# PROGRESS AND POTENTIAL IN SUGARCANE MOLECULAR PATHOLOGY

By

GRANT R. SMITH

*David North Plant Research Centre, Bureau of Sugar Experiment Stations, Brisbane*

## Abstract

Since the mid-1980s, significant progress has been made in the many fields of sugarcane molecular pathology. Diagnostic tests have been developed, complete viral genomes sequenced, resistance transgenes developed, and the causes of new and old diseases elucidated. From this substantial platform, the application of some of the new molecular technologies such as genomics and proteomics should allow a complete understanding of the interactions between sugarcane and its pests and pathogens, leading to new approaches to pathogen diagnosis and disease control.

## Introduction

Molecular biology has changed, and continues to change, our understanding of pathogens, how they interact with their hosts, and approaches to disease control. Sugarcane molecular pathology began in the mid 1980s when Skotnicki *et al.* (1986) developed cDNA probes for the diagnosis of *Fiji disease fijivirus* (FDV). Since then, the science and techniques of molecular biology have been increasingly applied in sugarcane pathology research including probe, PCR and serum-based diagnostics, analysis of pathogen genomes, development of resistance genes, identification and analysis of new pathogens, identification of the pathogens involved with old diseases, and the development of gene regulation sequences. Here, progress in these different areas is briefly reviewed and some future directions in sugarcane pathology research are predicted.

## Diagnostics

There have been four phases in diagnostic sugarcane pathology to date, namely symptomatology, serology, nucleic acid probe and polymerase chain reaction (PCR)-based. The next technology, biosensors, is already being applied in environmental and food applications (eg Glazier *et al.*, 1998) and should soon be applied in diagnostic plant pathology. As noted above, FDV was the first pathogen for which a nucleic acid test was developed. The genome of FDV consists of ten linear segments of double-stranded (ds) RNA. It is interesting to note, whilst reviewing this area of research, that dsRNA is the most difficult template for application of reverse transcription (RT)-PCR techniques. The early RT-PCR techniques for the PCR amplification of RNA did not work on dsRNA templates, and a new approach to priming the reaction had to be developed (Smith *et al.*, 1992). Subsequently, PCR-based assays were developed for a range of viral (eg Smith and Van de Velde, 1994; Braithwaite *et al.*, 1995), bacterial (eg Fegan *et al.*, 1998, Pan *et al.*, 1998, 1999) phytoplasma (eg Cronjé *et al.*, 1998) and fungal pathogens (eg Albert and Schenck, 1996) of sugarcane. The status of molecular tools for indexing sugarcane for a range of pathogens was reviewed by Braithwaite and Smith (1996). Dietzgen *et al.* (1999) reviewed the status of PCR-based diagnosis for sugarcane viruses, while the latest publication by Braithwaite and Smith (2001) reviewed the current state of diagnosis for viral pathogens of sugarcane.

An interesting application of molecular technology to established serum-based sugarcane diagnostic assays was published in 1995, when recombinant viral coat protein was used as an antigen for the production of a specific high-titre antiserum to *Sugarcane mosaic potyvirus* (SCMV) (Smith *et al.*, 1995). The recombinant coat protein had been inducibly expressed in *E. coli* as a fusion protein from a plasmid construct containing the cloned SCMV coat protein fused to the maltose binding protein (MBP), so that purification of the fusion protein from the bacterial lysate was facilitated by column chromatography. This approach to antiserum production was repeated in 1998 with the expression and purification of the proteins encoded by the two open reading frames of the cloned FDV segment 9 (Soo *et al.*, 1998).

Variation in pathogens remains a fundamental issue to diagnostics. Modern molecular techniques permit evaluation of potential areas of the genome to target for diagnostic tests, so that both generic tests to identify all strains/ races/ isolates of a pathogen, as well as specific tests to discriminate between those strains/ races/ isolates can be developed. Analysis of the internal transcribed spacer region (ITS) sequence of *Clavibacter xyli* ssp. *xyli* (Cxx) and *C. xyli* ssp. *cynodontis* (Cxc) led to the development of a PCR-based assay to discriminate these closely related bacterial pathogens (Fegan *et al.*, 1998). Yang and Mirkov (1997) developed a RT-PCR test that discriminated between the SCMV and *Sorghum mosaic potyvirus* (SrMV) isolates by sequencing and analysing a section of the viral genome, while Smith *et al.* (1996) reported on the consequences of the variation found in populations of *Sugarcane bacilliform badnavirus* (SCBV) for accurate diagnosis.

KEYWORDS: Diagnosis, Aetiology, Resistance, Transgenes, Genomes.

## Aetiology of old and new diseases

There are a number of diseases of sugarcane for which the pathogen is yet to be identified, including chlorotic streak, Ramu stunt and Sereh. Molecular techniques have identified a virus associated with striate mosaic, a disease limited to a small geographic region of Queensland (Thompson *et al.,* 1998, Choi *et al.,* 1999). Whilst it is important to note that the association of the viral sequences with the disease does not constitute causality, this is the first direct evidence of a pathogen for a disease that was first described by Hughes (1961).

In 1988, a leaf-yellowing symptom of sugarcane was noted in Hawaii (Schenck, 1990). This disease began to attract more scientific attention in the mid-90s as yellow leaf symptoms were noted in other countries and a range of causes proposed. In 1997, the first proof that a virus was involved was reported (Irey *et al.,* 1997, Vega *et al.,* 1997), whilst molecular evidence for a phtyoplasma was published the following year by Cronjé *et al.* (1998). In 2000, two groups (Moonan *et al.;* Smith *et al.*) published the complete genome of the virus, finding that Sugarcane yellow leaf virus (SCYLV) was a previously unreported member of the Luteoviridae that had arisen as a result of at least two independent recombinations between luteovirid genomes. The relative importance of the viral and phytoplasma forms of yellow leaf syndrome (YLS) are yet to elucidated.

## Pathogen genomes

The genomes of several viral pathogens of sugarcane have now been fully determined including *Sugarcane streak mastrevirus* (Hughes *et al.,* 1993), SCBV (Bouhida *et al.,* 1993), *Peanut clump pecluvirus* (Miller *et al.,* 1996, Naidu *et al.,* 1996), SCYLV as noted above and SCMV (Pickering *et al.,* 2001) and mostly determined for FDV (Soo *et al.,* 1998) and Sugarcane striate mosaic associated virus (Thompson *et al.,* 1998). Full or partial genomic sequences of sugarcane pathogens have found important uses in three distinct areas of sugarcane pathology and molecular biology research. Firstly, sequence data is the basis for the development of generic and/ or specific molecular assays. Secondly, the sequences are important sources of pathogen-derived resistance (PDR) genes that have been developed and proven in sugarcane against SCMV (Joyce *et al.,* 1998a,b), SrMV (Ingelbrecht *et al.,* 1999) and FDV (McQualter *et al.,* 2001). Molecular analysis of pathogen isolates to monitor and predict the possibility of resistance-transgene breaking isolates is an important component during the development of PDR genes. For the SCMV resistant transgenic plants developed in Australia, survey results of the SCMV strain A isolates suggested that the transgene sequence would provide resistance to all isolates found in Australia (Handley *et al.,* 1996, 1998). Thirdly, viral gene regulation sequences (promoters and terminators) have been developed from the genomes of DNA viruses including SCBV (Tzafrir *et al.,* 1998).

There has been less genomic progress with the other pathogens of sugarcane due to the size of these genomes. Limited genetic analysis on the leaf scald bacterium, *Xanthomonas albilineans* has indicated that at least two gene clusters are involved in albicidin production (Rott *et al.,* 1996). Resistance genes against *X. albilineans* have also been developed (Zhang *et al.,* 1999) and demonstrated to provide field resistance to leaf scald in transgenic sugarcane plants. The complete genome of the bacterial pathogen Cxx is currently being sequenced and will be the first non-viral sugarcane pathogen for which the full genomic sequence will be determined.

## The future

The next phase of diagnostic technology, biosensors, are being applied for the rapid and accurate detection of virtually any compound including microbial toxins and pathogens. This technology could be suited to the fast screening of important sugarcane germplasm, but requires considerable research and development to provide a basis for making a decision on its suitability. Our understanding of the molecular biology of the interactions between sugarcane and its pathogens should increase significantly in the next few years as genomics and proteomics research in sugarcane begins to deliver outcomes. The ability to monitor the way the plant responds at the molecular level to biotic or abiotic stimuli should lead to the conception and development of new approaches to the control of the numerous pests and pathogens of sugarcane and smarter management of the many environmental factors that influence the productivity of sugarcane.

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