Abstract

Coconut is a major source of revenue and an integral part of the livelihood of more than 0.8 million people of Sri Lanka. Currently, the main threat to the local coconut industry is a devastating outbreak of a rapidly spreading, phytoplasma borne, non-lethal disease syndrome, first reported in 2006 from the Weligama area in the Southern Province, aptly named "Weligama Coconut Leaf Wilt Disease (WCLWD)". The present study was undertaken to identify and confirm the causative agent of the WCLWD and to develop molecular and immuno diagnostic techniques for accurate and early diagnosis of WCLWD.

Scanning electron micrographs, for the first time recorded the presence of phytoplasma like bodies within the sieve tubes of pinnae mid rib of WCLWD infected spear leaves. Molecular diagnosis of WCLWD phytoplasma based on in-house established nested PCR of *SecA*, similarly confirmed the association of a phytoplasma strain with the disease. A phytoplasma strain with sequence similarity to WCLWD phytoplasma was identified, associated with the arecanut yellow leaf disease (AYLD), for the first time in Sri Lanka that was reiterated by SEM.

Phylogenetic analysis performed using maximum likelihood (ML) with General Time Reversible model with discrete gamma distribution (GTR + G) model confirmed that WCLWD phytoplasma was clustered in the 16SrXIV phytoplasma group, '*Candidatus* Phytoplasma cynodontis', evidencing nonconformity with the existing classification based on 16S rRNA gene. Bayesian inference (BI) based phylogenetic tree confirmed the ML phylogram. This relationship was further elucidated by *secA* based virtual restriction fragment length polymorphism (RFLP) analysis of partial sequences of the WCLWD phytoplasma secA gene. Accordingly, identical RFLP banding patterns observed for WCLWD, AYLD and Bermuda grass wilt lethal disease (BGWLD) phytoplasmas, were discerned from sugarcane white leaf disease (SCWLD) and sugarcane grassy shoot disease (SCGSD). This was confirmed by similarity coefficient (F) values of 1.0 for the former three phytoplasmas and 0.56 for the latter two.

The prototype population study based on Sri Lankan phytoplasma strains focused on understanding the factors determining evolutionary change, stasis, the amount and pattern of genetic variation of *secA*, within and between phytoplasma populations. All the phytoplasma populations possessed low genetic diversity and polymorphism, thus, forming a relatively homogeneous local population.

The in house established indirect ELISA as an immunodiagnostic tool was based on polyclonal antibodies, was validated with diagnostic sensitivity (93.9%), specificity (81.77%), false positivity (18.23%) and false negativity (6.1%). WCLWD polyclonal antiserum showed low cross reactivity with SCWLD (7%), SCGSD (8%) and noticeably high cross reactivity with BGWLD (69%) and AYLD (70%). Although Receiver operator characteristic curve revealed accuracy of 93% in differentiating diseased from symptomless palms, the high false positivity (18.23%) deterred the use of this ELISA for routine diagnosis of WCLWD.

The establishment of loop mediated isothermal amplification (LAMP) assay, a low cost sensitive, simple assay that could be used in the field, though unsuccessful for WCLWD was accomplished for SCWLD and SCGSD, where the latter diseases affect the economically viable sugarcane cultivation of the country.

In conclusion, *secA* based PCR, and specific polyclonal antibody based indirect ELISA, were established as molecular and immunodiagnostic tools for WCLWD. Elucidation of WCLWD phylogenetic relationships and population genetic structure of WCLWD phytoplasma will assist in the effective implementation of disease control and management measures. Importantly, the SCWLD and SCGSD specific LAMP assay developed as an off-shoot of the main study will benefit the sugarcane sector which is critically in need of revitalization to fill the gap that exists in the sugar requirement of the country.