

DEVELOPMENT OF NEW METHODS FOR DIAGNOSING YELLOW LEAF SYNDROME

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Introduction

A disorder of sugarcane (*Saccharum* species hybrid) characterised by yellowing of the leaf midrib and lamina was first reported in East Africa in the 1960s. This disorder was termed yellow wilt (Ricaud, 1968). A similar disorder was reported as autumn decline in Brazil (Hughes, 1964). Some thirty years later similar symptoms were observed on sugarcane in Hawaii (Schenk *et al.*, 1990) and Brazil (Comstock *et al.*, 1994) and described as yellow leaf syndrome (YLS). Today YLS is prevalent in all sugarcane growing regions of the world (Lockhart and Cronje, 2000). YLS first became apparent in the South African sugar industry in 1995 when large areas of sugarcane suddenly began exhibiting yellowing symptoms (Bailey *et al.*, 1996; Cronje *et al.*, 1998). In some cases, YLS has been associated with significant yield losses, although this depends largely on varietal resistance/susceptibility and causal agent (Vega *et al.*, 1997; Comstock *et al.*, 1998).

Two pathogens, a phytoplasma (sugarcane yellows phytoplasma, SCYP, Cronje *et al.*, 1998) and a luteovirus (sugarcane yellow leaf virus, SCYLV, Scagliusi and Lockhart, 2000) have been implicated in this disease. In South Africa it was found that SCYP was consistently associated with the disease and found throughout the industry, whereas SCYLV was found to a lesser degree and mainly occurred in the northern, irrigated areas (Cronje *et al.*, 1998). Diagnosis of YLS based on symptoms alone is not accurate since similar symptoms can be induced by abiotic factors such as moisture and nutrient stress, insect feeding, physiological stress, lodging of cane and damage to leaves (Bailey *et al.*, 1996; Matsuoka and Meneghin, 1999).

Keywords: YLS, SCYP, SCYLV, FTA, RT-PCR

Aims of this study

Continual monitoring of the occurrence of these two pathogens is ongoing, and newer, more sensitive methods are needed for screening. The aim of this work was to improve the currently available methods for testing for SCYP and SCYLV to reduce cost, improve sensitivity and specificity and decrease processing and handling time.

Materials and Methods

For detection of SCYP, DNA was extracted using two different methods, namely phytoplasma DNA enrichment method (Harrison *et al.*, 1994) and the FTATM method (Whatman BioScience FTATM). The phytoplasma DNA enrichment method is a modified CTAB method specifically used for phytoplasma DNA extraction. The FTATM method entails capturing DNA on a paper matrix, after which the DNA is washed with FTA Purification SolutionTM and 1x TE buffer and allowed to dry for 1h. PCRs were separately optimised for both extraction methods, altering parameters such as the number of cycles, additives, MgCl₂ concentration, different polymerase enzymes and different reaction volumes.

For detection of SCYLV, the classical method of tissue-blot immunoassay (TBIA) was used and performed according to Schenck *et al.*, 1997. Newer molecular techniques such as reverse transcriptase-PCR (RT-PCR) and immunocapture RT-PCR (IC-RT-PCR) were developed, optimised and compared with the TBIA method. The IC-RT-PCR consisted of coating PCR tubes with polyclonal antibody specific to SCYLV after which infected leaf sap was added and incubated overnight. PCR parameters such as annealing temperatures and additives were investigated.

Results and Discussion

The current method of diagnosis of SCYP involves a DNA enrichment method. However this method is expensive, time-consuming and only a few samples can be processed at a time. The FTA™ method was found to be useful for phytoplasma detection as well as the bacteria *Leifsonia xyli* subsp *xyli* (the causative agent of RSD) and the fungal pathogen *Glomerella tucumanensis* (causal agent of red rot). Using the FTA™ method rather than the DNA enrichment method for phytoplasma detection, it is now possible to process six times more samples per week with a 65% reduction in cost and a 300% increase in accuracy.

Current detection methods for SCYLV utilise a tissue-blot immunoassay (TBIA), which is easy-to-perform, rapid, cost effective and a large number of samples can be processed at a time. Newer methods such as RT-PCR and IC-RT-PCR were developed and compared with TBIA. Results suggest that the TBIA method is reliable and accurate for diagnosis. Using the TBIA method we can process three times more samples with a 93% reduction in cost and identical accuracy compared with the PCR methods. However the PCR methods developed are still useful as we are now able to obtain detailed molecular and sequence information of the genome of SCYLV.

These improved methods are now being used for surveys of YLS within the South African sugar industry to determine the spread and prevalence of both SCYP and SCYLV.

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