2.4	Granulate or striato-granulate
19.	<i>Rubus acuminatus</i>	20.8-25.6	23.04	12.8-16	14.72	156.52	Prolate	Tricolporate, zonoaperturate	17.6-12.4	19.2	1.2-2	-	83.33	1.2-2.4	-	1.2-1.6	Slightly perforated to reticulate
20.	<i>Rubus ellipticus</i>							Tricolporate, zonoaperturate								1.2-2.4	Perforated to reticulate
21.	<i>Rubus hypargyryus</i>	-	16	-	12.8	125	Subprolate	Tricolporate, zonoaperturate	-	10	-	.8	62.5	1.2-1.6	1.4	.6-8	'Y'-shaped striae
22.	<i>Rubus linearis</i>	20.8-22.4	21.44	14.4-16	15.68	136.73	Prolate	Tricolporate, zonoaperturate	16-17.6	16.96	1.2-1.6	-	79	2.4-3.2	-	1.6-2.4	Perforated to reticulate
23.	<i>Rubus paniculatus</i>	20.8-21.6	21.28	16.8-18.4	17.6	121	Subprolate	Tricolporate, zonoaperturate	16-17.6	16.8	1.2-1.6	-	78.96	2.4-3.2	-	1.6-2.4	Regularly striate
24.	<i>Rubus wardii</i>	27.2-28.8	28	24.8-25.6	25.28	106.76	Prolate-spheroidal	Tricolporate, zonoaperturate	22.4-14	23.68	1.6-2.4	-	84.57	2.4-3.2	-	1.6-2.4	Not detected

Sl.No. = Serial number; taxa = Name of the investigated taxa; P.A. = Polar axis; E.D. = Equatorial diameter; Shape = Based on the shape classes (Erdtman, 1952); P/E = Ratio of P.A and E.D; R = Range;

A = Average; μm = Micrometer; (CL/PA) x100 = Colpus length (average) divided by length of polar axis (average) and then multiplied with 100 (it is a percentage of the length of polar axis covered by each colpus) and also shown as discrete variables.

The striae ornamentation is consisting of elongated muri and separated by lamina. Muri may be arranged parallel and without any branching as found in *Pyranantha* sp., on the other hand the muri may be arranged in overlapping condition, wavy and discontinuous fashion (*Rosa sericea*, *Potentilla fulgens* and other species of *Potentilla*). Sometimes branched muri are also found (*Cotonester* sp.).

Pollen grains of some species (*Rosa*, *Rubus ellipticus*, *R. acuminatus*, *R. linearis* and *R. paniculatus*) have also been studied by Samanta and Das²². Their observations more or less support our view but differ in surface ornamentation as reticulate in *Rosa sericea* under LM but SEM investigation of *Rosa sericea* showed distinctly striae and other species represent faintly striae ornamentation. On the other hand Samanta and Das²² described surface ornamentation of two species of *Rubus linearis* and *Rubus paniculatus* as granulated. This differs from our observation and SEM analysis showed that surface of *Rubus* pollen either perforate-reticulated (*Rubus acuminatus*, *Rubus ellipticus* and *Rubus lineatus*), granulated to scabrate, though LM study exhibited result different features. According to many authors^{18,3} a high number of irregularly shaped and sterile pollen grain is a reliable apomictic indicator. It has also reflected in our investigation and present observation detected high number of ill-developed sterile pollen.

Under the genus *Rubus*, 4-species have been investigated using SEM e.g. *Rubus ellipticus*, *R. acuminatus*, *R. paniculatus* and *R. lineatus* of which *R. ellipticus*, *R. acuminatus* and *R. lineatus* have shown

similar pollen characters in size, shape, apertural patten and also in ornamentation (i.e. Perforate-reticulate type of ornamentation) but the pollen grains of *R. paniculatus* are totally different more or less in all parameters (size, shape, apertural patten and even in surface ornamentation). So palynologically, the placement of the species *R. paniculatus* under the genus *Rubus* cannot be supported and it is palynologically much more related with different species of *Rosa* sp., *Potentilla* and *Fragaria*. But before concluding anything we should consider the characters from other disciplines also and on the basis of ornamentation of the pollen grain two sub-taxa may be created under the genus *Rubus*. Elongated apertures with faintly striae (e.g. *Cotonester* sp. *Eriobotria elliptica*) or striae ornamentation (e.g. *Potentilla kleiniana*, *Potentilla fulgens* and *Potentilla atrosanguinea*) suggested that these species are palynologically primitive in comparison to scabrate or perforated-reticulate and granulated ornamentation as found in *Rubus acuminatus*, *Rubus ellipticus*, *Rubus lineatus*, *Rosa* sp., *Ribes* sp., *Pyrus* sp., *Pyrus sikkimensis* etc. and the reticulate and striae ornamentation helps in entomophily (advance character). So palynologically the family is an admixture of both primitive and advance characters.

The result of the present study demonstrated that surface sculpture of the pollen grains is a good criterion for the identification of different genera of the family Rosaceae but not for the different species under the same genus. But for the taxa *Rubus acuminatus*, *Rubus ellipticus* and *Rubus lineatus* are characterized by perforate-reticulate while

Rubus paniculatus shows regular-striae ornamentation. Other characters such as the shape of style, the petal to sepal length, the types of leaf division, the leaf pubescence as well as the sculpture of fruit, may be valuable criteria for this family¹⁴.

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