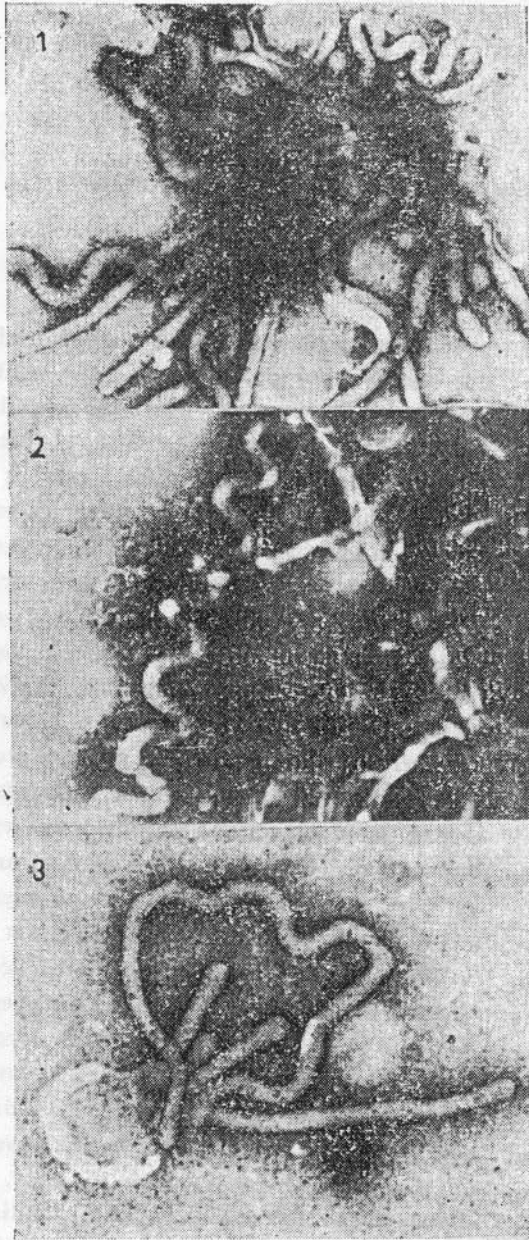


### Culturing of a helical Mycoplasma (*Spiroplasma*) from sesamum affected by phyllody

*Spiroplasmas* (Mollicutes) are associated with certain "yellows"—affected plants, vertebrates and invertebrates<sup>1-4</sup>. During the course of studying ultra-thin sections of phyllody-affected sesamum plants, some helical filaments were observed in sieve elements.

Petioles and mid-veins of tender leaves from phyllody-infected sesamum were surface sterilized in 1% sodium hypochlorite, rinsed in sterile water and triturated in small amounts of a horse serum-enriched C-3G liquid medium<sup>4</sup>. The extracts were centrifuged at low speed and the supernatants were filtered (0.45  $\mu$ m MF filter). Aliquot of diluted inoculum (0.1 ml/vial) was added to liquid medium in vials by serial passages (5 ml of medium/vial). Controls were prepared from the healthy plants and all the culture vials incubated at  $30 \pm 1^\circ\text{C}$ . The vials were monitored for any change in the medium. Turbidity was observed in some of the culture vials and the medium changed its colour slowly from red to yellow within 14 days. No change in colour was noticed in controls. Liquid cultures were centrifuged at 18,000 rpm for 60 min., and the pellets were resuspended in 1% ammonium acetate, droplets transferred to carbon coated copper grids, stained with 5% ammonium molybdate<sup>5</sup> and examined in a Carl Zeiss EM 109R. transmission electron microscope. Control samples similarly processed were also examined.

Changes generally occur in liquid medium due to activity of prokaryotes. Slow shift to acidity with faint turbidity mark the growth of Mollicutes<sup>6,7</sup>. Similar changes were observed in medium inoculated with extracts of phyllody-infected sesamum tissues indicating adaptation and *in vitro* growth of a Mollicute. The organism was serially sub-cultivable. Electron microscopy of cultures revealed pleomorphic organism with branching helical filaments devoid of true cell-walls. Sack-like blebs and spiral filaments were invariably observed in cultures in active phase of growth. Certain filaments were connected to irregularly shaped main bodies and filament branches had bulbous ends (Figs. 1-3). The control samples were free of organisms. The cultured organism was filterable (0.45  $\mu$ m MF filter), gram +ve, resistant to penicillin and thallium acetate, sensitive to Oxy-tetracycline, sterol-dependent and failed to revert to bacterial form in penicillin free medium. In the serum-enriched solid medium the organism behaved fastidiously and could not actively grow. However, in liquid medium it was amenable to grow. The characters of the organism are substantive<sup>6</sup> that it is a helical mycoplasma and identified as *Spiroplasma* sp. Although MLOs have been reported to be associated with sesamum phyllody disease<sup>8</sup>, identification of *Spiroplasma* with the disease and its successful cultivation



**Figs. 1-3 :** Electron micrographs of 5 day old cultures showing the helical mycoplasma cultured from sesamum plants affected with phyllody ; (1) The pleomorphic organism with typical spiral filaments ; (2) Sack like bleb and spiral filaments in actively growing *Spiroplasma* culture ; (3) Irregular main bodies attached with branching filaments of *Spiroplasma* (Note the bulbous ends of the filaments).

*in-vitro* were not reported so far. Successful recovery of helical filaments outside the phyllody affected tissues is suggestive that such structures are true form of spiral filaments. This is the first instance of a successful culturing of a *Spiroplasma* reported from India out of plant tissues.

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N. SRINIVASAN  
J. J. SOLOMON

C. P. C. R. I.,  
Regional Station,  
Kayangulam,  
Krishnapuram-690 533,  
Kerala.

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