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STUDIES ON THE ULTRA-STRUCTURAL CHANGES IN ROOT (WILT) DISEASED PALMS

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ABSTRACT

Coconut root (wilt) disease, a debilitating malady is characterized by external symptoms like flaccidity, yellowing and necrosis. Anatomy of the diseased roots revealed alterations in vascular tissues in the form of tylosis of xylem vessels, gummosis and phloem necrosis. In the course of the investigation to detect submicroscopic pathogen(s) associated with the disease, tissues of submeristem, bases of rudimentary leaves, rachilla of juvenile inflorescence , spear leaf, mature leaf and root tips were studied. The samples examined were from palms of various intensities of disease. The prominent structural changes noticed were an elaboration of the membrane system with unusually large number of cell organelles, presence of paramural bodies and accumulation of electron dense material filling the sieve tube indicating necrotic obliteration of the phloem cells and deposition of callose adjacent to the sieve pores. Disproportionately high number of phytoferritin particles and cuneate crystalline inclusions were the other prominent alterations observed. These structural anomalies in relation to the disease are discussed.

INTRODUCTION

Coconut root (wilt) disease is a debilitating malady exhibiting flaccidity of leaflets as the most consistent and diagnostic symptom and yellowing and marginal necrosis as other associated symptoms (Radha and Lal, 1972). Rajagopal *et al.* (1986) stated that an imbalance in the water economy formed as a result of damage to stomatal apparatus could ultimately be leading to an irreversible flaccidity symptom. Comparative histopathological studies on palms with different intensities of disease revealed structural alterations in vascular tissues in the form of tylosis in xylem vessels and gummosis. Phloem tissues showed increased chromophily and necrotic obliteration (Govindankutty and Vellaichamy, 1983). Solomon *et al.*(1983) reported the presence of a phloem limited mollicute in the sieve tubes of apical meristem, petiole of juvenile leaves and root tips. In the course of our investigation to establish the constant association of mycoplasma - like organisms (MLOs) with the disease, palms of various age groups and intensities of disease were studied. The ultrastructural changes observed in the vascular tissues of diseased palms especially in the phloem and the morphological details of the organism are discussed in this paper.

MATERIALS AND METHODS

Six West Coast Tall palms (20-25 yr. old), three each in the early and middle stage of disease were subjected to destructive sampling and tissues of sub-meristem, bases of rudimentary leaves, rechilla of juvenile inflorescence, leaflets of spear and mature leaf and tender root tipes were studied. In non-destructive samplings, rachilla of tender inflorescence and roots of nine diseased palms (30-35 yr. old), three each in early, middle and advanced stages of disease and similar tissues of ten healthy palms from a disease-free area (Kasaragod) were also studied.

The fixatives used were 2% Glutaraldehyde + Paraformaldehyde in 0.05 M cacodylate buffer pH 7.2 - 7.4 and 2% Glutaraldehyde in 0.1 M phosphate buffer pH 7.2. The tissues were postfixed in 2% osmium tetroxide in the respective buffer, dehydrated in a graded alcohol series followed by changes in acetone and embedded in Spurr's resin (Thomas, 1979).

Ultra-thin sections of 600 to 700 A cut in a LKB ultratome IV were transferred to uncoated 200 mesh copper grids. The sections were stained with uranyl acetate and Reynold's lead citrate as per the standard procedure and

examined under Carl Zeiss EM 109 Turbo transmission electron microscope operating at an accelerating voltage of 80 KV.

One micron sections of the blocks were also taken prior to ultra-thin sectioning for locating the desired tissues.

RESULTS AND DISCUSSION

Electron microscopic examination of the ultra-thin sections of sub-meristem, leaf bases of developing leaves, rachilla of juvenile inflorescence and root tips from diseased palms revealed the presence of MLOs in the sieve tubes. The phloem parenchyma and companion cells were not having the organisms. Pleomorphic forms ranging from circular to oval and occasionally beaded or filamentous were observed (Fig. 1). The cocoid forms were in the size range of 250-400 nm (Solomon and Govindankutty, 1991). The organisms were bound by a trilamellar unit membrane and contained well-defined internal structures such as DNA strands and peripherally distributed ribosomes. The cell walls of the invaded cells and those close to them were often thickened, the cytoplasm granulated and contained vesicle-like structures (Solomon *et al.* 1983).

Distribution of MLOs within the vascular bundles was rather sparse and not all sieve elements in a patch contained them. Some of the bundles were totally free of MLOs. They were found in parietal position when occurring in low concentration. Such uneven distribution of the organism have been reported in lethal yellowing disease of palms (Parthasarathy, 1974a; Thomas, 1979), X-disease of Prunus (Garnet and Gilmer, 1971) and certain other Witches' broom diseases (Hiruki and Shukla, 1973; Seliskar *et al.*, 1973). In the mature leaves only moribund forms lacking cytoplasmic contents were observed. Similar empty structures were described in MLO infected coconut tissues from West Africa (Dabek, 1977) and in yellow leaf diseased arecanut palms in India (Nayar and Seliskar, 1978). Parthasarathy (1974b) postulated that the apparent absence of MLOs in mature parts and their occurrence in tender tissues suggests the movement of the organism in the phloem assimilate to the "sink" region.

Sieve tubes which contained MLOs and the bordering cells often had fibrillar tubules (Fig. 2). The tubules occasionally had electron dense granules. In sieve tubes of rachilla the fibrils were found aggregating close to the cell wall (Fig. 3). Opinions differ as to the identity of these fibrils. According to Parthasarathy (1974 b, c), these structures did not morphologically resemble the P-proteins normally present in palms. However, Dollet *et al.*, (1976) have observed the fibrils in coconut palms affected by Kaincope disease and described them as P-proteins. Esau (1968) had noticed such overproduction of P-proteins in TMV infected tobacco plants. The P- proteins which are similar to microfilament are supposed to provide the motive force for assimilate movement in sieve elements (Hepler and Palevitz, 1974). Although P-proteins are normal host components, their massive accumulation in sieve cells of diseased palms may be part of the host-parasite interaction.

The protophloem elements in roots and rachilla of diseased palms were often compressed and had electron dense contents indicating necrotic obliteration. One of the consistent features observed was presence of callose deposits lining the sieve pores of MLO infected cells (Fig. 4). Callose deposition, a response to injury, is often observed in a number of plant diseases.

Another prominent change noticed in sieve cells was the elaboration of the membrane system. Plasmalemma which lines the cell wall makes deep intrusion into the cell lumen forming invaginations. Such elaboration was found to be very extensive in diseased palms. The invaginations often enclosed vesicular or membraneous structures lacking contents. These structures were described as paramural bodies (Merchant and Robards, 1968). Similarly, loamosomes, another type of paramural bodies with membraneous elements derived from cytoplasmic membranes were also observed. They were either empty or had sparsely distributed electron dense contents. Some of the empty vesicles seem to be associated with or to develop from golgi bodies (Fig. 5).

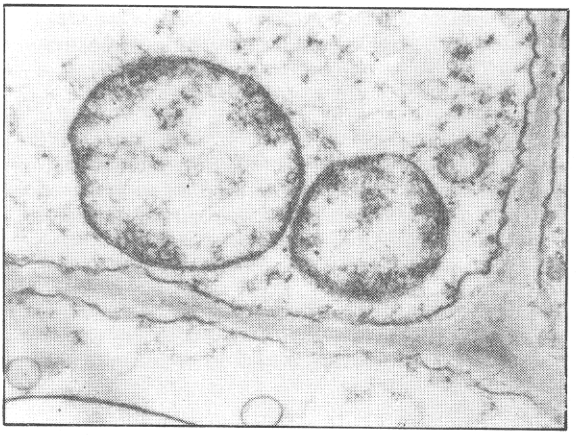

Fig. 1 An enlarged view of MLOs in sieve tubes of rachilla of a discased palm

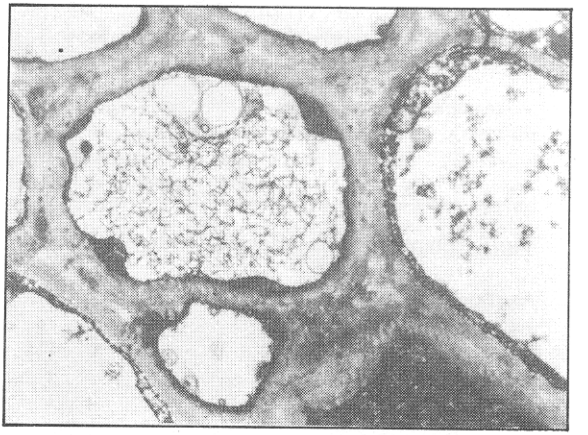

Fig. 2 P. protein fibrils filling the lumen of the sieve tube

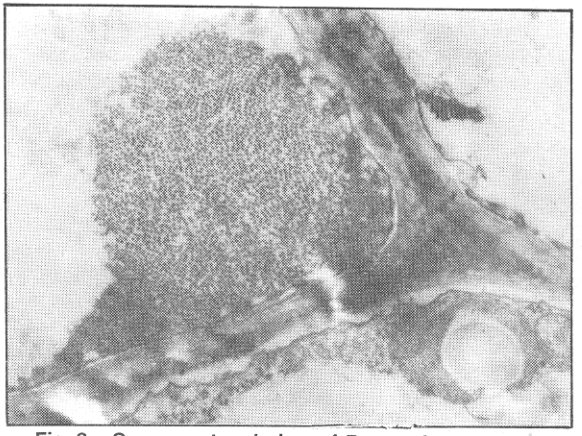

Fig. 3 Cross sectonal view of P. protein aggregates occupying position close to the sieve tube wall

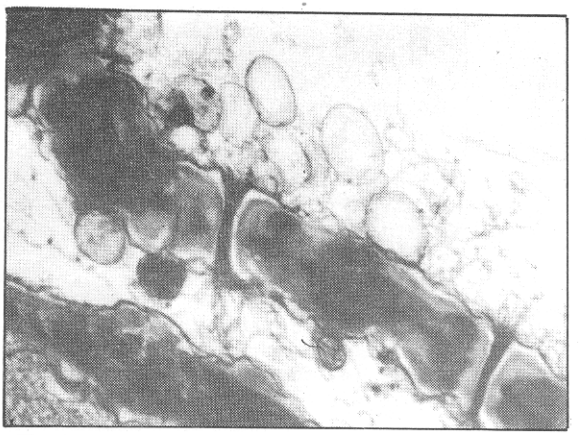

Fig. 4 Callose lining the sieve pore of a MLO infected cell

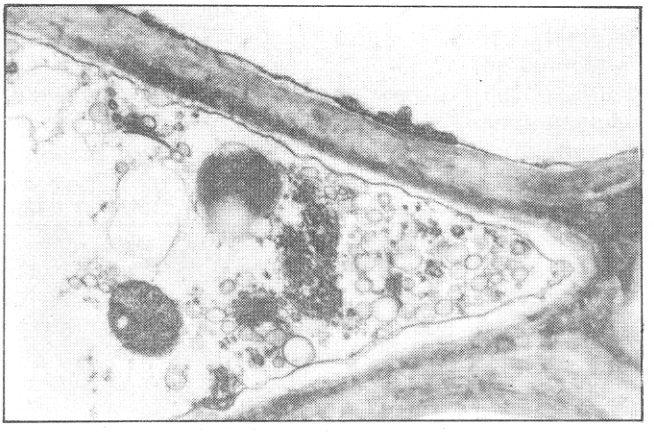

Fig. 5 Empty vesicles associated with golgi bodies

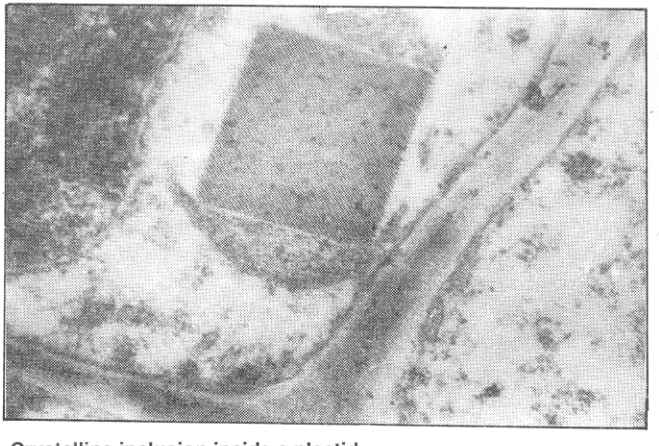

Fig. 6 Crystalline inclusion inside a plastid

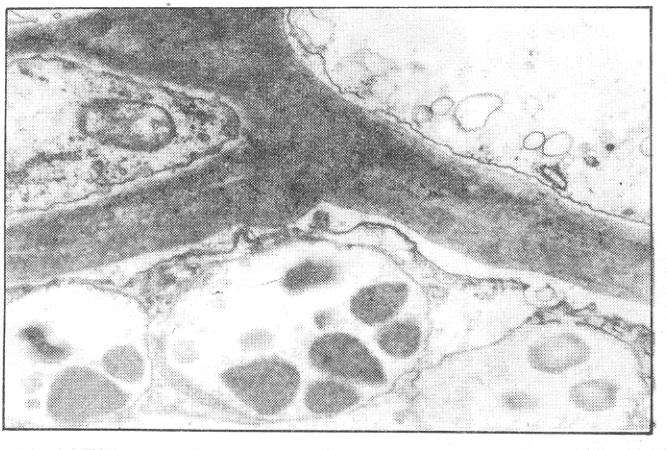

Fig. 7 Cross sectional view of a sieve tube having starch grains and phytoferritin particles encircled by a membrane

The sieve tube plastids contained crystalline inclusions with well defined crystal lattices (Fig. 6). They were interpreted as 'cuneate crystalline inclusions', a normal host component present in a number of palm species (Parthasarathy, 1973). Degenerative changes were also noticed in plastids. Accumulation of starch and protein crystals were observed in the plastids of rachilla. Clusters of electron dense crystals enclosed in large vesicles by a membrane were often observed. These membrane bound crystals were four sided or rhombic or sometimes hexagonal (Fig. 7). A gradual increase in the number of cells containing such cytoplasmic crystals and the number of crystals per cell was observed in rachilla of diseased palms. These crystalline structures were also observed in coconut leaves of palms affected by yellowing and was interpretted as phytoferritin (Wildman and Hunt, 1976). The phytoferritin, a normal host component often accumulates in plants infected with a number of biological agents.

Although no major alterations were noticed in cell organelles such as mitochondria, ER and Golgi bodies, the significant changes observed in the membrane system, plastids, P-proteins and the occurrence of large number of crystalline structures indicate the host response to mycoplasmal infection.

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REFERENCES

DABEK, A. J. 1977. Electron microscopy of Kaincope and Cape St. Paul Wilt diseased coconut tissue from West Africa. *Phytopath. Z.* **38** : 341-346.

DOLLET, M., GIANNOTI, J. and CZARNECKY, 1976. Kaincope Disease: Presence of mycoplasma in the phloem of diseased coconuts. *Oleagineux* **31** : 169-171.

ESAU, K. 1968. *Viruses in plant hosts - Form, distribution and pathologic effects.* The University of Wisconsin Press, London, 225 pp.

GARNET, A.L. and GILMER, R. M. 1971. Mycoplasmas associated with X-disease in various *Prunus* species. *Phytopathology,* **61**: 1036-1037.

GOVINDANKUTTY, M. P. and VELLAICHAMY, K. 1983. Histopathology of the roots of coconut palms affected with root (wilt) disease. pp. 421-425. In *Coconut Research and Development* (ed. N. M. Nayar), Wiley Eastern, New Delhi 518 pp.

HEPLER, P. K. and PALEVITZ, B. A. 1974. Microtubules and microfilaments. *Ann. Rev. Plant Physiol.* **25** : 309-362.

HIRUKI, C. and SHUKLA, P. 1973. Mycoplasma - like bodies associated with Witches'-broom of bleeding heart. *Phytopathology* **63** : 88-92.

MERCHANT, R. and ROBARDS, A. W. 1968. Membrane systems associated with the plasmalemma of plant cells. *Ann. Bot.* **32** : 457-471.

NAYAR, R. and SELISKAR, C. E. 1978. Mycoplasma-like organisms associated with yellow leaf disease of *Areca catechu L. Eur. J. For. Path.* **8** : 125-128.

PARTHASARATHY, M. V. 1973. Mycoplasma-like organisms in the phloem of palms in Florida affected by lethal yellowing disease. *Plant Dis. Reptr.* **57** : 861-862.

PARTHASARATHY, M. V. 1974a. Mycoplasma-like organisms associated with lethal yellowing disease of palms. *Phytopathology.* **64** : 667-674.

PARTHASARATHY, M. V. 1974b. Ultrastructure of phloem in palms I. Immature sieve elements and parenchymatic elements. *Protoplasma,* **79** : 59-92.

PARTHASARATHY, M. V. 1974c. Ultrastructure of phloem in palms. III. Mature phloem. *Protoplasma* **79** : 265- 315.

RADHA, K. and LAL, S. B. 1972. Diagnostic symptoms of root (wilt) disease of coconut. *Indian J. agric. Sci.* **42** : 410-413.

RAJAGOPAL, V., PATIL, K. D. and SUMATHY-KUTTYAMMA, B. 1986. Abnormal stomatal opening in coconut palms affected with root (wilt) disease. *J. Exptl. Bot.* **37** (182) : 1398-1405.

SELISKAR, C. E., WILSON, C. L. and BOURNE, C. E. 1973. Mycoplasma like bodies found in phloem of black locust affected with Witches' -broom. *Phytopathology,* **63** : 30-34.

SOLOMON, J. J. and GOVINDANKUTTY, M. P. 1991. Etiology e. Mycoplasma-like organisms in coconut root (wilt) disease. pp. 31-40. Monograph

Series No. 3 (ed. M. K. Nair and others). Central Plantation Crops Research Institute, India 92 pp.

SOLOMON, J. J., GOVINDANKUTTY, M. P. and NIENHAUS, F. 1983. Association of mycoplasma-like organisms with the coconut root(wilt) disease in India. *Z. Pflkrankh. Pflschutz.* **90** : 295-297.

THOMAS, D. L. 1979. Mycoplasma-like bodies associated with lethal decline of palms in Florida. *Phytopathology,* **69** : 928-934.

WILDMAN, R. B. and HUNT, P. 1976. Phytoferritin associated with yellowing in leaves of *Cocos nucifera* (Arecaceae). *Protoplasma,* **87** : 121-134.

DISCUSSION

K. JAYARATHINAM : Have you conducted similar electron microscopic studies on totally ematiated (very unhealthy) plants in disease free area? Is it not better to compare the diseased tree with that of the above?

J.J. SOLOMON : Yes, 10 healthy palms from a disease free area, Kasaragod were also studied for comparison.

P.R.V. SUBRAMANIA IYER : Is it possible to correlate these structures with some biochemical compounds?

J.J. SOLOMON : I do not know.

Y.R. SARMA : What will be the state of ultrastructure of the free palms which are positive for disease but did not express the symptom?

J.J. SOLOMON : We have not studied the ultrastructure of apparently healthy palms in a diseased area. The present investigation is between healthy and diseased palms only. However, it is worth examining the apparently health palms also.

R.D. IYER : What is the nature of the P-protein? Is it entirely comprising of host protein or is elaborated by MLO infection? Are you planning to characterize these proteins in terms of electrophoretic pattern and/or amino acid profile?

J.J. SOLOMON : P-proteins which are similar to microfilaments are normal host components. But over production of P-protein was observed in diseased palms. This may be as a result of host-parasite interaction. It is worth characterizing the proteins.

K.V. NAGARAJA : Does the accumulation of P-proteins in response to the infection?

J.J. SOLOMAN : Yes. The accumulation or over production of P-proteins is in response to infection.