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Transmission of a mycoplasma-like organism from Cocos nucifera with root (wilt) disease to Catharanthus roseus by Cassytha filiformis*

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Abstract

A mycoplasma-like organism (MLO) was successfully transmitted through dodder laurel (*Cassytha filiformis*) from root (wilt) diseased coconut to periwinkle (*Catharanthus roseus*) plants. Symptoms caused by the organism in periwinkle were chlorotic spots in the interveinal areas and at vein endings of fully opened leaves, later coalescing into yellow patches, and premature leaf abscission. Light and electron-microscopic examination revealed the presence of MLOs in diseased coconut palms, dodder laurels and periwinkles.

Additional keywords: electron microscopy, fluorescence light microscopy.

Introduction

About a hundred years ago a new disease of coconut palms (*Cocos nucifera*) was reported from three isolated areas in Kerala State, India. It was named 'root disease' because of the rotting of roots of infected trees. When inward curving or flaccidity of leaflets simulating wilting was described as the major diagnostic symptom, the disease was renamed as 'root (wilt)'. It has now become a serious threat to Indian economy. More than a third of the trees on 410 000 ha in the state are affected and annually about 968 million nuts are lost (Anonymous, 1986). Nagaraj and Menon (1956) surmised the disease to be due to a virus because of its systemic nature and transmissibility, but Solomon and Sasikala (1981) could not isolate a virus or virus-like agent. Solomon et al. (1983) reported the presence of mycoplasma-like organisms (MLOs) in the sieve tubes of developing petioles, inflorescences, root apexes and growing points of diseased palms. MLOs were absent in tissues of disease-free palms. We have tried to transmit the agent to *Catharanthus roseus* by dodder (*Cuscuta* spp.) and dodder laurel (Cassytha filiformis).

Materials and methods

Initial attempts to let phanerogamic parasites parasitize diseased coconut were restricted to species of *Cuscuta* (Convolvulaceae): *C. campestris*, *C. chinensis* and *C. subinclusa*.

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In subsequent trials dodder laurel (*Cassytha filiformis* (Lauraceae)), found parasitizing coconut seedlings in Minicoy island (Lakshadweep), was used. Coconut seedlings parasitized by the dodder laurel were brought over to Kayangulam from Lakshadweep and planted in steam-sterilized soil held in reinforced cement concrete tubs. Dodder laurel twines were trained on to coleus and periwinkle (*Catharanthus roseus*), meant as feeder plants in order to establish the parasite on diseased coconut seedlings growing in the open at the research station. Test seedlings of periwinkle, kept protected inside insect-proof cages covered with nylon gauze, were inoculated with dodder laurel connected to diseased palms, and kept there under observation for symptoms. Periwinkle seedlings connected via dodder laurel to disease-free palms served as control. Examination for MLOs in the donor coconut seedlings, dodder laurel bridge and recipient test plant was by light and electron microscopy according to Deeley et al. (1979), Russell et al. (1975) and Solomon et al. (1983).

Results

In our attempts the three species of *Cuscuta* tested failed to parasitize coconut. Strands of dodder laurel from the feeder plants made haustorial connections on coconut leaflets. The parasitic plant survived well on the diseased coconut seedlings if connection with the feeder plants had been severed after twine establishment on the diseased coconut seedlings. Establishment of haustoria occurred within 4-8 days during the wet season (June to December) and 22-25 days during the dry season. Serial sections of a haustorium revealed vascular connections between the host periwinkle and the parasitic dodder laurel. Within 14 to 21 days after the establishment of haustorial connections, the periwinkle test plants showed symptoms, which appeared as diffuse chlorotic flecks in the interveinal areas and at vein endings of fully opened leaves. The spots later enlarged and coalesced to form yellow patches eventually covering the entire leaf. Premature leaf abscission was another symptom. Out of 38 plants artificially submitted to infection from eight diseased palm trees since July 1984, 30 exhibited symptoms. Periwinkle plants connected with healthy palm trees by dodder laurel remained symptomless.

A second periwinkle, connected with twines of dodder laurel to a first symptom-bearing periwinkle still in connection with the diseased coconut seedling, developed comparable symptoms 11 days after the establishment of the haustorial connections. Symptoms in a third periwinkle, connected with the second test plant after severing connection between the diseased coconut seedling and the first periwinkle showed chlorotic flecks. Symptoms were not induced in a fourth periwinkle plant connected serially.

Top cleft grafting (Bos, 1967) between infected and healthy periwinkle plants also resulted in symptoms in the leaves of healthy stock plants. Symptoms appearing in leaves of *C. roseus* test plants after grafting were less severe than those appearing after transmission by *C. filiformis*. This was true also of symptoms in test plant leaves produced after the dodder laurel bridge from the diseased coconut tree had been disconnected and the plants had been removed to shade. However, five out of eight such plants developed severe symptoms seven to eight months later.

Abnormal bluish colouration in the sieve tubes of fresh leaf sections made soon after the appearance of initial symptoms and treated with Dienes' stain (Deeley et al., 1979) indicated infection in the phloem. DAPI and Hoechst 33258 fluorochromes (Russell

et al., 1975) also induced abnormal fluorescence in the phloem of the diseased coconuts, the dodder laurel and *C. roseus* test plants; control plants were free of these reactions.

Electron-microscopic examination of ultrathin sections of leaflets of diseased coconut, petiolar and mid vein tissues of periwinkle and the connecting dodder laurel strands revealed typical MLO cells in the sieve tubes. MLO cells were absent in the tissues of dodder laurel strands and of periwinkles sampled prior to connection with diseased coconut seedlings.

Discussion

The results provide evidence for the successful transmission of an MLO from root (wilt)-diseased coconut to periwinkle through a plant vector. So far, virus and MLO were known to be transmitted by *Cuscuta* spp. Dijkstra and Lee (1972) transmitted sandal spike disease to periwinkle test plants via *Cuscuta subinclusa* and back to healthy sandal plants from symptom-bearing periwinkle plants. Muniyappa et al. (1980) transmitted these organisms from sandal to periwinkle and from periwinkle to periwinkle using the same species of dodder but did not succeed to transmit the disease incitant to sandal from diseased sandal or periwinkle. Attempts to transmit lethal yellowing, an MLO-associated disease of coconut palm in Florida (USA), through *Cuscuta campestris* were unsuccessful (Tsai, 1976). Reddy et al. (1985) reported transmission of citrus mosaic through dodder laurel from Sathugudi orange (*Citrus sinensis*) to acid lime (*Citrus aurantifolia*). The penetration of coconut leaflets by haustoria and the establishment of vascular connection prove parasitization of coconut by the dodder laurel. Serial sections showed haustorial connections on coconut pinnae and periwinkle foliage. Wellman (1964) reported the wilde range of host plants including palms for both *Cuscuta* and *Cassytha* and observed that *C. filiformis* is spread by ocean currents and occurs most frequently near beaches and in coastal plains, which explains the natural parasitizing of coconut in Minicoy by the dodder laurel.

Although Koch's postulates have not yet been fulfilled, the experimental transmission of the disease and observation of MLO cells in naturally diseased and artificially infected plants make the mycoplasma etiology highly likely.

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Samenvatting

Overdracht door Cassytha filiformis van een mycoplasma-achtig organisme van 'root (wilt)'-zieke Cocos nucifera naar Catharanthus roseus

Door de als warkruid groeiende lauracee *Cassytha filiformis* werd van kokosnootpalm (*Cocos nucifera*), aangetast door 'root(wilt)', een mycoplasma-achtig organisme (MLO) overgebracht naar de toetsplant *Catharanthus roseus*. Hierop werden op de bladeren tussennervige, later ineenvloeiende vlekken en vroegtijdige bladval waargenomen. Uit onderzoek met de lichtmicroscoop en de elektronenmicroscoop kon de aanwezigheid

van MLO's in *Cocos nucifera*, *Cassytha filiformis* en *Catharanthus roseus* worden vastgesteld.

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