ON THE OPERCULATE APERTURE IN THE POLLEN OF *SANCHEZIA PARVIBRACTEATA*

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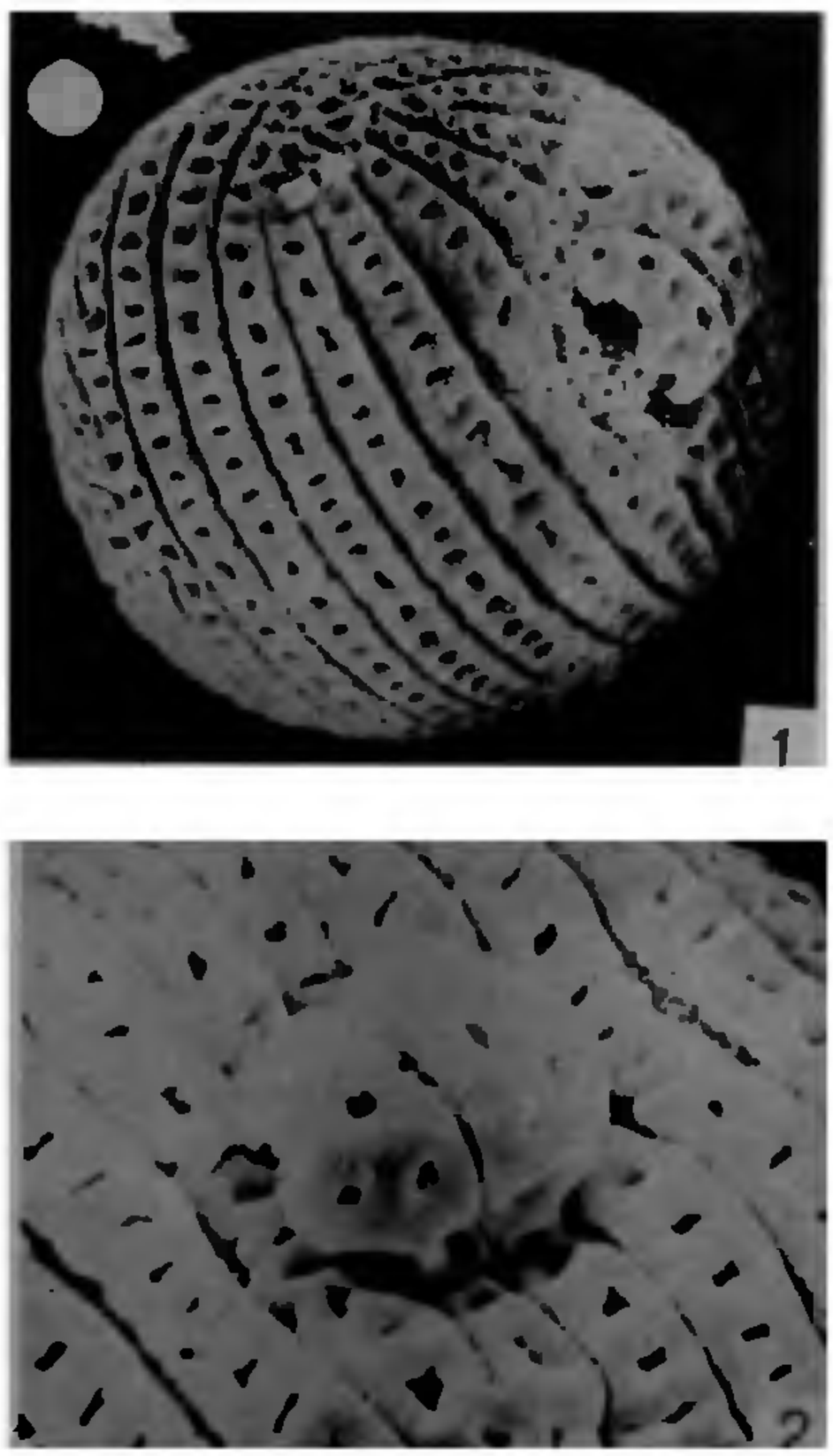
POLLINIFEROUS materials of *Sanchezia parvibracteata* Sprague and Hutchin. (Acanthaceae) were procured from plants grown at the public gardens, Trivandrum. Routine acetolysis method[1] was used and standard terminology[2] was followed. The pollen descriptions are based on 100 grains viewed under light microscope as well as SEM photographs.

The average pollen size is 104.6×100.9 μ (range 86–113×76–106 μ), shape prolate-spheroidal, and exine 6.5 μ thick. The exine surface is striato-reticulate with prominent ridges (striae) running along the polar diameter on one face which separate at the two poles, curving along the equatorial diameter and converging at the equator on the other face (figure 1). The ridges (3.35 μ broad) are beset with marginal rows of columellae islands, and bear a linear row of lumina which are square/rectangualrly-shaped. The depressions (lirae) between ridges (0.69 μ) are deep and devoid of columellae. The grains are 2-porate, one each on opposite face along the equatorial zone of the grain. Each pore is characteristically provided with a bi-flanged operculum, each measuring 9.2 (P)×4.5 (E) μ (figure 2).



Figures 1 & 2. SEM pictures of the pollen grains of *Sanchezia parvibracteata*. **1.** A 2-porate grain at the equatorial view (×1400) **2.** Portion of the exine surface showing an operculate aperture (×2800)

Pollen morphology of eight species of *Sanchezia*[3,4] are known so far, but the type of exine ornamentation of *S. parvibracteata* is not known in these. Further, its characteristic operculate aperture morphoform is unique, and this is not comparable with the two semi-circular sexinous patches around the aperture reported in *S. klugii* where it appeared as a narrow short colpus[3].

The occurrence of operculate apertures is a rare feature in Dicotyledons, but in the Gramineae this is a diagnostic feature[1]. Thus, occurrence of the operculate aperture in *S. parvibracteata* may represent a highly evolved or specialised condition.

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INFLUENCE OF METHIONINE AND THREONINE ON LYSINE OVERPRODUCERS

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THE biosynthetic pathway of lysine in bacteria is known to be interrelated with methionine and threonine. In most of the lysine overproducers, it has been shown that exogenous supply of these amino acids in the growth media has influence on the yield of lysine[1-3] The effect of methionine and threonine on some

isolated lysine overproducing organisms[4] has now been studied.

The organisms (*Arthrobacter globiformis* Lb, *Micrococcus luteus* B6 and *M. varians* 2Fa) were cultivated in the medium of Makula and Finnerty[5] with glucose (1% v/v) as carbon source. The medium was taken in 50 ml quantities in 250 ml Erlenmeyer flasks and sterilised by autoclaving. The amino acids were sterilised separately by filtration (Jena G5). The flasks in triplicate for each set were inoculated with a 24 hr old cell of the microorganisms and incubated on a rotary shaker (120 r m^{-1}) at 30°C ± 1° for 60 hr. Growth was measured by constant dry weight and the extracellular lysine was estimated by the method of Work[6]

It is evident from table 1 that in almost all the concentrations tested methionine stimulated the growth and lysine accumulation. Threonine at concentrations upto 50 μg ml^{-1} stimulated growth but lysine accumulation was limited. For the isolate Lb, threonine was inhibitory for lysine excretion at a concentration of 500 μg ml^{-1}, whereas for the isolate B6 and 2Fa threonine was inhibitory from the level of 100 μg ml^{-1}. At the concentration of 500 μg ml^{-1} threonine completely inhibited extracellular lysine accumulation in all the three cases. When methionine and threonine were added together in different amounts the results were closely similar to those obtained with different concentrations of threonine alone.

As methionine alone upto a concentration of 500 μg ml^{-1} showed no inhibition in all the three cases, it appears that the aspartokinase (E. C. 2.7.2.4, the first enzyme of the lysine pathway) is insensitive to methionine alone. Sine these organisms excrete lysine, and lysine alone, obviously they cannot repress the aspartokinase. The inhibition of lysine excretion by threonine suggests that these organisms possibly possess aspartokinase which is subjected to repression either by a high concentration of threonine alone or by threonine plus lysine (accumulated intracellularly) in a concerted manner. That the lysine overproducers possess a single aspartokinase subject to a multivalent feed-back inhibition, has been shown by various workers[1,7]. Inhibition of lysine accumulation by threonine was shown in *Micrococcus glutamicus* by other workers[1,2,8]

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TABLE 1

Growth and lysine production by B_6, Lb and 2Fa.

Amino acid added to the medium	Concentration μg ml^{-1}	Isolate B_6		Isolate Lb		Isolate 2Fa	
		Growth[a]	Lysine[b]	Growth[a]	Lysine[b]	Growth[a]	Lysine[b]
Methionine	10	114	61	52	35	116	72
	50	119	65	56	41	122	76
	100	123	66	55	40	126	77
	200	123	66	52	40	127	77
	500	122	64	49	38	125	74
Threonine	10	113	61	56	39	115	71
	50	116	63	58	40	116	74
	100	117	48	57	39	118	56
	200	118	26	56	39	119	39
	500	117	—	48	—	116	—
Methionine + Threonine	10 each	115	62	58	43	118	73
	50 each	120	65	62	41	123	78
	100 each	120	44	61	39	124	51
	200 each	122	25	58	31	125	37
	500 each	118	—	48	—	120	—
Control	No. amino acid	110	60	49	35	112	70

[a] growth, dry weight, mg/100 ml [b] lysine, mg/100 ml.

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LACTOPROPIONIC ORCEIN AS A SUITABLE STAIN FOR MITOTIC CHROMOSOMES OF OLEACEAE

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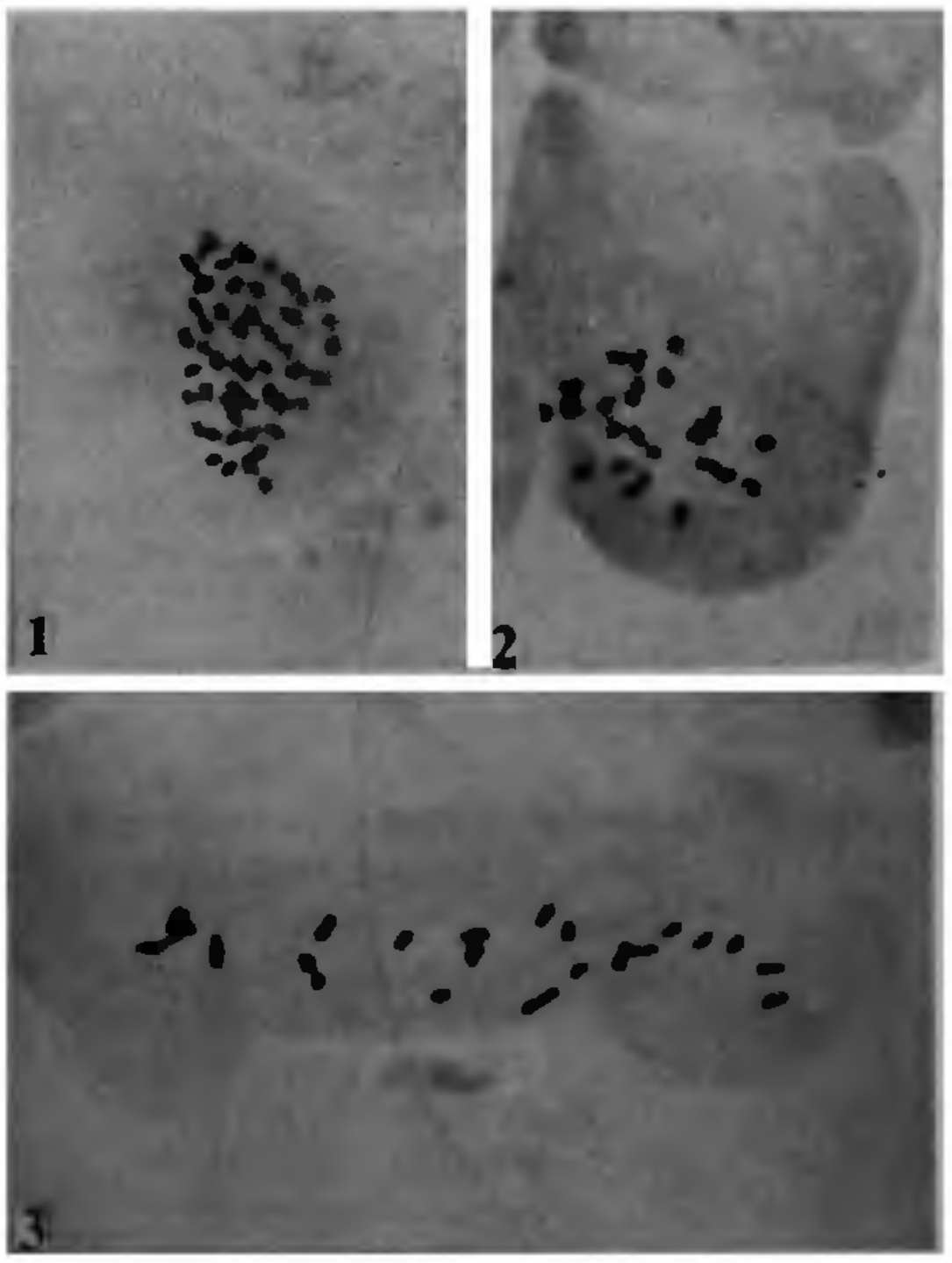
THE jasmine family, Oleaceae with 7 genera and 37 species in South India[1] is cytologically one of the least investigated. The chromosomes of these plants are extremely small and the $2n$ number varies from 24 to 52. No easy and rapid staining technique has been standardised for their chromosome study. Their mitotic cells, when stained by conventional acetocarmine etc staining methods[2-7], show intensely stained cytoplasm which reduces the contrast between the chromosomes and the background cytoplasm. Since the outline of the chromosomes is not clearly visible it does not permit detailed study of their morphology. In view of these, attempts were made to develop a staining technique which removes the disadvantages of the conventional methods.

The materials for the study were the root tips of species of *Jasminum*, *Olea* and *Myxopyrum* collected for fixation at 4.30 p.m. All the conventional methods[2-7] were tried. The results on comparison with regard to the quality of preparation especially for the contrast between chromosomes and cytoplasm and for the easiness and rapidity of procedure showed that lactopropionic orcein was the most suitable stain for root tip mitotic chromosomes. However, the method of Dyer[6] using this stain has been modified to suit the chromosome study in this set of plants and the procedure followed is given below.

Detached root tips were pretreated by immersing in 0.03% 8-hydroxyquinoline at 4° C for 3 hr, washed for 3 min in distilled water. Pretreated root tips were fixed at 4.30 p.m. in Carnoy's fluid (1:1:3 chloroform:glacial acetic acid:ethyl alcohol) with addition of a few drops of ferric acetate (8 min). Fixed root tip was stained by keeping it on a slide in two drops of lactopropionic orcein (previously prepared by dissolving 2 gm Gurr's natural orcein in 100 ml 1:1 lactic acid and propionic acid and diluting it to 45% with water) and warmed at 50° C (1 min). Stained root tip was then squashed in a fresh drop of lactopropionic orcein as quickly as possible (1 min). The squashed material was covered under a coverslip and pressed between blotting papers to wipe out excess stain. The slide was then ready for observation. Chromosome numbers of the following plants were determined for the first time. *Jasminum flexile* (from Courtallum), *J. primulinum*, *J. bignoniaceum*, *J. chinensis* (from Ooty) had $2n = 26$ while *Olea fragrans* (from Ooty) had $2n = 52$ and *Myxopyrum smilacifolium* (from Trivandrum) had $2n = 24$.

The present method differs from that of Dyer[6] by the use of 8-hydroxyquinoline instead of colchicine for pretreatment, the addition of ferric acetate and elimination of formalin in the fixative, the avoidance of maceration of the fixed material in HCL and consequent washing and the achievement of more rapid and



Figures 1-3. 1. $2n = 52$ chromosomes of *Olea fragrans* **2.** $2n = 26$ chromosomes of *Jasminum bignoniaceum* **3.** $2n = 24$ chromosomes of *Myxopyrum smilacifolium* (×1500)