Detection of Phytoplasma in Selected Agricultural Crops using Antiserum Seveloped Against Phytoplasma Causing Weligama Coconut Leaf Wilt (WCLW) Disease

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Abstract

Phytoplasmas are specialized bacteria that are obligate parasites of plant phloem tissues, which are found, associated with numerous plant diseases worldwide. There were no any remedial measures after infection of phytoplasma. Therefore, it is important to destroy infected plants to avoid further spreading of the pathogen. For that, early detection of infected plants are needed. Enzyme Linked Immunosorbent Assay (ELISA) is one of the sensitive detection technique that can be used for the detection of phytoplasma infection. But there was no any commercially produced antiserum available at present. Plant Virus Indexing Center, Homagama has produced an antiserum for Weligama Coconut Leaf Wilt (WCLW) phytoplasma. Therefore, this study was done to find the possibility of detection of other phytoplasma infection using this antiserum. For this study SCGS and SCWL phytoplasma infected sugarcane samples and avocado malformed inflorescence samples collected from various locations, were tested by indirect ELISA using locally produced antiserum for WCLW phytoplasma. All SCGS and SCWL infected samples gave positive reaction for the test. All avocado samples gave negative reaction for the test. It can be concluded that SCGS and SCWL phytoplasmas can be detected successfully using locally produced polyclonal antiserum raised against WCLW phytoplasma. However, this antiserum is not suitable to detect board spectrum of phytoplasma.

Key words: Antiserum, Phytoplasma, Scgs, Scwl, Wclw

Introduction

Phytoplsama are bacterial pathogens that cause devastating yield losses in diverse low and high yield crops worldwide. They are obligate parasites of plant phloem tissues, cannot cultured in cell free media, characterized by their lack of cell wall. Phytoplasma diseases are most prevalent in tropical and sub tropical regions in the world. It requires a vector to be transmitted from plant to plant. Insects are the main vectors, which feed on the phloem tissues of the infected plants, picking up the phytoplasma and transmitting them to the next plant they feed on. For this reason, host range of phytoplasma is strongly depend on feeding behavior of its insect vectors.

A common symptom caused by phytoplsama infection is called "phyllody", which is the production of leaf like structures in place of flowers. Many phytoplsama infected plants gain a bushy or witches' broom appearance due to changes in normal growth patterns caused by the infection. Most plants show apical dominance but phytoplsama infection can cause the proliferation of axillaries (side) shoots and increase in size of the internodes.

Sugarcane white leaf disease (SCWLD), sugarcane grassy shoot disease (SCGSD), avocado malformation, papaya die back, Weligama Coconut Leaf Wilt (WCLW) disease are the common phytoplasma diseases recorded in Sri Lanka (Sarathchandra et al. 2009). Like virus diseases, phytoplasma diseases also do not have any recommended control measures. Only measure is avoidance of further spread by various methods. Therefore, early and sensitive detection and diagnosis of phytoplasma is of paramount importance for effective prevention strategy, particularly because phytoplasma may have a very long latency period.

Enzyme Linked Immunosorbent Assay (ELISA) is one of the sensitive techniques that can be used for diagnosis phytoplasma associated with plants. Commercially produced antisera are not available for phytoplasma in the world. There is an antiserum produced locally for WCLW phytoplasma at Plant

Virus Indexing Center, (PVIC) Homagama (Ranasinghe et al. 2010). Therefore, this study was done to find the possibility of detection of other phytoplsama diseases using this locally produced antiserum for WCLW phytoplasma.

Materials and Methods

Sample collection

Phytoplsama like infected sugarcane and avocado samples were collected from various locations. SCWL and SCGS phytoplasma infected samples were collected from the cultivated fields of Galoya plantations (Pvt) Ltd, Hingurana site, Hingurana in Eastern province in Sri Lanka. Avocado malformation samples were collected at the Fruit Crops Research and Development Center (FCRDC) at Horana. Healthy samples of above crops were also collected from Homagama area. WCLW infected samples were collected from Weligama area as positive controls.

Laboratory testing

Protocol developed for WCLW antiserum (Ranasinghe, et al, 2010) was followed with minor modification. Samples weighing of 0.1 g were crushed with the extraction buffer (0.05M carbonate buffer + 1% gelatine + 0.2% egg albumin, pH 9.6) in 1:10 (W/V) dilution ratio. Extracts were centrifuged at 5000rpm for 5 minutes. Two hundred micro liter of the supernatant was pippetted in to the micro wells of the ELISA plate and incubated overnight at 4°C.

After the incubation period, the plate was washed with the Phosphate Buffer Saline with Tween 20 (PBST) for two quick washes and three washes with 5 minutes prolonged period. Then the antiserum diluted in PBSTPO buffer (PBST+2%pvp+0.2%Egg albumin) in 1:500 ratios was pippetted in to the micro wells. Then the plate was incubated at 37 °C for 2 hours. After the incubation period the plate was washed again with PBST buffer as explained above. Then the protein A conjugate diluted in PBSTPO in 1:500 ratio, was pippetted in to the micro wells. After the incubation period, the plate was washed again with PBST buffer. Then pnytrophenyl substrate tablet diluted in substrate buffer in 5mg/5ml ratio was pippetted in to the micro wells and the plate was incubated for 1 hour at 37 °C. After the incubation, the absorbance was taken with ELISA plate reader at 405 mn wave length. Then the plate was kept at 4 °C over night and again the absorbance was recorded.

Results and Discussion

Antisera raised against phloem – limited phytoplsama generally react only with the phytoplsama strain used to produce the antigen (Lipman et al. 2008). This antiserum was produced against the purified WCLW phytoplsam using Percoll Density Gradient Centrifugation method described by Ranasinghe et al. (2010). There was no evidence in literature about the specific membrane protein of WCLW phytoplsama, targeted for raising antibodies.

According to the results, detection of SCGS and SCWL phytoplasma is possible with the locally produced polyclonal antiserum, but detection of phytoplasmas associated with avocado malformation is impossible.

Sample	Location	Absorbance	THV	Results
Sugarcane-1	Hingurana	0.229	0.15	+
Sugarcane-2	Hingurana	0.226	0.15	+
Sugarcane-3	Hingurana	0.210	0.15	+
Sugarcane-4	Hingurana	0.225	0.15	+
Sugarcane-5	Hingurana	0.496	0.27	+
Sugarcane-6	Hingurana	0.600	0.27	+
Sugarcane-7	Hingurana	0.187	0.095	+
Sugarcane-8	Hingurana	0.175	0.095	+
Sugarcane-9	Hingurana	0.177	0.095	+
Sugarcane-10	Hingurana	0.148	0.095	+
Avocado-1	Horana	0.051	0.061	-
Avocado-2	Horana	0.042	0.061	-
Avocado-3	Horana	0.066	0.061	-
Avocado-4	Horana	0.018	0.061	-
WCLW infected Coconut	Weligama	0.314	0.15	+
WCLW infected Coconut	Weligama	0.426	0.27	+
WCLW infected Coconut	Weligama	0.224	0.095	+

This probably gives the evidence for closely relation of WCLW phytoplasma SCGS and SCWL phytoplasma. Therefore, it can be considered that there is a cross reaction of locally produced WCLW polyclonal antiserum with SCGS and SCWL phytoplasma.

A similar study was carried out by Wei et al. (2004) to evaluate the reactivity of eight different phytoplasma from few distinct 16Sr groups with the antiserum raised against sec A membrane protein of Onion Yellow phytoplasma.

According to that study, reported that the antiserum raised against the Sec A membrane protein of Onion Yellow phytoplasma, which belongs to the Aster Yellow group (16Sr 1 group) could detect eight phytoplasma strains from four distinct 16Sr groups. *Sec* A protein is a component of the *Sec* protein translocation system, which is universally present in bacteria and indispensable for cell viability. Amino acid sequence motifs are highly conserved among Sec A proteins from widely ranging species of bacteria (Kakizawa et al. 2001). Therefore, Sec A is a viable candidate immunogen for the production of antibodies that react with many different phytoplasmas (Wei et al. 2004).

The immunodominant membrane protein (highly antigenic membrane protein) is the major portion of the total cellular membrane proteins in most phytoplasmas (Bertaccini and Duduk, 2009). Wei et al. (2004) reported that immunodominant membrane protein from different phytoplasma strains have limited amino acid sequence identify, such that antibodies raised against each immunodominant membrane proteins are generally strain specific.

In this study there was no way to differentiate SCGSD and SCWLD, because they are known to cause symptoms in infected plants that are often indubitable. However, serological comparisons did not allow discrimination between SCGSD and SCWLD (Saindu and Clark., 1993). Wongkaew et al. (1997) and shoodee et al. (1999) reported that the SCGS phytoplasma can be distinguished from the SCWL phytoplasma using RFLP analysis with restriction endonucleases.

Classification of SCGS, SCWL and WCLW phytoplasma in same phytoplasma group (16Sr XI group) revealed that those are phylogenetically closely related phytoplsama strains (Wongkaew et al., 1997. Therefore, it is possible to share a significant similarity by these strains. Due to their phylogenetically close relatedness same epitopes can be present in their antigens. Sometimes they might be able to share major epitopes and sometimes to share minor epitopes.

Lipman et al. (2005) reported that, antibodies can cross react with similar epitopes on other antigens but usually with less affinity and this antibody cross reaction may serve as a useful tool for identifying related antigens. However this method can be confounding when recognizing epitopes on unrelated antigens.

Conclusion

SCGS and SCWL phytoplasma can be detected successfully using locally produced polyclonal antiserum against WCLW phytoplasma. However, it cannot use to detect broad spectra of phytoplasma. Production of specific monoclonal antisera against different phytoplasmas seems to be more suitable for an effective detection.

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