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Phylogenetic relationships of sugarcane grassy shoot phytoplasma with closely related agents

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Abstract

The phylogenetic relationships of sugarcane grassy shoot phytoplasma strains to each other and to closely related phytoplasmas infecting mainly gramineous plant worldwide, at both 16S rRNA gene and 16S/23S rDNA spacer region sequence level were examined. Most of the rDNA sequences used were retrieved from GenBanks whereas additional sequences of two Indian strains were determined in this work. The sugarcane grassy shoot phytoplasma, which is known to be a member of the rice yellow dwarf (RYD) phytoplasma group or 16SrXI group, proved to be very closely related to the sugarcane white leaf agent. More distantly related to it, are sorghum grassy shoot phytoplasma, '*Candidatus* Phytoplasma oryzae' and '*Candidatus* Phytoplasma cynodontis'.

Key words: sugarcane grassy shoot, rice yellow dwarf group, ribosomal DNA, phylogenetic analysis.

Introduction

Sugarcane (*Saccharum* sp. and hybrids) is affected by two lethal phytoplasmal diseases, i.e., sugarcane grassy shoot (SCGS) and sugarcane white leaf (SCWL) (Rao *et al*., 2005). SCGS disease has been reported to occur in India, Bangladesh, Malaysia, Nepal and Pakistan whereas SCWL is predominant in Taiwan, Sri Lanka and Thailand (Rao *et al*., 2005). They are caused by SCGS and SCWL phytoplasmas, respectively. These non-helical mollicutes form, together with a few other phytoplasmas infecting mainly gramineous plants, the ribosomal rice yellow dwarf (RYD) phytoplasma group or 16SrXI group, also named SCWL group (Jung *et al*., 2003; Marcone *et al*., 2004). However, there is very little information on the phylogenetic relationships of SCGS phytoplasma with other members of this group.

In this work, the phylogenetic relationships of SCGS phytoplasma strains to each other and to closely related phytoplasmas infecting mainly gramineous plant worldwide, at both 16S rRNA gene and 16S/23S rDNA spacer region sequence level, were examined.

Materials and methods

Most of the ribosomal DNA (rDNA) sequences used in this study were retrieved from GenBanks. Additional rDNA sequences were generated as follows. From SCGS-affected sugarcane plants collected in India, phytoplasmal DNA was amplified by polymerase chain reaction (PCR) assays using the primer pair P1/P7 followed by P4/P7 as nested primers (Schneider *et al*., 1995). The P4/P7 PCR products, approximately 530 bp in length which include the 16S/23S rDNA spacer region and part of 16S rRNA gene, were separated by electrophoresis in 1.5% agarose gel, excised from the gel and eluted using the QIAquick gel extraction kit (Qiagen). DNA fragments were either sequenced directly or cloned prior to sequencing. Sequences were then assembled and edited using DNASTAR's LaserGene software (DNASTAR) and consensus sequences were generated. Sequence alignments were performed by using CLUSTAL, version 5, using DNASTAR's LaserGene software (DNASTAR). The GenBank accession numbers of sequences determined in this study are AB239611 and AB243298.

Phylogenetic and molecular evolutionary analyses were conducted using the neighbor-joining program of the genetic analysis software MEGA, version 3.1 (Kumar *et al*., 2004). The data were resampled 1,000 times and the bootstrap percentage values are given at the nodes of the tree. Phylogenetic distances were calculated by pairwise comparison.

Results

Nucleotide sequence analysis of 16S rRNA genes revealed that SCGS phytoplasma is very closely related to the SCWL agent sharing a sequence similarity which varied from of 97.5 [SCGS-Bid (DQ459438) *versus* SCWL-Ud (AB052874)] to 98.8% [SCGS-Pun (DQ459439) *versus* SCWL-Ud]. Both strains of SCGS phytoplasma, namely SCGS-Bid and SCGS-Pun, for which the full-length 16S rDNA sequences are available from GenBanks, lack the *Hin*fI restriction site following position 901, which is present in the 16S rDNA sequences of strains SCWL (X76432) and SCWL-Ud of the SCWL agent. These findings largely confirm the results of previous work which showed that the SCGS phytoplasma can be distinguished from the SCWL agent using restriction fragment length polymorphism (RFLP) analysis of PCR-amplified 16S rDNA sequences with *Hin*fI restriction endonuclease (Marcone *et al*., 2004). More distantly related to SCGS agent, are the sorghum grassy shoot (SGS), RYD ('*Candidatus* Phytoplasma oryzae') and leafhopper (*Psammotettix caphalothes*)-

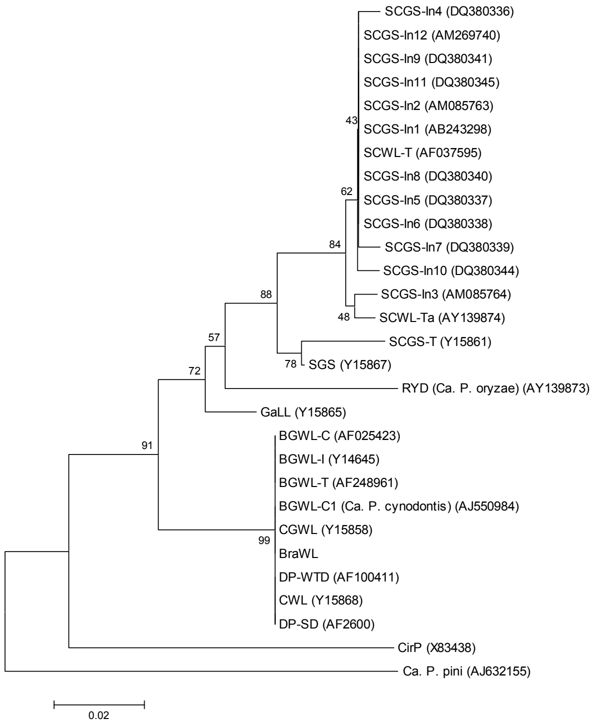

Figure 1. Phylogenetic tree constructed using the neighbor-joining method with 16S/23S rDNA spacer region sequences from strains of the sugarcane grassy shoot (SCGS) agent and related phytoplasmas. Bar represents a phylogenetic distance of 2%. GenBank accession numbers and bootstrap values are shown in parentheses and on branches, respectively.

borne (BVK) phytoplasmas sharing 16S rDNA sequence similarity values of 96.4 – 97.8, 95- 96.1 and 95 – 96%, respectively. These phytoplasmas are members of the RYD phytoplasma group as well (Jung *et al.*, 2003; Marcone *et al.*, 2004). By showing divergences of 5 – 5.7 and 4 – 5%, respectively, cirsium phyllody (CirP) and galactia little leaf (GaLL) phytoplasmas are most distantly related to the SCGS agent in the RYD group. Of the phytoplasmas that cluster in other phylogenetic groups, those most closely related to SCGS phytoplasma are the Bermuda grass white leaf (BGWL) (‘*Candidatus* Phytoplasma cynodontis’) and brachiaria grass white leaf (BraWL) agents, which share 97.3 and 97.1% 16S rDNA sequence similarity, respectively. Phytoplasmas from the remaining phylogenetic groups differ from the SCGS agent in more than 6% of 16S rDNA positions.

At 16S/23S rDNA spacer region sequence level, SCGS phytoplasma strains from India (SCGS-Ind1 through SCGS-Ind12) were identical or nearly identical, with similarity values between 98.7 and 100%. The Thai strain SCGS-T had sequence similarity of 86.5 - 87.3% with them (figure 1).

The closest relatives, sharing between 86.9 and 97.9% sequence identity with SCGS phytoplasma strains, are the SCWL and SGS phytoplasmas. More distantly related to SCGS agent, are RYD, GaLL, BraWL and BGWL phytoplasmas, with similarity values ranging from 79.8 to 86.9%. The 16S/23S rDNA spacer region of SCGS phytoplasma is about 240 bp in length. The gene encoding tRNA$^{\mathrm{Ile}}$ was present in all strains examined and the sequence of this gene was identical in all strains but one, the SCGS-In4 strain, where A was replaced by a T residue.

Discussion

Prior to this work, the phylogenetic position of SCGS phytoplasma and its relatedness to closely related phytoplasmas were based only on the sequence analysis of 16S/23S rDNA spacer region of the Thai strain SCGS-T and RFLP data of PCR-amplified 16S rDNA and 16S/23S rDNA spacer sequences of a few Thai and Indian isolates (Marcone *et al.*, 2004). Thus, the phylogenetic relationships of SCGS phytoplasma strains to each other and to the most related phytoplasmas, at both 16S rRNA gene and 16S/23S rDNA spacer region sequence level were examined for the first time in the present work.

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