A simple, cost effective and sensitiveImmuno– dot blot assay for detection of Weligama Coconut Leaf Wilt Disease associated phytoplasma

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Phytoplasma borne Weligama Coconut Leaf Wilt Disease (WCLWD), the current major threat to the coconut cultivation of Sri Lanka was first reported in 2006 from Weligama, in Southern Sri Lanka. There would be dire consequences if it spreads to the coconut triangle which consists of more than 70% of the total land under local coconut cultivation. As a sensitive, specific and rapid diagnostic test for WCLWD is an imperative need, we undertook to raise diagnostic monoclonal antibodies to be used in immunologic assays for WCLWD. This study reports the establishment of a specific immuno dot blot assay based on polyclonal serum raised in experimental animals to purified phytoplasma to be used for subsequent screening of hybridomas.

Immuno – dot blot was established by optimizing reagent concentrations; antigen(1/5 - 1/50 w/v), phytoplasma specific antibody(1:100 – 1:2000), enzyme conjugated secondary antibody (1:250 – 1:2000) and antigen volume per spot (1 µL – 6 µL) using checker board titration to achieve maximum specificity and sensitivity. Optimized conditions for sensitive detection of WCLWD was achieved using 1:20 (w/v)antigen, 1:250 antibody and1:500 conjugate dilutions. Briefly, Polyvinylidenedifluoride (PVDF) membrane was spotted with 4 µL of test antigen, blocked, incubated with phytoplasma specific polyclonal antibody, then with enzyme conjugate and finally with substrate, 3-amino-9-ethylcarbazole. In between each step, the membrane was washed with washing buffer. The optimized assay enabled visual differentiation of infected coconut palms from apparently healthy samples. The cross reactivity of the assay was determined using sugarcane leaf extract infected with white leaf disease caused by another phytoplasma. This assay, that did not cross react with crude leaf extract of Sugarcane white leaf disease, will be validated using a significant number of disease positive and negative coconut samples. Cross reactivity of the assay will be further analysed using arecanut and bermuda grass leaf samples infected with closely related phytoplasma strains.

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