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RESEARCH ARTICLE

POLLEN MORPHOLOGICAL STUDIES IN TWENTY ACCESSIONS OF *JASMINUM SAMBAC* (L.)AIT. FROM KERALA.

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Abstract

The pollen morphology of 20 accessions of *Jasminum sambac* L.(Ait) collected from different parts of Kerala State were studied. The major palynological features relating to the pollen shape, aperture and exine ornamentation are conserved within the species. The pollen grains were in general spheroidal, medium-sized and 3(4) – zonocolporate with reticulate exine ornamentation. SEM analysis revealed microvariations relating to the exine components such as muri and lumina. Although these variations were not adequate enough to classify the taxa into palynological groups, certain character associations were observed such as the link between homobrochate condition and oval or ellipsoidal lumina. Four accessions with very short lumina and almost porous exine had spiny projections or pyramidal tapering from their upper mural ends. The phylogenetic significance of such microstructural variations in the pollen exine remain to be evaluated based on SEM studies of the exine architecture including more taxa belonging to the group.

Introduction:-

Pollen morphological features relating to the aperture, exine ornamentation, exine strata, pollen size and pollen shape have been used as 'palynological markers' in the study of systematic relationships and phylogeny of angiosperms at all taxonomic levels. The pollen grains of the Oleaceae in general are tricolpate with a reticulate exine, exhibiting consistency in the expression of major palynological traits. Erdtman (1971) and Nilsson (1988) opined that pollen traits of the taxa are of little taxonomic significance in the family. Earlier studies on the pollen of the genus *Jasminum* include those by Nair (1965), Raman *et al*. (1972), Jin-Tan (1982), Zhongxin et al (1988) etc.

Jasminum sambac (L.) Ait., commonly referred to as Arabian Jasmine, is native to southern Asia (Green and Miller, 2009). The species exhibits a wide range of morphological variation relating to its vegetative and floral features, and has a number of cultivars known by differing vernacular names such as *kuttimulla, kudamulla, arimulla, iruvachimulla, moonnadukkumulla, adukkumulla* etc., grown all over the State of Kerala as garden ornamentals. Earlier studies on the pollen morphology of the species have focused largely on light microscopic observations. But the advent of high end tools such as the Scanning Electron Microscope has enabled in-depth analysis of the intricate patterns of exine ornamentation, opening up new vistas of exploration. Nair and Kapoor (1974) demonstrated that statistical evaluation of pollen variations can be used as an effective tool in the categorization of varieties, and also in the understanding of the hybridity status of the cultivars within a taxon. Hence the present study is undertaken to

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make a detailed comparative intraspecific analysis of the pollen from twenty accessions of *Jasminum sambac* from Kerala using LM and SEM studies, and to evaluate the significance of palynology in the systematics of the species-complex.

Materials and Methods:-

Twenty accessions of *Jasminum sambac* (L.) Ait. were collected from various locations in the State of Kerala. Polliniferous materials from the collected specimen were fixed in glacial acetic acid. The details regarding the 20 taxa included in the present study are furnished in Table 1.

Table 1:- List of *Jasminum sambac* (L.)Ait. accessions collected from different parts of Kerala with locality

Sl.No	Accession Name	No. of petal tiers	Locality	District	N	E
1	AC-1	1	Chirayinkeezhu	Thiruvananthapuram	8° 39' 54"	76° 47' 54"
2	AC-2	2-3	Anathalavattom	Thiruvananthapuram	8° 39' 56"	76° 46' 19"
3	AC-3	1	Koonthalloor	Thiruvananthapuram	8° 39' 54"	76° 47' 53"
4	AC-4	2	Neyyattinkara	Thiruvananthapuram	8° 23' 20"	77° 5' 60"
5	AC-5	2-3	Mamom, Attingal	Thiruvananthapuram	8° 41' 11"	76° 49' 21"
6	AC-6	several	Moonnumukku, Attingal	Thiruvananthapuram	8° 41' 12"	76° 49' 21"
7	AC-7	several	Palace Road, Attingal	Thiruvananthapuram	8° 41' 38"	76° 48' 44"
8	AC-8	2-5	Mamom, Attingal	Thiruvananthapuram	8° 41' 12"	76° 49' 21"
9	AC-9	1	Moonnumukku, Attingal	Thiruvananthapuram	8° 41' 12"	76° 49' 21"
10	AC-10	2-5	Kariavattom	Thiruvananthapuram	8° 33' 54"	76° 53' 2"
11	AC-11	2-3	Kariavattom	Thiruvananthapuram	8° 33' 54"	76° 53' 3"
12	AC-12	2-5	Kacherinada, Attingal	Thiruvananthapuram	8° 41' 56"	76° 48' 59"
13	AC-13	1	Kulanada, Pandalam	Pathanamthitta	9° 13' 22"	76° 40' 38"
14	AC-14	2-3	Chalakkudy	Thrissur	10° 18' 5"	76° 9' 19"
15	AC-15	2-3	Pallikkara	Ernakulam	10° 1' 8"	76° 22' 59"
16	AC-16	2	Azheekkal	Alappuzha	9° 29' 54"	76° 20' 56"
17	AC-17	2-3	Pattambi	Palakkad	10° 47' 2"	76° 9' 40"
18	AC-18	1	ThazheChowva	Kannur	11° 52' 33"	75° 21' 46"
19	AC-19	2-3	Thalankara	Kasargod	12° 29' 29"	74° 59' 17"
20	AC-20	1	Kozhikode Beach	Kozhikode	11° 14' 46"	75° 46' 49"

Palynological analysis:-

Fresh anthers from mature unopened flower buds were fixed in glacial acetic acid. Pollen preparations were made by the acetolysis method standardized by Erdtman (1952) and modified by Nair (1970). The pollen shape and size classes were ascertained following the classifications suggested by Erdtman(1966) and Walker & Doyle (1975) respectively. The terminologies suggested by Punt *et al.* (1994) were adopted for describing the aperture types and exine ornamentation patterns. Measurements relating to the following characters were taken using an ocular micrometer. In each case, the mean values of the measurements were taken from a random sample of 30 pollen grains.

Quantitative characters:-

1. Polar diameter of pollen grain (μm) -P
2. Equatorial diameter of pollen grain (μm)-E
3. P/E ratio
4. Thickness of exine (μm)
5. Number of apertures

Qualitative characters:-

1 Pollen shape
2 Pollen type
3 Pollen size class
8 Muri height
9 Lumina depth
10 Lumina floor nature

4 Exine ornamentation
5 Muri shape
6 Muri wall nature
7 Muri width
11 Lumina shape
12 Wax plugging in lumina
13 Aperture shape

LM photomicrographs of the pollen preparations were taken using an Olympus CH - 20 Research Microscope with Digital camera attachment facility. The slides of pollen preparation are deposited in the Department of Botany, Sree Narayana College, Kollam. SEM pictures were taken at the NIIST, Pappanamcode, Trivandrum using a JEOL – JSM – 5600LV scanning electron microscope.

Results and Discussion:-

Details of pollen morphological characters observed from 20 accessions of *Jasminum sambac* L.(Ait) collected from different parts of Kerala State are represented in Tables 2a-b and 3a-b. Among the twenty accessions studied, only fourteen yielded adequate pollen material for the study (Figs. 1-38 in Plates I and II). Pollen from the remaining taxa appeared to be disfigured in the palynological preparations and hence could not be used for the comparative analysis.

The pollen are in general medium-sized, 3(4) – zonocolporate and spheroidal with the polar and equatorial diameters ranging from 49.8±8.63 µm and 46.8±10.20 µm to 35.1±9.80 µm and 33.60±9.14 µm respectively (Tables 2a-b). The largest pollen grains were observed in AC 10 and the smallest in AC 15 (Figs. 17, 27). Exine 3.3±0.22 µm to 4.98µm ±0.51 µm thick, thinner towards the colpi margins and reticulate. The aperture number was 3 in almost all accessions except in AC 5 which showed 4 colpi in some of its pollen grains (Fig. 10).

Table 2a:- Quantitative pollen morphological characters in 14 accessions of *Jasminum sambac* L.(Ait) from Kerala

Characters	**AC 2**	**AC3**	**AC4**	**AC5**	**AC6**	**AC8**	**AC10**
Polar diameter –P (µm)	43.5±8.86	42±7.35	42.3±6.7	39.3 ±5.38	42.9±4.48	45.6±6.45	49.8±8.63
Equatorial diam.- E (µm)	41.4±8.46	40.5±7.78	41.1±6.33	36.6±5.62	41.1±4.01	42±7.07	46.8±9.08
P/E	1.05±0.07	1.04 ±0.11	1.03±0.07	1.08±0.09	1.05±0.06	1.09±0.05	1.07±0.06
Exine thickness (µm)	3.33±0.22	3.9±0.76	3.45±0.38	3.3±0.32	3.18±0.29	4.11±0.8	4.98±0.51
Aperture number	3	3	3	3 - 4	3	3	3

Table 2b:- Quantitative pollen morphological characters in 14 accessions of *Jasminum sambac* L.(Ait) from Kerala

Characters	**AC12**	**AC 14**	**AC 15**	**AC 16**	**AC17**	**AC 19**	**AC 20**
Polar diameter –P (µm)	47.1±8.61	45.9±5.84	35.1±9.8	42.6±8.34	40.5±5.34	48.6±11.7	44.1±7.49
Equatorial diam.- E (µm)	44.7±10.2	45±6.48	33.6±9.14	41.4±8.1	39.9±6.49	46.8±10.2	40.2±5.87
P/E	1.07±0.1	1.03±0.11	1.06±0.1	1.03±0.1	1.02±0.08	1.04±0.11	1.1±0.1
Exine thickness (µm)	3.87±0.5	3.48±0.29	3.42±0.32	4.14±0.9	3.3±0.32	4.68±0.78	4.14±0.75
Aperture number	3	3	3	3	3	3	3

The major qualitative palynological features were also conserved within the species with the members having mostly spheroidal, 3-zonocolporate, medium-sized pollen with reticulate exine ornamentation (Tables 3a-b). Only one accession (AC 5) showed occasionally 4-zonocolporate pollen in addition to the more frequent 3-zonocolporate ones. Although the lumina were irregular in size due to varying mural traits, the pollen could be broadly categorized into homobrochate and heterobrochate types (Figs. 1, 20). The former was observed in only three taxa (AC 2, 3 and 10). Muri were very tall, narrow or broad, with upper end flat and smooth in most accessions, except in AC 5 where it was pyramidal (Fig. 11). Small granular warts were observed on the mural surface in some taxa – AC 2, 3, 4, and 17. The accession AC 12 showed slight granulation on its mural surfaces, with the granules not assuming the form of warts. The accessions AC 8, 14 and 15 showed spiny projections from the mural wall (Plate III).

The lumina were very deep with the luminal floor not clearly visible due to the tallness of the mural walls in almost all cases, except in AC 20 where few warts and perforations were noted in the lumina. Even in this case, the mural walls were tall. In homobrochate forms the lumina appeared oval or ellipsoidal, while the heterobrochate pollen had narrow and irregularly elongated lumina. Interestingly, the four taxa with pyramidal projection or spiny outgrowths from the mural wall had very short and irregularly narrow lumina giving a porous appearance to the pollen exine. In all other taxa, the lumina were more prominent giving a reticulate appearance. The lumina appeared to be plugged with waxy material in three accessions – AC 4, 5 and 15.

Table 3a:– Qualitative pollen morphological characters in in 14 accessions of *Jasminum sambac* (L.) Ait. from Kerala

Characters	AC 2	AC3	AC4	AC5	AC6	AC8	AC10
Pollen shape	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal
Pollen type	3-zonocolporate	3-zonocolporate	3-zonocolporate	3- 4 zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate
Pollen size-class	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Exine ornamentation	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
Muri shape	Homobrochate	Homobrochate	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate	Homobrochate
Muri wall nature	Flat, smooth, outer surface often with small granular warts	Flat, smooth, outer surface often with small granular warts	Flat, smooth, outer surface often with small granular warts	Pyramidal, smooth	Flat, smooth	Flat, smooth with spiny projections from corners	Flat, smooth
Muri width	Less broad	Less broad	Less broad	Narrow	Broad	Narrow	Less broad
Muri height	Very tall	Very tall	Very tall	Very tall	Very tall	Very tall	Very tall
Lumina depth	Very deep	Very deep	Very deep	Very deep	Very deep	Very deep	Very deep
Lumina floor nature	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Granular, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri
Lumina shape	Oval or ellipsoidal	Oval or ellipsoidal	Narrow irregularly elongated	Very short and irregularly narrow, appearing porous	Irregularly elongated	Short, irregular, appearing slightly porous	Oval or ellipsoidal
Wax plugging in lumina	Absent	Absent	Present	Present	Absent	Absent	Absent
Aperture shape	Colporate	Colporate	Colporate	Colporate	Colporate	Colporate	Colporate

Table 3b:– Qualitative pollen morphological characters in in 14 accessions of *Jasminum sambac* (L.) Ait. from Kerala

Characters	AC12	AC 14	AC 15	AC 16	AC17	AC 19	AC 20
Pollen shape	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal	Spheroidal
Pollen type	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate	3-zonocolporate
Pollen size-class	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Exine ornamentation	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
Muri shape	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate	Heterobrochate
Muri wall nature	Flat, smooth, outer surface slightly granular	Flat, smooth, with occasional spiny projections	Flat, smooth, with occasional spiny projections	Flat, smooth	Flat, smooth, outer surface with small granular warts	Flat, smooth	Flat, smooth
Muri width	Broad	Narrow	Narrow	Less broad	Less broad	Broad	Broad
Muri height	Very tall	Very tall	Very tall	Very tall	Very tall	Very tall	Very tall
Lumina depth	Very deep	Very deep	Very deep	Very deep	Very deep	Very deep	Very deep
Lumina floor nature	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	Smooth, not clearly visible due to the height of muri	With few warts and perforations
Lumina shape	Narrow irregularly elongated	Short, irregular, appearing	Short, irregular, appearing	Narrow irregularly elongated	Narrow irregularly elongated	Narrow irregularly elongated	Narrow irregularly elongated

		slightly porous	slightly porous				
Wax plugging in lumina	Absent	Absent	Present	Absent	Absent	Absent	Absent
Aperture shape	Colporate	Colporate	Colporate	Colporate	Colporate	Colporate	Colporate

Conclusions:-

The pollen observed from 20 accessions of *Jasminum sambac* L.(Ait) collected from different parts of Kerala State were in general spheroidal, medium-sized and 3(4) – zonocolporate with reticulate exine ornamentation. These characters are largely consistent with the pollen features reported for the genus and its family, the Oleaceae. Thus the major palynological features are conserved within the species and its higher level groups. Erdtman (1971) and Nilsson (1988) had earlier opined that pollen characters are not good taxonomic markers for the family Oleaceae. The results from the present study corroborate their findings. However, the fine exine architecture revealed through SEM analysis reflected the microvariations with regard to exine components such as the muri and lumina. These microvariations were not adequate enough to group the taxa studied into palynological classes. Certain character associations were also observed such as the correlation between homobrochate condition and oval or ellipsoidal lumina. In addition, spiny projections or pyramidal tapering from the upper mural ends were noted in the four accessions with very short lumina and almost porous exines. The phylogenetic significance of such microstructural variations in the pollen exine remain to be evaluated based on SEM studies on more taxa belonging to the group.

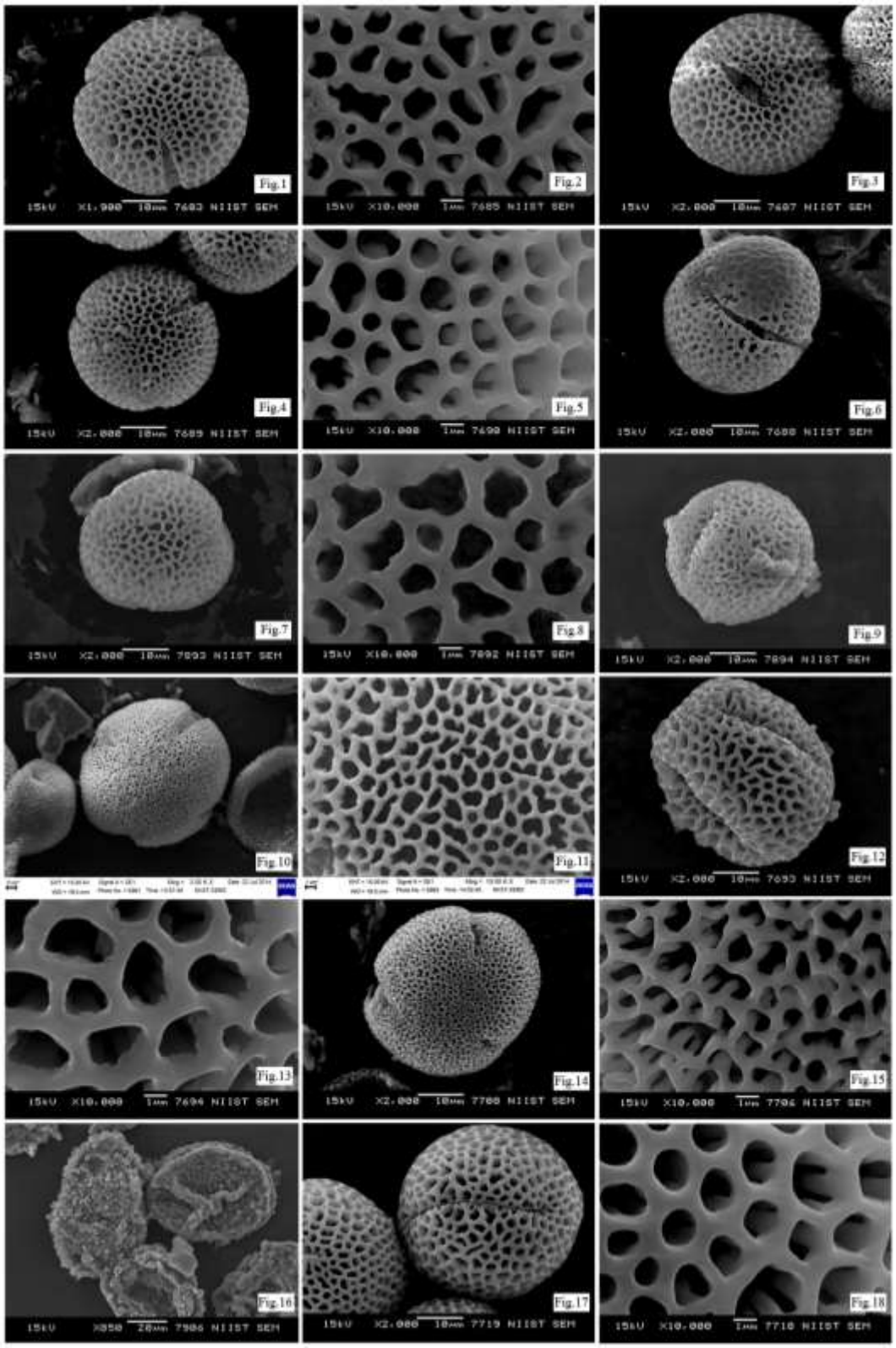

Plate I:- Figs.1-3:AC 2 pollen- 1,900x ,10,000x,2,000x; Figs.4-6:AC 3 pollen-2000x, 10,000x,2000x; Figs.7-9:AC 4 pollen-2000x,10,000x,2,000x; Figs.10-11:AC 5 pollen- 3000x, 10,000x; Figs.12-13:AC 6pollen- 2,000x, 10,000x; Figs.14-15:AC 8 pollen- 2,000x,10,000x; Figs.16:AC 9 pollen-850x; Figs.17-18:AC10pollen-2,000x,10,000x.

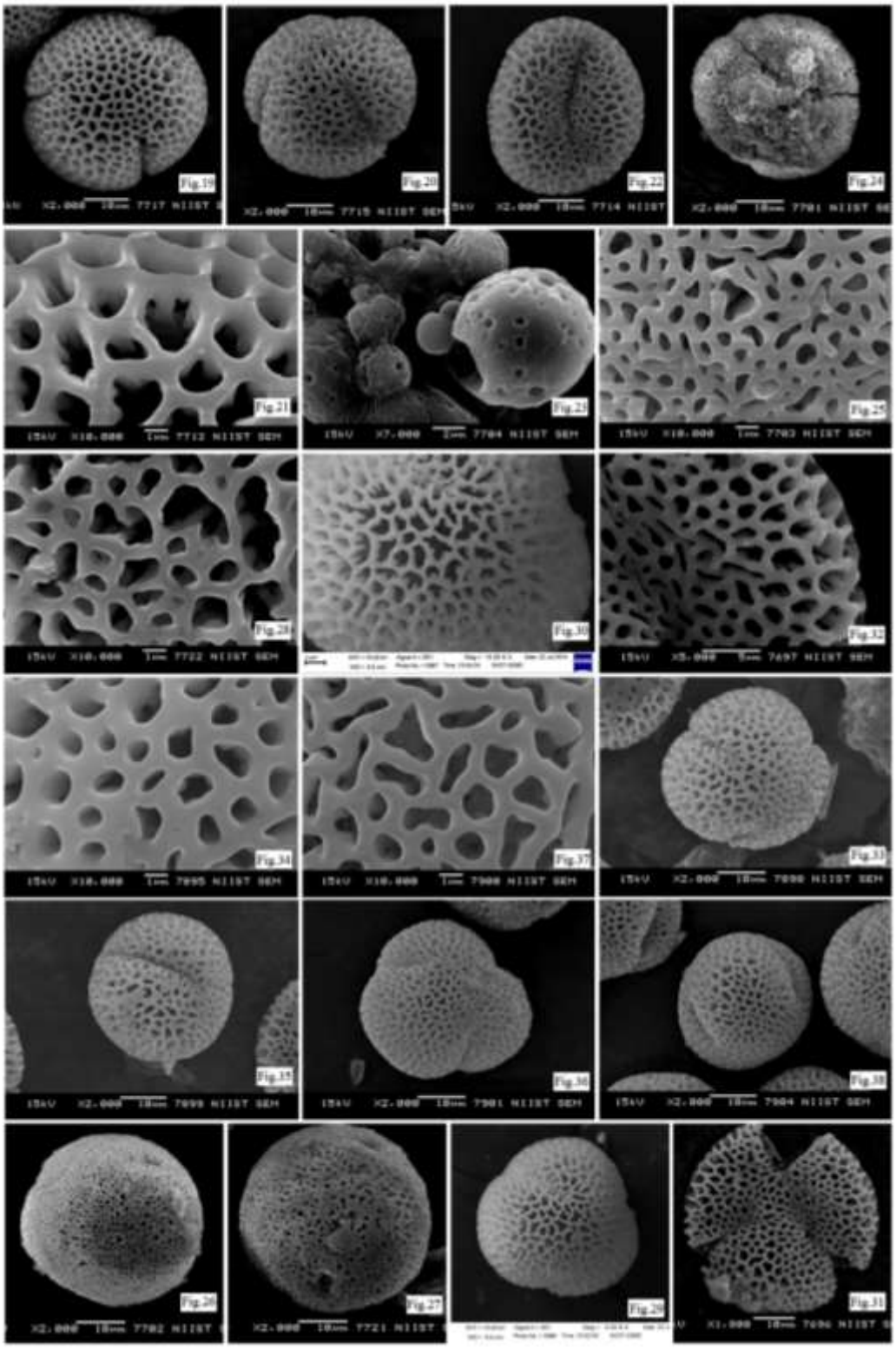

Plate II:- Fig.19:AC10 pollen-,2,000x; Figs.20-22:AC12pollen-2,000x,10,000x,2,000x; Figs.23:AC13 pollen-7,000x; Figs 24-26:AC 14 pollen-2,000x,10,000x,2,000x; Figs.27-28:AC 15 pollen-2,000x,10,000x; Figs.29-30: AC16 pollen-5000x,10,000x; Figs.31-32:AC17 pollen -1800x,5000x; Figs.33-35:AC19 pollen-2,000x,10,000x,2,000x; Figs.36-38:AC 20 pollen-2,000x,10,000x,2,000x.

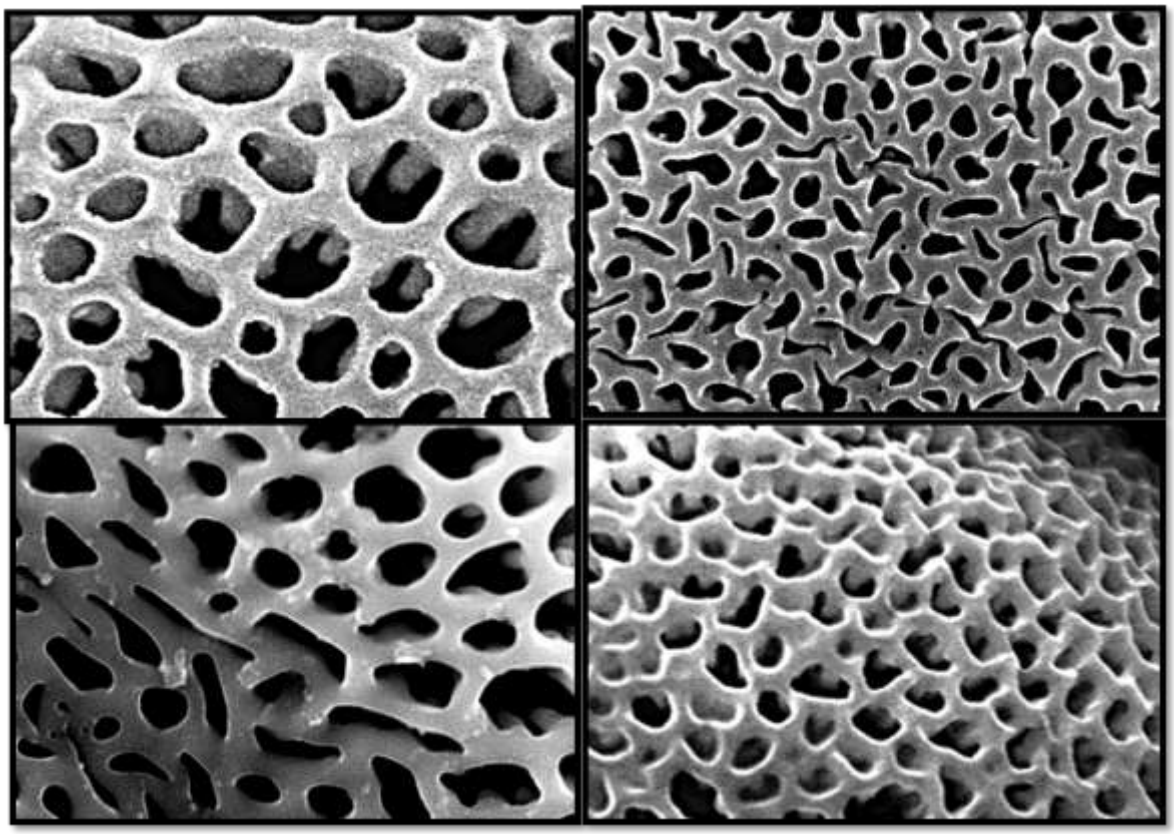

Plate III:- Muri walls showing small granular warts, spiny projections and pyramidal tapering in accessions of *Jasminum sambac*

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