

LIGHT AND FLUORESCENT MICROSCOPIC STUDIES ON KOKKAN DISEASE OF BANANA [*MUSA AAB NENDRAN*]

Kokkan disease, a serious threat to banana in Kerala, still remains to be a disease of unknown etiology. It was first reported by Samraj *et al.* (1966) in Trichur district. The infestation is seen throughout in Kerala causing serious damage to the crop, with the intensity of the disease and extent of damage being maximum in Nendran variety, which is largely cultivated on commercial scale in this state.

Several efforts have been made to detect the causal organism and to evolve effective control measures for this menace. But none of the trials conducted so far could confirm the etiology of the disease. However, it was revealed that the cause of the disease was not any toxicity or deficiency of major or minor elements and association with any fungi or nematode. Use of any antibiotic was not helpful. Virus or mycoplasma like organism is now suspected to be the causal factor for the disease (Pushkaran *et al.*, 1994).

An investigation was conducted in this regard using light and fluorescent microscopic studies to find out the etiology of kokkan disease. Histopathological and cytopathological detection techniques, using light and fluorescent microscopy have been used extensively in recent times for the diagnosis of yellow disease caused by molecules and allied pathogens (Schneider, 1973; Ghosh, 1982). Ultrastructural morphology of the diseased tissue and remission of the symptoms by tetracycline therapy are the two evidences for the mycoplasmal etiology of these diseases. Since kokkan plants exhibited auto-remission of the symptoms (summer months), an attempt was made for the detection of mycoplasma or allied pathogen.

Experiments were conducted at the Banana Research Station, Kannara, Trichur for studying the etiology of kokkan disease in collaboration with the Kerala Forest Research Institute, Peechi. General anatomical studies did not show any necrosis or excessive formation of phloem vessels. Fluorescent microscopy

was also conducted for studying the phloem cells of diseased plants. For that fluorochrome, aniline blue which is used for detecting callus in the phloem of mycoplasma infected plant tissues was prepared according to Hiruki and Shukla (1973). Free hand sections of fresh tissues were immediately heat killed in boiling water for 8 min and stained in 0.1% aniline blue (Ghosh and Raychaudhuri, 1974). Stained sections were viewed and photographed under a Leitz Dialux microscope with epifluorescence attachment.

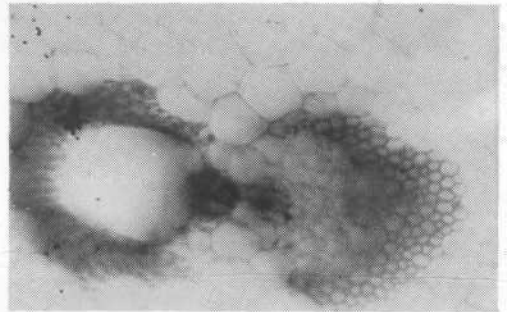


Plate 1. Vascular bundle of healthy banana (x 165)

The results indicated that the phloem was not disturbed whereas more number of mechanical tissues i.e., the xylem and fibres (sclerides) were seen in diseased plants (Plates 1 and 2). In the case of vascular bundles also there was not much reduction in number from both healthy and diseased plant parts (midribs, pseudosheath, roots etc).

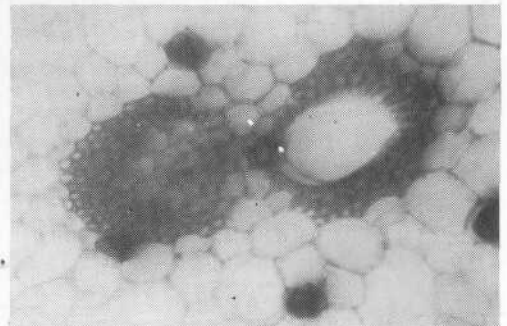


Plate 2. Vascular bundle of kokkan affected banana (x 165)

The quantitative changes in phloem were studied with the sections viewed under projection microscope and the section images were drawn on a graph paper. The percentage area occupied by phloem out of the total area gave no marked difference between healthy and diseased plants.

Diene's staining reaction

Diene's stain normally used for detection of animal mycoplasmas (Dienes *et al.*, 1948) contains 2.5 g methylene blue, 1.25 g azure II, 10 g maltose and 0.25 g sodium carbonate in 100 ml distilled water. Hand sections of fresh tissues were directly stained in 0.2% solution of filtered Diene's stain for 10 min after washing in distilled water and viewed under the

microscope gave negative results showing that no such distinct area was seen with diseased tissues.

During the study, a check was made with a mycoplasma disease namely, little leaf disease of eucalyptus by Diene's reaction and fluorescent microscopy and in that case the results were positive. This obviously indicated that mycoplasma association was not involved in the case of kokkan.

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