Preliminary Investigation on Weligama Coconut Leaf Wilt Disease: A New Disease in Southern Sri Lanka

H.T.R. Wijesekara*, L. Perera, I.R. Wickramananda, I. Herath, M.K. Meegahakumbura, W.B.S. Fernando and P.H.P.R. de Silva,
Coconut Research Institute, Bandirippuwa Estate, Lunuwila 61150.
** corresponding author. email: cpd@cri.lk*

ABSTRACT

An unusual yellowing of leaflets in coconut palms in the Galle, Hambantota and Matara districts in the Southern Province of Sri Lanka was observed in late 2006. The symptoms associated with affected palms are flattening and downward bending of leaflets, yellowing of lower fronds followed by drying of leaflets starting from the margins. Symptoms resemble that of Kerala wilt disease in India. This condition was first reported from Weligama in Matara district and, hence called the "Weligama Coconut Leaf Wilt Disease" (WCLWD).

Analysis of nutrients N, P, K, Ca and Mg in leaflets of affected and healthy palms revealed that, yellowing of leaflets has no relationship with nutrients. DNA extracts of bud leaves subjected to Nested PCR with phytoplasma specific primer pair combinations R16F2n / R16R2 with rU3 / fU5 and R16mF2 /R16R2 with fU5 /rU3 yielded an amplification of 773 base pair product in 3 of 24 palms tested. Sequencing of the PCR product confirmed phytoplasma as the causative agent of WCLWD. The sequence of the WCLWD was compared with the phytoplasma sequences deposited in the NCBI database and WCLWD sequence was found to be 98% similar to sugarcane grassy shoot phytoplasma and sugarcane white leaf phytoplasma. Apart from WCLWD, another fungal disease of coconut leaf rot disease was also observed in the same locations, sometimes in the same palm. This is the first report of association of phytoplasma in coconut disease in Sri Lanka.

Key words: *Coconut phytoplasma, Weligam Coconut Leaf Wilt Disease (WCLWD)*

INTRODUCTION

Coconut is an important plantation crop grown in many tropical parts of the world. It provides considerable amount of daily caloric requirement for the people of these countries. In Sri Lanka coconut is grown widely in many parts of the island, but large scale plantings are located in areas bordering Kalutara, Kurunegala, and Puttalam districts, which, is termed "the coconut triangle". Many medium scale plantations and several home garden level palms are found in the Southern Province of Sri Lanka, especially, in the "Mini Coconut Triangle," which is bordered by the towns Middeniya, Beliatta and Ranna.

Production of coconut in Sri Lanka is hampered by many diseases, such as bud rot, stem bleeding, leaf blight, and Ganoderma root and bole rot which, occur in small patches. In 2006, an unusual yellowing and flaccidity in leaflets of coconut palms in Weligama area in the Matara district was observed. Hence, the name "Weligama Coconut Leaf Wilt Disease" (WCLWD) was given to this disease. The symptoms of affected palms were similar to that of root (wilt) disease in Kerala, India (Mathewkutty, 1998; Koshy *et al.*, 2001). Apart from WCLWD, another disease, leaf rot, which was first reported in late 1999 in Weligama area was also observed both in some WCLWD affected palms and in some unaffected palms.

Hence, different methods were used to identify the causative organism of the WCLWD.

MATERIALS AND METHODS

A brief survey was carried out to demarcate the boundary of the affected area. The symptoms of the affected palms and their progressive development were recorded. Cross sections of trunks, roots and inflorescences were examined for any abnormalities. Oil content of mature nuts and sugar content of water of tender nuts were measured. Leaf nutrient content was analyzed to study the reason for yellowing of fronds. DNA from tender leaflets was subjected to Nested PCR with phytoplasma specific primers to confirm the involvement of phytoplasma in the disease.

RESULTS AND DISCUSSION

Disease Symptoms

The earliest symptom of the disease is the flattening and downward bending of leaflets giving the frond a ribbed or flaccid appearance. Crowns of such palms appear darker (Fig. 1a). This symptom is first seen in the younger leaves and becomes more prominent when the fronds are fully opened. The degree of flaccidity of leaflets varies among the fronds in a single palm, which is not readily recognizable to an untrained eye. The most striking feature of this disease is the intense yellowing of lower whorls of fronds up to about the 12th frond (Fig. 1b). The yellowing becomes more prominent after the rains. Occasionally, yellowing of mid whorls of fronds is seen in some palms. In such cases, yellowing is restricted to about 6 – 8 fronds in the middle whorl. Subsequently, drying up of the leaflets starts from the margins of the affected fronds (Fig. 1c). Dried up fronds hang on the crown for sometime before falling. In some severely affected palms, the fronds also curl downward giving a clumsy appearance to the crown. The tips of fronds become twisted or break and hang down in some palms (Fig. 1d). Unopened bud leaf looses its rigidity and bends downward in severely affected palms.

Necrosis in unopened inflorescences or immature nut fall, have not been observed in affected palms. With the reduction in the number of fronds, the crown becomes smaller and trunk begins to taper. As the disease progresses, female flower production declines and the palm becomes unproductive.

High degree of necrosis of root tips was observed in moderately affected palms, while no young roots were observed in severely affected palms. The roots show intense branching followed by necrosis of stelar region of young white roots (Fig. 2a and 2b).

Majority of the WCLWD affected palms were in the bearing stage, while few were pre-bearing. Palms of any stage were found to be susceptible to the disease, palms over 3 years of age most commonly affected. Flaccidity of leaflets is seen even in seedlings below 3 years of age, where as yellowing is always seen only in older palms. No other palm species in the area were observed with similar symptoms.

There was no difference in the sugar content of nut water moderately affected and apparently healthy palms from the same area. Nut water from both in WCLWD affected and apparently healthy palms had sugar content of 5.86% and 5.59% respectively, which is in the normal range. The kernel of nuts from moderately affected palms were similar to that of apparently healthy palms but the oil content was 7.4% lower in nuts from moderately affected palms

There was no necrosis or colour change or change in hardness in the trunk tissues of affected palms.

Prevalence of diseased palms

Weligama coconut leaf wilt disease is currently prevalent in Divisional Secretariat Regions Akuressa, Athuraliya, Devinuwara, Dickwella, Hakmana, Kamburupitiya, Kirinda-Puhulwella, Malimboda, Matara, Pitabeddera, Thihagoda, Weligama and Welipitiya of Matara district, Galle and Habaraduwa in the Galle district and in Beliatta, Ookewela, Tangalle and Walasmulla in the

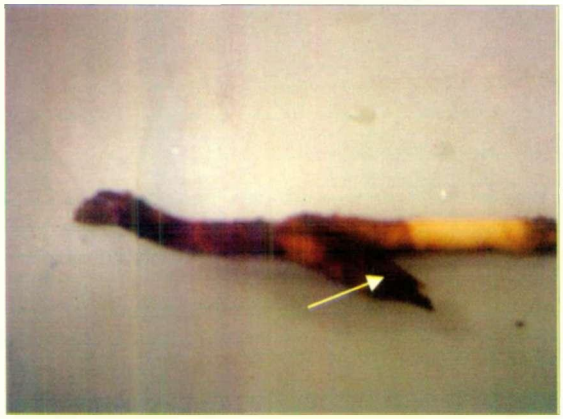
(a) Flaccidity

(b) Yellowing

(c) Drying of leaflets from margin

(d) Breaking tips of fronds

Fig. 1. *Progressive development of symptoms in palms affected by Weligama Coconut Leaf Wilt Disease (WCLWD)*



(a) Necrosis in root tip area

(b) Extensive branching

Fig.2. *Symptoms seen in root system of palms affected by WCLWD*

Hambantota district in the Southern Province (Fig. 3). As the disease is confined to a limited area, it is reasonable to assume that the disease is of fairly recent origin in Sri Lanka. The disease is prevalent in all types of soils from coastal areas to sloppy lands. The affected palms are found in pockets, sometimes several kilometers away from each other. They are in small clusters even in affected lands or scattered throughout.

black and fall off, while partially affected leaflets with blackish shriveled tips, and fronds with completely rotten leaflets appear as blackish "fishing rods" (Fig. 4). The fungi responsible for the leaf rot disease are *Ceratocystis paradoxa*, *Colletotrichum gloeosporioids* and *Fusarium solani* (Fernando *et al.*, 2002). Currently leaf rot disease is found in all areas where WCLWD prevails. Most of the leaf rot affected palms had

Fig. 3. *Map of Sri Lanka showing Weligama coconut leaf wilt disease (WCLWD) affected areas in the Southern Province*

Leaf rot disease

Leaf rot disease was also observed to be associated with some of the WCLWD affected palms. The symptoms of leaf rot disease are produced by a fungal infection in the unopened fronds. After opening of affected fronds, the rotten portions turn

flattened leaflets as in the case of WCLWD but yellowing and marginal necrosis could not be observed as the part of leaflets are already rotten.

(a) Rotten leaflets of affected bud leaf

(b) Affected fronds of adult palms

Fig. 4. *Symptoms of Leaf rot disease in coconut*

Detection

Analysis of leaflets from affected palms grown in fertilized lands revealed that, the yellowing is not related to nutrient deficiency, particularly, Magnesium, Potassium and Calcium (Table 1).

Since, the symptoms of the WCLWD resemble that of root (wilt) disease in Kerala, India, which is claimed to be caused by a phytoplasma, studies were conducted to investigate whether WCWLD is associated with a phytoplasma.

Confirmation of causative agent of WCLWD

Milky white to ivory coloured bud leaf samples were collected from mildly or moderately WCLWD affected palms and 10 g of chopped ekel pieces were used to extract DNA. Total DNA from bud leaves was extracted by CTAB method (Sambrook *et al.*, 1982), and used for nested PCR with phytoplasma specific primer pairs R16F2n / R16R2 and fU5 / rU3. Similarly, primer pair combinations R16mF2 / R16mR1 and fU5 / rU3 were also used and a PCR product of expected size of 773 bp was obtained (Fig. 5). PCR product was purified by

	Nutrient content			
Category	**N%**	**K%**	**Mg%**	**Ca%**
Affected palms	2.18	1.87	0.34	0.43
Healthy palms	2.21	1.78	0.27	0.33
SD	0.281	0.691	0.328	0.144
Sufficiency range	1.9 – 2.1	1.2 – 1.5	0.25– 0.35	0.35 – 0.5

(Mean of 9 palms)

Table 1. *Percent nutrient levels in leaflets from 14th frond in moderately WCLWD affected palms and appararently healthy palms*

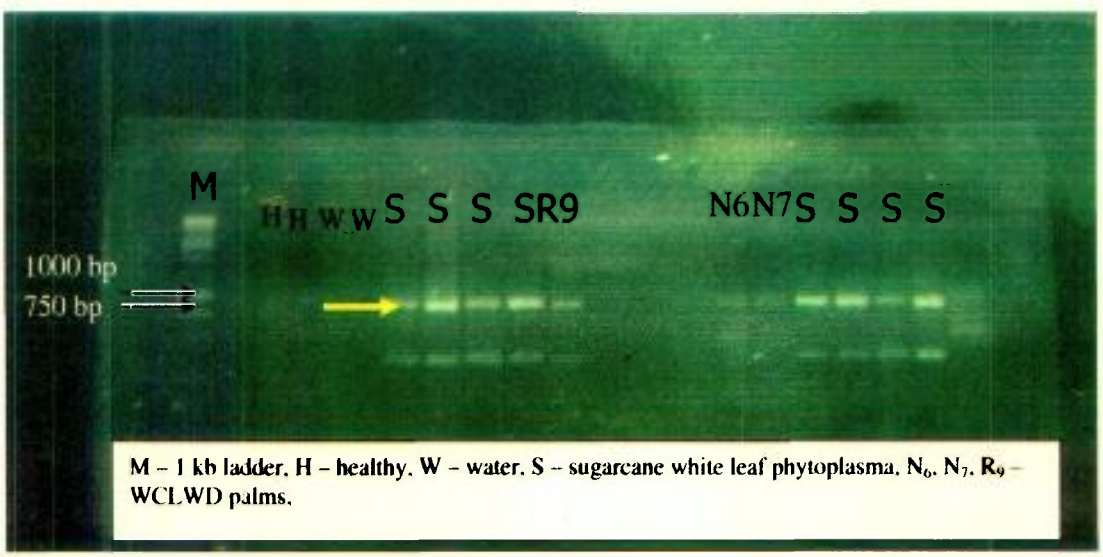


Fig. 5. *Agarose gel photograph of nested PCR products with phytoplasma specific primer pairs R16F2n / R16R2 and fU5 / rU3.*

"Wizard SV gel and PCR clean-up system" kit (Promega, USA) and subjected to direct sequencing. The sequence was deposited in the NCBI data base (Accession number: EU 635503). It showed 98% sequence similarity with the sugarcane white leaf and sugarcane grassy shoot phytoplasmas. This is the first report of a coconut disease associated with phytoplasma in Sri Lanka.

Preliminary investigations on the effect of oxytetracycline on the affected palms showed remission of symptoms confirming the above results. Several hypotheses have been postulated to explain the etiology of the WCLWD.

(1) Disease has been introduced to the country from symptom-less ornamental palm imported to the country.

(2) Pathogen has expanded its host range from sugarcane to coconut

(3) Pathogen has expanded its host range from wild grasses to coconut

Carefully planned experiments are necessary to test these hypotheses.

The distribution pattern of WCLWD affected palms suggests involvement of air borne vector/s.

It is suspected that the disease is transmitted by the plant hopper *Proutista moesta* and the lace bug *Stephanitis typica* as they are the most common sucking insects on coconut in Sri Lanka as in the case of root wilt disease in India (Koshy *et al.*, 2001).

FUTURE RESEARCH

As the WCLWD was detected recently, hardly any information is available on its epidemiology, etiology, symptomatology, vectors and alternative hosts. Therefore, future research are aimed at understanding on the above aspects and thereby to design proper disease management strategies. A readily diagnostic PCR method through designing of WCLWD phytoplasma specific primers for the PCR and identification of best tissue type for the detection is currently underway.

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