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IDENTIFICATION OF ALTERNATE HOSTS OF WELIGAMA COCONUT LEAF WILT (WCLW) PHYTOPLASMA

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ABSTRACT

The Weligama Coconut Leaf Wilt (WCLW) disease is a serious problem of the coconut industry in Sri Lanka and is caused by a phytoplasma. In addition to the infected coconut palms there may be other alternate host plants, which harbor the same pathogenthus aggravating the spread of disease further. Identification of alternate hosts of the pathogen will help understanding the spread of the disease and support identifying or developing sound integrated management strategies against the spread of the disease. In this investigation 100 individual plants of different species, sampled from WCLW infected areas in the southern part of Sri Lanka and few commercial nurseries, were indexed for their host status to the WCLW pathogen. The presence of phytoplasma in these samples were tested by indirect enzyme linked immunosorbant assay (ELISA) using locally produced polyclonal antiserum to WCLW pathogen. Arecanut (*Areca catechu*), Ranthambili (*Cocos nucifera*), Dothalu (*Loxococus rupicola*), Red palm (*Acanthophoenix rubra*), Fan palm (*Hyphaene petersiana*), Vinca palm (*Rhapis humulis*), Bermuda grass (*Cynodon dactylon*), Catharanthus (*Catharanthus spp*), Heliconia (*Heliconia latispatha*) and Alstonia (*Alstonia macrophylla*) were confirmed as alternate hosts. Date palm (*Phoenix dactylifera*), some *Heliconia* spp and Cane palm (*Dypsis lutescence*) showed a boarder line reaction to the test.

KEYWORDS: Coconut, Hosts, Leaf wilt, Phytoplasma disease

INTRODUCTION

Coconut (*Cocos nucifera*) is the most useful tropical palm in the world. It is extensively cultivated in more than 93 countries in the hot humid tropics and is the main source of livelihood for more than 1000 million people in the world. In Sri Lanka, coconut is one of the major plantation crops, contributing 1.4% to the Gross Domestic Product. The land extent under coconut cultivation is about 400,000 ha (Anon, 2010a). Weligama Coconut Leaf Wilt (WCLW) disease is similar to Kerala wilt disease, and was first recorded in the Weligama area of Sri Lanka in late 2006. The causal agent of this disease has been identified and confirmed as a phytoplasma (Wijesekera *et al.*, 2008). Phytoplasma are specialized bacteria that are obligate parasites of plant phloem tissue and transmitted by phloem-sucking insects such as leaf hoppers, plant hoppers, psyllids and lace bugs in a persistent manner. They were first named mycoplasma-like organisms or MLOs. They are characterized by their lack of a cell wall, a pleiomorphic or filamentous shape, normally with a diameter less than 1 micrometer, and their very small genomes.

They are Titer of phytoplasma cells in the phloem of infected plants may vary according to the season and plant species. Since they cannot be cultured *in-vitro*, serological and moleculer-based diagnostic techniques are useful for detection of phytoplasma. The WCLW disease is common in the southern region of Sri Lanka, especially in Matara, Galle and Hambanthota districts. It has become a national problem and at present, nearly 400,000 coconut palms have been infected (Anon, 2010b). Symptoms of WCLW disease are flattering of leaf laminar, yellowing of fronds, ribbing like appearance, marginal scorching of some leaves, abnormal branches of roots, decaying of roots, retarded growth and yield reduction. At present this disease is confined to the southern area and precautions have been taken to control its' spread to other areas where coconuts are extensively grown.

Many phytoplasma diseases have weeds or other alternate hosts that provide a reservoir of phytoplasma from which economically important crop plants may get infected. Bermuda grass (*Cyndon dactylon*) suffered from a destructive phytoplasmal disease known as "Bermuda grass white leaf" detected in Thailand, India and Malayasia (Nejat and Vadamlai, 2010). Kallmanthen wilt is similar to the phytoplasma disease in rice plants and sugarcane (Nejat *et al.*, 2010). Sugarcane grassy shoot disease and Ramu stunt disease can be seen in gardens in areas affected by Kerala wilt (Nejat *et al.*, 2009). Similarly, other plant species in the family Palmae to which coconut belongs to may harbor WCLW phytoplasma. Disease control strategies should thus be formulated considering the alternate hosts of the WCLW disease.

In addition, it is important to identify the initial source (foci) of infection from which phytoplasma would start spreading, in order to formulate effective disease management strategies. . Three principle foci of infection are usually recognized (Thresh, 1981); spread may occur from infected weeds within a crop, from weeds to other plants growing adjacent to the crop or from infected hosts in areas remote from the crop. The occurrence of infected plants decreases with the increasing distance from the focus of infection.

The objective of the present investigation was to identify weeds and other alternate hosts of WCLW disease by using serological methods in order to help manage the spread of the disease.

MATERIALS AND METHODS

Sample collection

Sampling of plant species were carried out in disease affected locations in Weligama area in the Matara district. Sampling was done during rainy and dry seasons. The weed and other alternate host species were collected randomly from coconut cultivations in areas that had high as well as low

incidence of WCLW disease. Furthermore, various ornamental palm species in the family Palmae were also collected from some of the nurseries located adjacent to the disease affected areas. Healthy samples of each species were collected from Homagama and Horana in the western province where disease has not been reported to-date. The species sampled were edible palms, ornamental palms, grasses, rice, few shrubs and trees. One hundred samples were collected during the study period in 2009/2010 covering approximately 30 species, with two to three samples per species.

Disease diagnosis

Pathogen identification was done by indirect Enzyme Linked Immunosorbant Assay (ELISA) as reported by Mouwat and Dowson (1987). The WCLW phytoplasma pathogen was purified according to method described by Mayilvaganan *et al.* (2001), with minor modifications (Wijerathna *et al.*, 2008). Subsequently, the polyclonal antiserum for WCLW disease was produced locally and its specificity and sensitivity in detecting the pathogen was assayed (Ranasinghe *et al.*, 2010).

Enzyme Linked Immunosorbant Assay (ELISA)

Plants of each species were taken separately at a given sampling location. . One hundred mg of each leaf sample containing mid ribs were ground in a sample extraction buffer (0.5M carbonate buffer, pH 9.6 + 1 % gelatine + 0.2 % ovalbumin; 1:10 w/v) with a mortar and pestle. The ELISA two wells of micro plates were first coated from each samples by filling the wells with 200 µl of crude sap extracted from different plant species. Two WCLW infected coconut samples, two healthy coconut samples and two healthy samples (from disease free areas) for each plant species sampled were included in the ELISA plate. Then the plates were incubated overnight at 4°C. In the following morning, the plates were washed with phosphate buffer saline with tween 20 (PBST buffer) as two quick washes and three prolonged washes at five minute intervals. This was followed by filling each well with 200 µl of polyclonal antiserum for WCLW phytoplasma diluted in PBSTPO buffer (PBST + 2% PVP + 0.2% ovalbumin; 1:400). Incubation was done 2 hr at 37°C. After washing the plates as above, they were filled with 200 µl of protein A conjugate (enzyme labeled anti rabbit) diluted in 1:1000 PBSTPO buffer. Incubation was done for 2 hrs at 37°C and the standard washing steps were followed. Finally, the wells were filled with 200 µl of freshly prepared substrate solution containing 5 mg/5 ml P nitrophenyl substrate in diethanolamine buffer (pH 9.8). After incubation periods 1 hr at 37°C and 18 hrs at 4°C, the plates were read at 405 nm wave length using ELISA plate reader.

The threshold value

Samples with absorbance values (at 405 nm) greater than twice the absorbance values of healthy samples were considered as positive for the test as described by Sutula *et al.* (1986).

RESULTS AND DISCUSSION

Test results for edible palm species

The indirect ELISA absorbance values for edible palm species are given in Table 1.

Table 1. Absorbance values of edible palm species at 405 nm wave length for 18 hrs at 4°C

Collected location	Plant species	Absorbance value	Threshold value	Test results
Bissawila	Arecanut (*Areca catechu*)	0.280	0.170	+
Bissawila	Arecanut (*Areca catechu*)	0.179	0.170	+
Nugagahawattha	Arecanut (*Areca catechu*)	0.105	0.150	-
Bissawila	Arecanut (*Areca catechu*)	0.045	0.142	-
Bissawila	Arecanut (*Areca catechu*)	0.110	0.150	-
Nugagahawattha	Ranthambili (King coconut) (*Cocos nucifera*)	0.385	0.142	+
Nugagahawattha	Kithul (*Caryota urnes*)	0.113	0.175	-
Godagama	Date palm (*Phoenix dactylifera* L)	0.057	0.030	+/-
Nugagahawattha	Dothalu (*Loxococus rupicola*)	0.180	0.130	+
Nugagahawattha	WCLW infected coconut	0.180	0.130	+
Nugagahawattha	WCLW infected coconut	0.160	0.130	+

Abbreviations: + Positive for the test, - Negative for the test, +/- Boarder line reaction

The results indicated that, out of 9 plant samples of edible palm species tested, only two arecanut (*Areca catechu*) samples, one "dothalu" (*Loxococus rupicola*) sample and one "ranthambili" (*Cocos nucifera*) sample gave positive results. Further, a faint color reaction was observed with date palm (*Phoenix dactylifera*) sample tested.

Test results for ornamental palm species

Absorbance values (405 nm) for different ornamental palm species are given in Table 2.

Table 2. Absorbance values of different ornamental palm species at 405 nm wave length for 18 hrs at 4°C

Collected location	Plant species	Absorbance value	Threshold value	Test results
Godagame	Red palm (*Acanthophoenix rubra)*	0.125	0.072	+
Godagame	Red palm (*Acanthophoenix rubra)*	0.080	0.077	+/-
Thudawa	Cane palm (*Dypsis lutescens)*	0.067	0.037	+/-
Godagama	Vinca palm (*Rhapis humuli*)	0.120	0.026	+
Near University of Ruhuna	Vinca palm (*Rhapis humulis*)	0.150	0.120	+
Hemantha Nursery, Matara	Merely palm (*Adonidia merrilii)*	0.024	0.04	-
Near University of Ruhuna	Fan palm (*Hyphaene petersiana)*	0.141	0.105	+
Thudawa	Fan palm (*Hyphaene petersiana)*	0.073	0.150	-
Godagama	Champion palm (*Hyophorbe lagenicaulis*)	0.070	0.082	-
Hemantha nursery, Matara	Royal palm (*Roystonea regia*)	0.048	0.090	-
Bissawila	WCLW infected coconut	0.290	0.280	+
Bissawila	WCLW infected coconut	0.500	0.280	+

Abbreviations: + Positive for the test, - Negative for the test, +/- Boarder line reaction

The results revealed that several ornamental palm species such as Fan palm (*Hyphaene petersiana)*, Vinca palm and Red palm (*Acanthophoenix rubra)* are also infected with WCLW phytoplasma disease. However, the cane palm (*Dypsis lutescens)* showed boarder line reactions to the test.

ELISA Test results for the common phytoplasma host plants

The samples of Bermuda grass (*Cynodon dactylon)*, one sample of catharanthus (*Catharanthus roseus)* and one sample of "Mahaundupiyaliya" (*Desmodium heterocarpon*) gave visible color reaction and was positive for the test (Table 3).

Table 3. Absorbance values of common phytoplasma host plant species at 405 nm wave length for 18 hrs at 4°C

Location	Plant species	Absorbance value	Threshold value	Test results
Bisawila	Bermuda grass (*Cynodon dactylon)*	0.213	0.041	+
Bissawila	Bermuda grass (*Cynodon dactylon)*	0.180	0.041	+
Bissawila	Grass spp	0.030	0.041	-
Thudawa	Mahaundupiyaliya (*Desmodium heterocarpon)*	0.187	0.110	+
Thudawa	Charanthaus spp (*Catharanthus roseus)*	0.045	0.062	-
Godagama	Charanthus spp (*Catharanthus roseus)*	0.086	0.140	-
Near Ruhuna University	Charanthus spp (*Catharanthus roseus)*	0.679	0.140	+
Bissawila	Rice *(Oryza sativa)*	0.054	0.019	-
Bissawila	Bamboo (*Bambusa vulgaris)*	0.060	0.160	-
Bissawila	WCLW infected coconut	0.410	0.210	+
Bissawila	WCLW infected coconut	0.480	0.210	+

Abbreviations: + Positive for the test, - Negative for the test, +/- Boarder line reaction

ELISA Test results for other tree crops and cultivated crops

The ELISA absorbance values for other trees and cultivated crops are shown in Table 4.

Both Alstonia (*Alstonia macrophylla*) and Heliconia (*Heliconia latispatha*) gave positive results for the test. Some of the Heliconia species collected from different locations showed boarder line reactions (Table 4).

Table 4. Absorbance values of other tree crops and cultivated crops at 405 nm wave length for 18 hrs at 4°C

Location	Plant species	Absorbance value	Threshold value	Test results
Bissawila	Alstonia (*Alstonia macrophylla*)	0.088	0.046	+
Bissawila	Alstonia (*Alstonia macrophylla*)	0.047	0.048	+/-
Near Ruhuna University	Alstonia (*Alstonia macrophylla*)	0.350	0.120	-
Bissawila	Alstonia (*Alstonia macrophylla*)	0.134	0.124	+
Hemantha nursery, Matara	Heliconia (*Heliconia spp*)	0.039	0.009	+/-
Bissawila	Heliconia (*Heliconia latispatha*)	0.135	0.009	+
Bissawila	Heliconia (*Heliconia spp*)	0.057	0.048	+/-
Nugagaha waththa	Heliconia (*Heliconia latispatha*)	0.300	0.057	+
Nugagaha watha	Heliconia (*Heliconia latispatha*)	0.133	0.009	+
Bissawila	Citrus (*Citrus limon*)	0.420	1.230	-
Godagama	Banana (*Musa acuminata*)	0.025	0.038	-
Bissawila	WCLW infected coconut	0.217	0.120	+
Bissawila	WCLW infected coconut	0.180	0.120	+

Abbreviations: + Positive for the test, - Negative for the test, +/- Boarder line reaction

CONCLUSIONS

Locally produced polyclonal antiserum for WCLW phytoplasma helped identifying some hosts that can harbor the same pathogen. Arecanut (*Areca catechu*), Ranthambili (*Coconut nucifera*), Dothalu (*Loxococus rupicola*), Red palm (*Acanthophoenix rubra*), Fan palm (*Hyphaene petersiana*), Vinca palm (*Rhapis humulis*), Bermuda grass (*Cynodon dactylon*), Catharanthus (*Catharanthus* spp) Heliconia (*Heliconia latispatha*), Alstonia (*Alstonia macrophylla*) gave a strong color reaction, while Date palm (*Phoenix dactylifera*), some Heliconia spp and Cane palm (*Dypsis lutescense*) gave boarder line color reactions when tested with WCLW phytoplasma

antiserum. Recognition of weeds and other alternative hosts of WCLW phytoplasma in Sri Lanka has epidemiological significance and suggest that weed control in coconut plantations may benefit the disease management process management in the long term. Moreover, identification of alternative hosts will increase the understanding of the spread of the disease and help evolve sound integrated control measures against the spread of the disease. Confirmation of these alternative hosts by using PCR-based methodology with specific primers to WCLW phytoplasma is suggested.

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