Phylogenetic evaluation of phytoplasma causing Sugarcane White Leaf and Sugarcane Grassy Shoot Diseases in Sri Lanka

Chamini Kanatiwela-de Silva[1], Matthew Dickinson[2], Sumeda Thushari[3], Preethi V. Udagama[1*]

[1]Department of Zoology, Faculty of Science, University of Colombo, Colombo 03

[2] School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, UK

[3] Crop Protection Division, Sugarcane Research Institute, Udawalawe

Sugarcane (*Saccharum officinarum* L.) is a high potential cash crop in Sri Lanka that occupies 0.6% of the land extent. At present, the local sugar industry supplies only 5% of the domestic requirement. Therefore, sugarcane cultivation must be invigorated in order to reduce the adverse impact of incurring revenue to import the bulk sugar requirement of the country. Phytoplasma are associated with two major lethal diseases of sugarcane; sugarcane white leaf (SCWL) and sugarcane grassy shoot (SCGS) diseases. Implementation of proper disease management strategies is facilitated by the correct identification of the pathogen and its phylogenetic position. Nucleotide analysis of 16S rRNA gene has previously revealed that the two strains of phytoplasma causing SCWL and SCGS share a sequence similarity of 97.5 – 98.8%, and are thus closely related.Both phytoplasma strains are categorized under 16SrXI "*Candidatus* Phytoplasma oryzae" group. This study was undertaken to investigate the phylogenetic relationship of SCWL and SCGS disease causing phytoplasma in Sri Lanka, using highly conserved 16S rRNA and the less conserved secA genes.

Total DNA extracted from mid ribs of leaves infected with SCWL and SCGS were subjected to nested PCR using a pre–optimized PCR master mix (Bioline, UK). 16SrRNA gene was amplified first with universal primers P1/P7 followed by nested PCR with R16F2n/R16R2. Likewise, *secA* was amplified, with universal primers SecAfor2/SecArev3 followed by RicesecAfor2/RiceseAcrev3, specific for certain 16Sr XI and XIV phytoplasmas. Purified PCR products were sequenced using the single read sequencing method at Eurofinsmwg Operon (UK).

Partial nucleotide sequences of 16SrRNA (1240 bp) and secA (396 bp) genes of both SCWL and SCGS diseases thus generated, were subjected to NCBI nucleotide blast, that revealed 100% identity between these two phytoplasmas. A phylogenetic tree was constructed using maximum likelihood method with of MEGA 6 software. The reliability of the tree was assessed by bootstrap analysis with 1000 replications and a remarkable similarity in their clustering was observed. Moreover, *in silico* RFLP analysis performed using the virtual gel plotting program, pDRAW32 and similarity coefficient calculated by Perl (practical extraction and report language) disclosed that there is no difference between the banding pattern in SCWL and SCGS disease causing phytoplasma for both genes, confirmed by the similarity co efficient value (1.0).

It is evident that the phylogenetic evaluation based on 16SrRNA and secA genes, and *in silico* RFLP analysis confirmed that both SCWL and SCGS diseases are caused by the identical strain of phytoplasma in Sri Lanka. Hence, similar management strategies can be applied to control both diseases.

Financial assistance by the National Science Foundation (NSF/SCH/2012/01) is acknowledged.