

VIROLOGICAL INVESTIGATIONS

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NATURE OF THE PATHOGEN

The exact nature of the pathogen associated with the disease and which is transmissible through soil, sap, insect vector and possibly seed borne is still not established beyond doubt. Nagaraj, Davis and Menon (1954), based on systemic nature and resemblance of symptoms to other known plant viruses, suggested it to be a virus disease. Positive transmission of the disease on to the main host by mechanical inoculation of sap and through the insect vector Stephanitis typica in the field condition (Nagaraj and Menon 1956) and under insect-proof condition (Shanta, Thomas Joseph and Lal 1964) further contributed to the possible viral etiology of the disease. Shanta and Menon (1961) and Shanta (1971) studied the physical properties of the coconut wilt virus - the virus associated with the disease using cowpea as indicator plant. It was reported to have a wide host range, when artificially transmitted, in the natural orders Leguminosae, Solanceae and Palmae, causing systemic symptoms in all. Necrosis and malformation of leaves and stunting of plant are the major symptoms in general. The virus was also reported to occur in nature in the roots of many weeds that are found to be common in coconut gardens and also systematically in coconut, cowpea and Physalis. Physical properties of the pathogen in leaf extracts of coconut were, thermal death point of 76°c, dilution end point of 10⁻⁴ with maximum infectivity in the range of pH 5-9 in the supernatant solution of the extract. The longevity in vitro of the sap was 8 weeks when stored frozen and 3 weeks at room temp. (28-30°c) at a dilution of 1/2.5. Summanwar, Raychaudhuri, Jagadish Chandra, Nam Prakash and Lal (1969) reported the isolation of a virus from diseased leaves and roots of coconut. The purified fraction on inoculation produced few chlorotic lesions on Chenopodium amaranticolor and systemic mosaic on Nicotiana tabacum CV. white Burley. Purified fractions from C. amaranticolor on examination under EM showed rod shaped particles in the range of 320-360 nm length and 24-25 nm width. Similar particles of identical size and shape were also observed in purified fractions from diseased coconut leaves and roots. However, no rod shaped particles were observed in samples of leaves, roots and nut water from healthy palms. The virus was identified

as a strain of TMV based on its positive serological reaction to three different strains of TMV antisera. It was also reported to have a wide host range, longevity in vitro of about one year, dilution end point between 10^{-5} and 10^{-6} and thermal inactivation point of 90°C (Summanwar, Raychaudhuri and Jagadish Chandra 1971). Only purified fractions from diseased leaves and roots were found to be infectious on C. amaranticolor and crude extracts from the same were found to be non-infectious. The non-infectious nature of the crude sap was attributed to the presence of high tannin content in the sap, acting as inhibitor of viral infection, which on purification was either eliminated or reduced so as to express infectivity. Summanwar and Gupta (1976) using specific viral inhibitors concluded that the percentage of infection on test plants could be increased upto 50% when protective chemicals like lead acetate and Polyclar were incorporated in the sap to 15% and 30% concentration respectively. However, Shanta et al (1975) investigating on the possible association of TMV with the disease reported that purified preparation of TMV when mixed with coconut sap even at a dilution of 10^{-4} was still infectious and also could easily be distinguished in negatively stained preparations under EM. They also reported the presence of long tubular particles of 1-2 μ long and 19-24 μm wide in large amounts in young root and leaf tissues of palms from diseased as well as diseased-free areas. Such particles were either totally absent or, if present, only in low concentrations in mature green leaves and older leaf and root tissues. The rod shaped particles were recognized as of host origin not associated with any disease (Randles 1975). Shanta et al (1975) suggested that these particles breaking into rods of shorter size during the process of purification and resembling TMV might have been identified so.

An antiserum prepared against the partially purified root (wilt) sample on testing reacted only against its homologous antigens namely, the partially purified fractions and leaf extracts of diseased coconut samples. No reaction was observed against leaf extracts of healthy coconut and extracts from TMV infected tobacco. Similarly, the TMV Coconut isolate antiserum reacted only against purified TMV and leaf extracts from TMV infected tobacco and no reaction was evident against either, extracts of healthy and diseased or partially purified fractions of root (wilt) diseased coconut samples (Solomon and Sasikala 1980). This conclusively proves that TMV (coconut isolate) is not associated with the root (wilt) disease of coconut. Tissue samples of leaf, root, inflorescence and anther from diseased

palms fractionated by different methods meant for rod/spherical ss, ds RNA and DNA viruses did not yield any infectious preparations. However, preparations fractionated for ss and ds RNA virus had specific extinction coefficient at 260 nm with a minimum at 280 and had 260/280 ratio ranging from 1.13 to 1.4 suggesting the presence of a nucleoprotein similar uv absorbing, fraction were not obtained from samples collected from healthy trees processed by identical methods. Long rods were observed when these fractions were studied by electron microscope. No viral particles could be identified on preliminary examination of eluted precipitin gels. Maramcrusch and Kondo (1977) reported the presence of icosahedral virus-like particles with a diameter of 56 nm in the ultrathin sections of diseased leaf tissues. Similar particles have not been described associated with either healthy or any of the known coconut palm diseases. This has been later pointed out by Parthasarathy (1978) as transverse to slightly oblique section of plasmodesmata. The possible association of spherical bodies with the disease was indicated based on preliminary examination of diseased leaf sap coconut water from nuts of diseased palm as well as cowpea with symptoms (Ann. Rep. 1966). Recently a limited number of purified fractions on examination under EM revealed large bacilliform particles. Such particles were not seen in any of the apparently healthy samples tested. Since these reports are based on the examination of limited number of samples they warrant further extensive work before any association is suggested. Holmes (1965) pointed out that although the pathogen resembles a virus, it might be a related organism like a spirochete or a sporozoan. More concentrated effort on Electron Microscopy and serologically specific Electron Microscopy may throw light on the nature of the pathogen.

No mycoplasma like organism (MLO) could be cultured from roots, leaves and inflorescence of diseased trees although a number of attempts were made with different culturing media. Tender and mature tissues of root, leaf and inflorescence of apparently healthy and diseased palms of limited number of samples examined did not indicate the presence of MLO in the ultrathin section. This work was done in collaboration with Dr. M.V. Parthasarathy of Cornell University, U.S.A.

DIAGNOSIS

Root (wilt) diseased palms are diagnosed on the basis of visual symptoms considering flaccidity as the primary symptom and yellowing and necrosis as other associated symptoms. Since

these symptoms appear singly or in combination under strained environmental conditions too, identification of a disease is rather difficult. Hence the need for a more reliable and sensitive diagnostic test which could detect the palms at a very early stage of disease before the foliar symptoms are apparent was felt. This would also help in formulating suitable control measures.

A colour test based on differential dehydrogenase activity of leaf tissues developed after testing 1100 samples from 323 trees in different stages of disease was however, found to be not diagnostic since the test when applied to healthy samples did not yield conclusive and consistent results. This could obviously be due to the influence of environmental factors on the enzymatic activities of the plant.

Accumulation of aminoacids notably, asparagin and other ninhydrin positive free amino acids in the tender leaves of diseased coconut trees promised the developing of a color test. However, this again gave inconsistent results under varying environmental conditions.

A third test, based on tannin content was tried following the observation that diseased trees in general had low tannin content in their leaves. Although tannin or similar coloring substance, get gradually reduced/depleted as disease progresses the change is not so marked on the onset of the disease to use it as a diagnostic tool.

Paper chromatographic analysis of proteins revealed the presence of an abnormal protein associated with the disease which was found to be totally absent in the healthy. However, this analytical work could not be pursued further. Presence of such abnormal protein either pathogen induced or host induced as a response to infection is well known in other plant diseases and has been successfully used as a tool diagnosing the diseased plants. Studies on the Isozyme pattern is another line worth investigating.

A collaborative project between the Indian Space Research Organisation, Indian Agricultural Research Institute and Central Plantation Crops Research Institute with NASA of U.S.A. was undertaken for the early detection of the root (wilt) disease by 'remote sensing' technique using false infra-red aerial photography. Findings, by and large, indicated that the crown of healthy palms appeared red and those of diseased showed paleness as a result of weaker infra red reflectance on the film as

measured by microphotometer. Samples from some of the apparently healthy looking palms recording a weaker infra red reflectance when tested for infectivity as well as examined under EM revealed the presence of the virus thus confirming that this technique could be used to detect the diseased palms before visual symptoms appeared (Dakshinamoorthy, Krishnamoorthy, Summanwar, Shanta and Pisharody 1971; Dakshinamoorthy and Summanwar, 1972).

All these biochemical tests investigated so far were based on reflections of altered host metabolism perceptible in the form of either accumulation or depletion of substances consequent to differential enzymatic activity which could be induced under varying conditions. But a sero-diagnostic test would be of more specific, highly sensitive and reliable in detecting the disease even at an incipient stage. Since the suspected virus could not be isolated to reasonable purity, antisera were prepared against either alcohol/heat clarified and dialysed or partially purified fraction which proved to yield non specific reaction. Shanta *et al* (1971) observed that agglutination tests were unreliable with coconut leaf and root extracts because of non specific reaction obtained with normal serum proteins. Similarly non specific reactions were obtained with diseased coconut leaf extracts against all the tested antisera in Ouchterlony's double diffusion test. It was presumed that some materials in the coconut sap possibly tannins diffuse and reacts non specifically with serum proteins. However, antiserum prepared against clarified extract from cowpea failed to induce any antibodies.

Recently Solomon, Sasikala and Shanta (1976) developed a sero-diagnostic test for the early detection of the disease before the foliar symptoms are apparent. Diseased palms of all age groups growing in different soil types could easily be diagnosed and the severity of the disease assessed by the intensity of reaction. The antiserum is so specific to root (wilt) that no reaction was observed against healthy and samples of other coconut diseases. The specificity and the sensitivity of the antiserum promises wider application in serologically specific Electron Microscopy for a better understanding of the etiology.

REFERENCES

- ANONYMOUS 1966 Annual Report of the Central Coconut Research Station, Kayangulam. pp. 17-18.
- DAKSHINAMOORTHY, C., KRISHNAMOORTHY, B, SUMMANWAR, A.S., SHANTA, P. and PISHARODY, P.R. 1971. Remote sensing for coconut wilt. 7th Internat. Symp. on Remote sensing of environment, Ann. Arbor.

- DAKSHINAMOORTHY, C., and SUMMANWAR, A.S. 1972. Remote sensing of coconut palm in Kerala (India). 8th Internat. Symp. on Remote sensing of environment held at the University of Michigan Ann. Arbor. Mich., U.S.A.
- HOLMES, F.O. 1965. Report to the Government of India on investigations on the etiology of coconut root (wilt) disease. Report No. 1958, Project No. India/TE/OL.
- MARAMOROSCH, K, and KONDO, F. 1977. Electron Microscopy of leaf sections from Kerala wilt diseased coconut palm. J. Plant. Crops 5 : 20-22.
- NAGARAJ, A.N., DAVIS, T.A. and MENON, K.P.V. 1954. Sap transfusion, a new device for transmission trials in palm. Indian Cocon. J. 9 : 1961-1965.
- PARTHASARATHY, M.V. 1978. Have virus like particles been found leaf of Kerala wilt diseased coconut palms? Not yet. J. Plant. Crops 6 : 87-89.
- RANGLES, J.W. 1975. Detection in coconut of rod shaped particles which are not associated with disease. Plant. Dis. Reprtr. 59 : 349-352
- SHANTA, P. 1971. Studies on transmission and nature of the pathogen associated with root (wilt) disease of coconut of Kerala. Internat. Symp. on pathological wilting in plants, Madras.
- SHANTA, P. JOSEPH, T. and LAL, S.B. 1964. Transmission of root (wilt) disease of coconut. Indian Cocon. J. 18 : 25-28.
- SHANTA, P. and MENON, K.P.V. 1961. Studies on some properties of the coconut wilt virus. Indian Cocon. J. 15: 36-46.
- SHANTA, P., HARIHARASUBRAMANIAN, V. and GOPINATHAN PILLAI, N. 1975. Possible association of tobacco mosaic virus with the root (wilt) disease of coconut. J. Plant. Crops 3 (2) : 77-80.
- SOLOMON, J.J. and SASIKALA, M. 1980. A serological appraisal of the connection of the tobacco mosaic virus coconut isolate with the root (wilt) disease of coconut. Phytopath. Z. 99, 26-32
- SOLOMON, J.J., SASIKALA? M. and SHANTA, P. 1976. A serological test for the detection of the Root (wilt) disease of coconut. Internat. Symp. on Coconut Research and Development held at Central Plantation Crops Research Institute, Kasaragod during December, 1976
- SUMMANWAR, A.S. and GUPTA, M.D. 1976. A quick method for isolating coconut root (wilt) virus from the diseased palms. Indian Phytopath. 29 : 108.
- SUMMANWAR, A.S., RAYCHAUDHURY, S.P., JAGADISH CHANDRA, K, NAMPRAKASH and LAL, S.B. 1969. Virus associated with coconut root (wilt) disease. Curr. Sci. 38 : 208-210.
- SUMMANWAR, A.S., RAYCHAUDHURY, S.P. and JAGADISH CHANDRA, K. 1971. Further studies on coconut root (wilt) disease. 2nd Internat. Symp. on Pl. Path. January 27th - Feb. 3