

YELLOW LEAF VIRUS STRAIN DIVERSITY WITHIN THE SOUTH AFRICAN SUGARCANE INDUSTRY

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Abstract

Yellow Leaf Syndrome is a relatively new disease of sugarcane caused by Sugarcane Yellow Leaf Virus (SCYLV) and/or Sugarcane Yellows Phytoplasma (SCYP). Common symptoms include yellowing of the leaf midrib, sucrose accumulation in the leaves and leaf necrosis. Tissue-blot immunoassay and Titan One Tube RT-PCR are routinely used to determine the presence of SCYLV. Leaf samples taken from six imported varieties, held in the SASRI variety collection, and four quarantined imported varieties, all tested positive for the virus. The genotypic diversity of SCYLV in varieties from five countries, including South Africa, was determined through cloning and sequencing part of the viral genome. Using Clustal X sequence alignment and Neighbor-Joining phylogenetic methods, four different strains of virus were detected. Sequences were compared with known sequences from GenBank. Closely related viral sequences from varieties CP72/1210, CP79/1348, 94Z/0155, 94Z/0144, N30 and N32 represent one strain that was found to be similar to a Guatemalan strain G2. Sequences from CP78/2114 and the quarantined import CC87/505 from Colombia represent a second strain, similar to Colombian strains C1 and C3, and the three quarantined SP varieties were similar to the Brazilian strain B1. One putative strain has been found in SP71/3146. It has been observed that genetic variability exists in SCYLV.

Keywords: sugarcane, genotypic diversity, pathogenicity, strain, yellow leaf virus

Introduction

Yellow leaf syndrome (YLS) is a relatively new disease of sugarcane. Symptoms described as 'yellow wilt' (Ricaud, 1968) are similar to those of yellow leaf syndrome. YLS of sugarcane has been reported in 34 sugarcane growing regions around the world (Lockhart and Cronje, 2000). Two etiological agents have been associated with the syndrome. One is the sugarcane yellow leaf luteovirus (SCYLV) (Lockhart *et al.*, 1996) and the other a sugarcane yellows phytoplasma (SCYP) (Cronje *et al.*, 1996). Mixed infections of SCYP and SCYLV are common in South Africa (Cronje and Bailey, 1999).

Transmission of SCYLV occurs through sugarcane aphids such as *Melanaphis sacchari* and *Rhopalosiphum maidis* (Schenck and Lehrer, 2000), whereas SCYP is transmitted by leaf hoppers.

Symptoms of this syndrome include yellowing of the leaf midrib, which occurs in all forms of the disease, shortening of terminal internodes, yellowing of terminal leaves and sucrose accumulation in midribs (Comstock *et al.*, 1994). Yellow discoloration may also spread

laterally from the midrib, while necrosis begins from the leaf tip and progresses towards the leaf base. Cronje *et al.* (1998) reported that symptoms occur from leaf 3 to leaf 5, taking the first visible dewlap as leaf one. Severe cases have shown that veins can become reddish, necrosis develops along the leaf edges and there is a distinct reduction in the growth rate.

Current detection methods for SCYLV include tissue blot immunoassay (TBIA) and Titan One Tube Reverse Transcription Polymerase Chain Reaction (RT-PCR). Effective elimination of the syndrome can be achieved by using tissue culture. Various explants can be used; for example meristems, leaf-roll discs and auxiliary buds. However, the culturing of immature leaf-roll discs proved to be more efficient than culturing meristems, as the virus persisted after meristem culture (Pillay *et al.*, 2003).

Materials and Methods

Leaf samples were taken from six imported varieties (held in SASRI variety collection), namely 94Z/0141, 94Z/0155, CP72/1210, CP79/1348, CP78/2114, and SP71/3146, four imported quarantine varieties, CC87/505, SP86/155, SP87/365 and SP91/1049, and two South African varieties, N30 and N32.

Detection of SCYLV was carried out using the tissue blot immunoassay according to Schenck *et al.* (1997). An imprint of the midrib was made on a nitro-cellulose membrane, and an immunoassay was carried out using SCYLV specific antibodies. Positive results were indicated by the presence of blue dots in the vascular bundles.

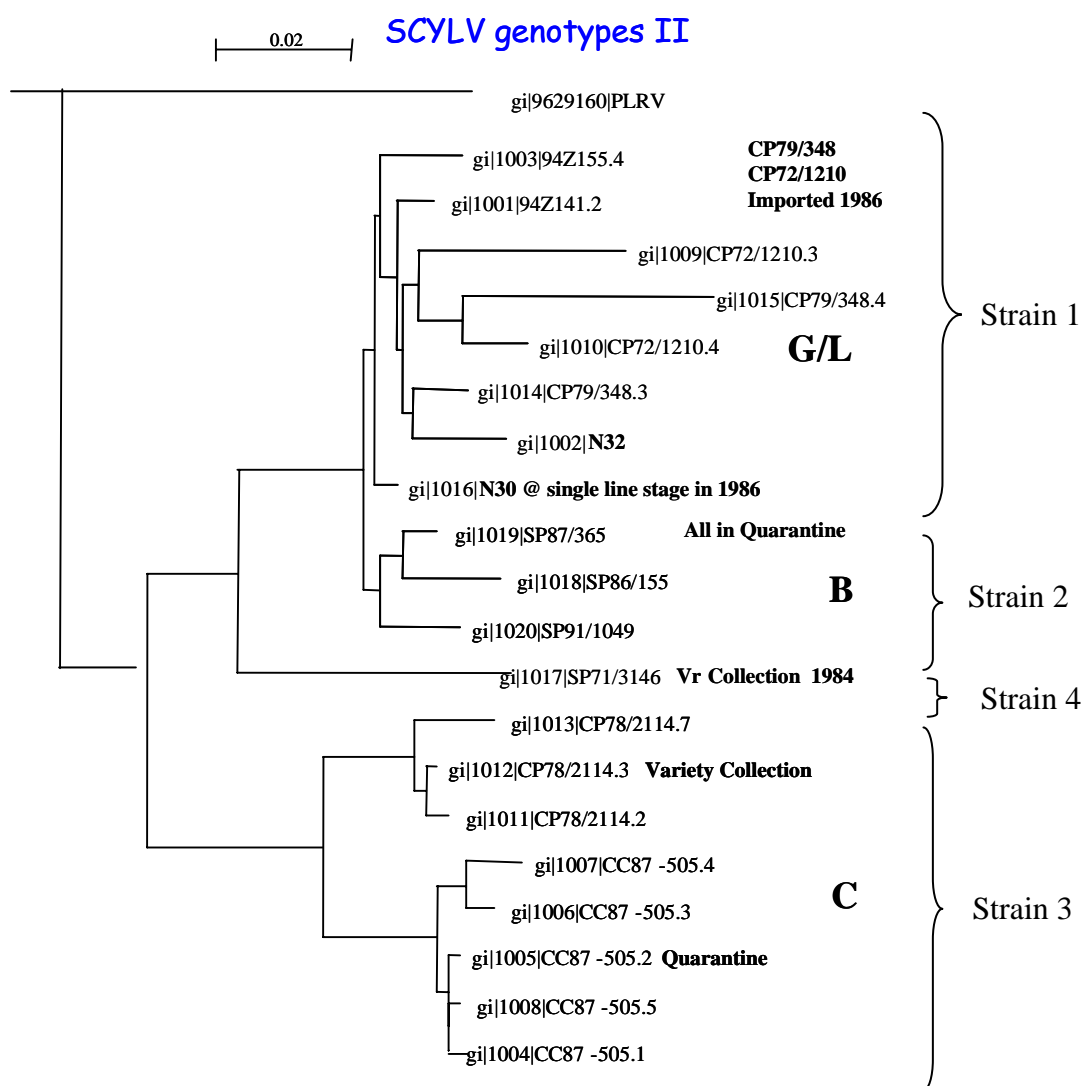
These varieties were re-tested for SCYLV using RNA extraction and RT-PCR. RNA was extracted using the Qiagen RNA-Easy Plant Mini Kit. Sugarcane yellow leaf virus was detected using Titan One Tube RT-PCR System. The REPUTR region was produced and amplified using virus specific primers, IALW-Forward and SRRNV-Reverse.

Positive virus fragments were excised and cloned using the pGEM-T Easy Vector Kit. Transformants containing plasmids with inserts were identified using blue and white screening. Colonies on a Luria Bertani plate that carried the insert were white, and untransformed plasmids were blue. White colonies were selected and their DNA amplified to verify the presence of the insert. Plasmid DNA was purified using the QIAprep® Spin Miniprep Kit (Qiagen). DNA was sequenced using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequences were analysed and aligned using Clustal X and Neighbor-Joining phylogenetic methods.

Results and Discussion

Leaf samples taken from these varieties all tested positive for the virus, using both tissue blot immunoassay and RT-PCR. Analysis of sequences indicated that three previously recognised viral strains (Moonan and Mirkov, 2002) and one putative new strain exist.

Closely related viral sequences from varieties CP72/1210, CP79/348, 94Z/0155, 94Z/0144, N30 and N32 represent one strain that is found to be similar to G2 from Guatemala and L1 from Louisiana (Strain 1). Sequences from CP78/2114 in the variety collection and the quarantined import CC87-505 from Colombia represent a second strain, similar to C1 and C3 from Colombia (Strain 2). The three quarantined SP varieties SP/86/155, SP87/365 and SP91/1049, represent the third strain, which is similar to B1 from Brazil (Strain 3). A putative fourth strain is present in the variety collection in SP71/3146 (Strain 4), which is different from the Brazilian strain B1 found in the three quarantined SP varieties.



Conclusions

It has been observed that genetic variability exists in SCYLV. However it is not yet known whether genetic diversity is related to differences in pathogenicity. Further studies will have to be undertaken to determine the effects of different strains of virus on yield loss, difference in symptoms and pathogenicity.

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