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LACTOPROPIONIC ORCEIN AS A SUITABLE STAIN FOR MITOTIC CHROMOSOMES OF OLEACEAE

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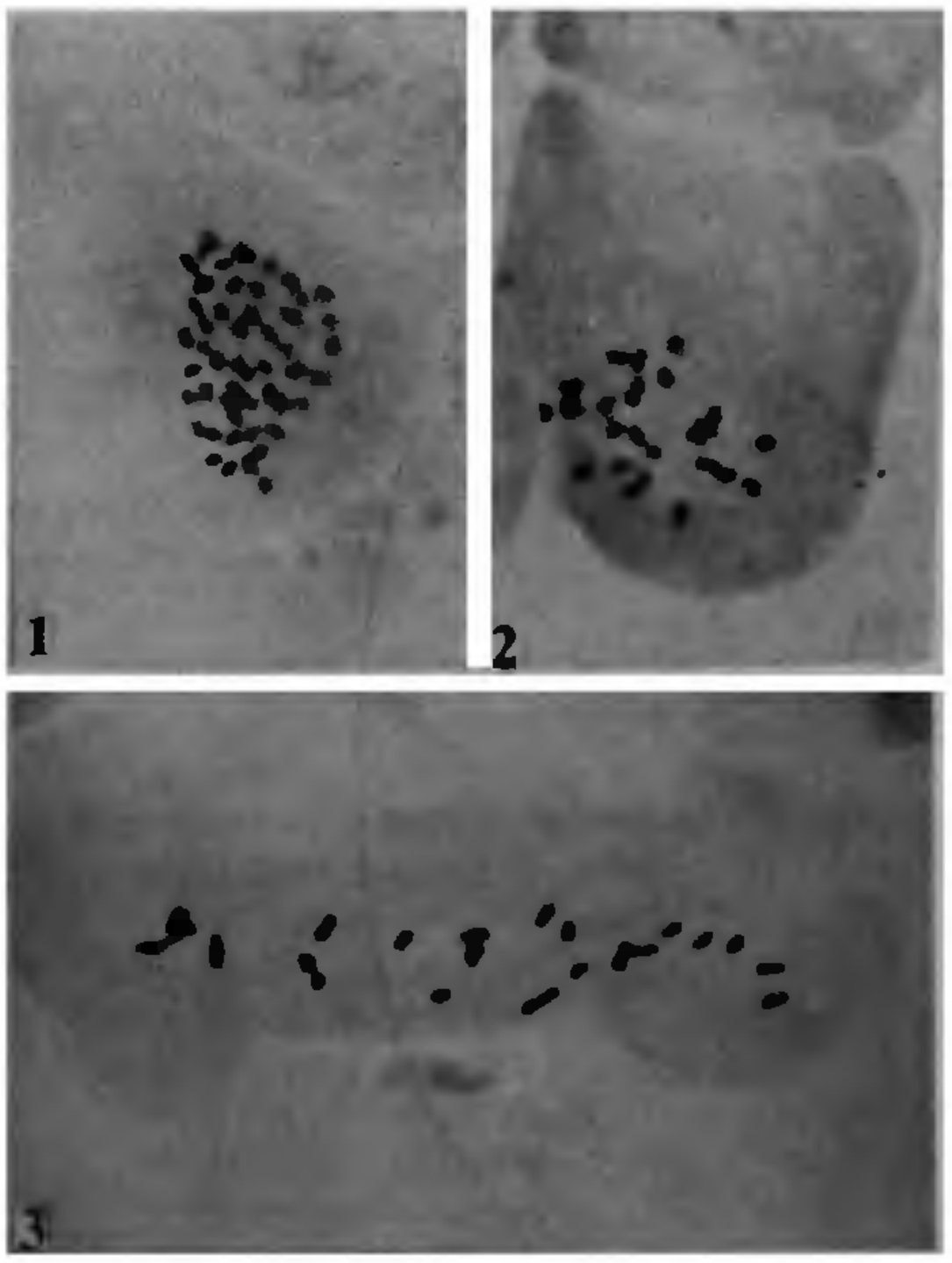
THE jasmine family, Oleaceae with 7 genera and 37 species in South India[1] is cytologically one of the least investigated. The chromosomes of these plants are extremely small and the $2n$ number varies from 24 to 52. No easy and rapid staining technique has been standardised for their chromosome study. 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)

easy staining and squashing. The whole process of fixation and staining requires not more than 12 min. The staining of chromosomes was intense preserving their structure without producing any artefacts. It reduced the staining of cytoplasm rendering a clear background for viewing the chromosomes with increased contrast (figures 1–3). As the chromosomes of these plants are small addition of formalin to the fixative is not essential for reducing bubble effect. Further, formalin caused hardening of tissue. The root meristems of these plants are soft enough for squashing after fixation in Carnoy's fluid and further softening by maceration in HCL is not necessary. Further elimination of cytoplasmic stain is achieved by warming the material in lactopropionic orcein during squashing. Addition of ferric acetate during fixation facilitates increased stainability of chromosomes providing greater contrast. Lactopropionic orcein is recognized as a more useful alternative to acetic orcein for delicate materials[4,8]. Propionic acid readily dissolves orcein and the stain penetrates cytoplasm without staining it. At the same time it stains the chromosomes more effectively and uniformly. Rupturing of cell membranes, scattering of chromosomes and deterioration of stain are avoided by addition of lactic acid as observed by Dyer[6]. The fixed material should not be stored for more than 12 hr as it increases staining of cytoplasm. Best results are obtained by staining immediately after 8 min fixation.

The modified lactopropionic orcein staining method is rapid, convenient and particularly useful for chromosome counts in root tips of all species of Oleaceae investigated presently.

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CONOCHAETE COMOSA KLEBAHN (CHLOROPHYTA)—A NEW ADDITION TO THE INDIAN FLORA

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THE genus, *Conochaete* Klebahn of the order Chaetophorales, Chlorophyta is rare in occurrence and known to have only four species[1]. Only *C. klebahnii* Schmidle has been reported from India[2]. *C. comosa* Klebahn communicated in this note is the first report from India. The alga collected in January 1983 from a permanent pond near Bihar village in Pratapgarh (UP) was found growing as an epiphyte on leaves and stems of certain aquatic angiosperms.

This alga formed scattered, mucilaginous patches of loosely aggregated cells and is usually grouped in 4 to 16 cells. The cells are globose and each of them has 2–3 delicate long setae radiating in different directions. The setae arise from protruded and prolonged conical

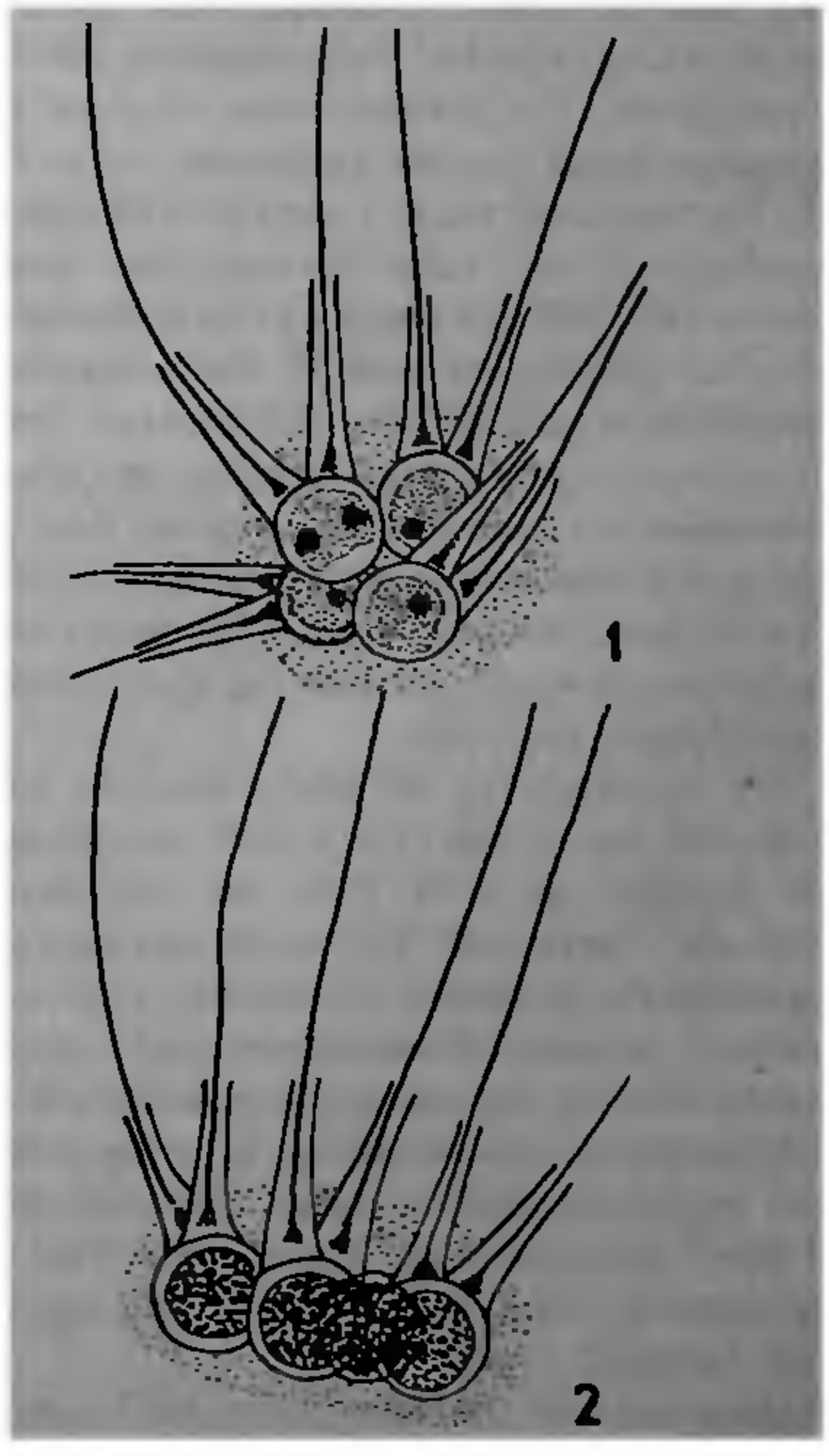

Figure 1 & 2 *Conochaete comosa* Klebahn (×350) **1.** A thallus showing vegetative cells **2.** A thallus showing perennating cells.