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Biotechnological Applications to Sugarcane Pathogens

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ABSTRACT

Sugarcane (*Saccharum* spp.) is an important cash crop accounting for nearly two thirds of the world sugar production. Much effort has and is still being devoted to breeding resistant varieties, as the crop is vulnerable to a number of diseases that affect production. These constraints have triggered the adoption of biotechnological tools to counteract the impact of diseases on sugarcane. The increasing use of biotechnology is enabling early detection of pathogens of fungal, viral, bacterial and phytoplasma origin, allowing preventive measures to be adopted much earlier, before the appearance of disease symptoms as well as enabling the increased use of clean seed for establishment of nurseries and for the exchange of germplasm. The strategy of developing transgenic sugarcane varieties for resistance to several diseases is now well established and several transgenic lines are being evaluated in the field in a number of countries, although no commercial transgenic cane is grown thus far. Recent advances in sugarcane genomics as well as the genomics of sugarcane pathogens will soon provide information on disease resistance genes enabling new measures to be adopted in the breeding for disease resistance.

Keywords: biosecurity, clean seed production, disease resistance, genetic transformation, molecular markers, molecular tools, *Saccharum* spp., sugarcane diseases

Abbreviations: **PCR**, polymerase chain reaction; **RFLP**, restriction fragment length polymorphism; **RT-PCR**, reverse transcriptase polymerase chain reaction; **SCMV**, *Sugarcane mosaic virus*; **SCYLV**, *Sugarcane yellow leaf virus*; **SrMV**; *Sorghum mosaic virus*; **TBIA**, tissue blot immunoassay

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INTRODUCTION

Sugarcane (*Saccharum* spp. *Poaceae*) is a major cash crop grown in tropical and sub-tropical countries around the world (Cheavegatti-Gianotto *et al*. 2011). With its soft and watery culm, it has been exploited primarily for sugar production, with a world production of about 160 million tonnes for the 2009-2010 period (USDA 2009) and accounting for nearly two third of global sugar production (D'Hont *et al*. 2008), the remaining being obtained from sugar beet (*Beta vulgaris* L., *Chenopodiaceae*). Recently, sugarcane has enhanced its importance as an energy crop (Cheavegatti-Gianotto *et al*. 2011) for ethanol production and as a source of energy through burning of bagasse, a

Received: 12 April, 2011. Accepted: 1 August, 2011.

Invited Review

major by-product generated in the mill after extraction of sucrose from the stalk (Menossi *et al*. 2008).

Commercial sugarcane varieties cultivated nowadays are hybrids derived mainly from crosses involving *S. officinarum* – also known as noble canes – the high sugar content species with poor disease resistance and vigour, and *S. spontaneum* – the wild species with high disease resistance and high vigour. Despite progress made in genetic improvement of sugarcane, the crop is still confronted with diverse diseases that still pose major constraints to its production. Some 120 sugarcane diseases have been reported (Rott *et al*. 2000a) and can be categorized as of bacterial, viral, fungal and phytoplasmal origin. Before the release of new improved cultivars in any breeding programme, their evaluation to major diseases is essential. In addition, management through cultural practices, crop hygiene and use of disease-free planting material produced by heat treatment is necessary. The use of chemicals in sugarcane disease management is limited to specific cases. As in all crops, diagnostics play a major role in early detection of pathogens, their identification and characterization, which in turn allow better disease management.

This review considers the major applications of biotechnology to seek for disease resistance and the application of molecular diagnostics in early, specific and rapid characterization of sugarcane pathogens.

BIOSECURITY

Movement of genetic resources (stalks pieces, rhizome pieces, tissue culture plantlets) among sugarcane-producing countries is fundamental to increase productivity. For countries with a breeding programme, introduced varieties are used in crossing or considered for commercial cultivation after evaluation. The transfer of sugarcane from one region or from one country to another involves risks that should be thoroughly assessed, given that the introduction of a disease may have a devastating effect on the industry. Rooted plant material (except for tissue culture plantlets) is not recommended. Diseases of concern during movement of germplasm are some fungal and bacterial ones, as well as viruses and phytoplasmas. Major diseases transmitted through cuttings are given in **Table 1**.

In general, imported planting material usually undergoes quarantine in confinement for a period of two years. Prior to the application of laboratory diagnostic techniques, the presence of diseases was based on the appearance of symptoms. It is likely that in the past, several undetected sett-borne diseases have owing to latency of the pathogen, e.g. ratoon stunt, leaf scald, or due to the fact that they were not previously recognized, e.g. yellow leaf, leaf yellows, leaf fleck and mild mosaic. Measures that have now been taken to reduce risks of disease introduction include:

- No authorization to import from high-risk areas
- Pre-export quarantine procedures
- Heat treatment of cuttings
- Movement of disease-free tissue culture plantlets
- Quarantine at a site away from sugarcane areas
- Comprehensive disease detection methods, with emphasis on molecular tools.

Procedures for the safe movement of sugarcane germplasm have been described by Frison and Putter (1993) and Bailey *et al*. (2000). It is to note, that a disease such as leaf fleck caused by a ds-DNA virus cannot be eliminated from the infected plant by any means. Its presence is consequently not given much importance during quarantine by many countries, the more so that currently no impact of the disease has been shown in commercial cultivars.

Biosecurity is more and more being treated as a global or regional issue. The South African Sugarcane Research Institute (SASRI) provides quarantine services for other African countries (van Antwerpen *et al*. 2001). Australia, a major sugar producer, has examined the threat posed by exotic diseases from Indonesia and Papua New Guinea (Magarey *et al*. 2008). As a result, screening tests for sugarcane have been developed against Ramu stunt and smut and information collected on sugarcane mosaic disease present across the region. The Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) has a fully equipped international quarantine unit, recently given a new name, Visacane that distributes clones from a number of different sources (international varieties, clones created by CIRAD in Guadeloupe and by the West Indies Central Sugarcane Breeding Station in Barbados). After two growth cycles, healthy cuttings are sent to sugarcane growing regions in West and Central Africa and to sugarcane breeding stations in the Guadeloupe and Réunion (Rott *et al*. 1997). Visacane is meant for both import and export of varieties from and to most sugarcane countries in the world (Girard *et al*. 2011).

Among the various sugarcane diseases intercepted in quarantine in the past, mosaic and ratoon stunt were commonly encountered. Currently, the most common one is yellow leaf caused by *Sugarcane yellow leaf virus* (SCYLV). Although a quite recently identified virus (Lockhart *et al*. 1996; Vega *et al*. 1997; Scagliusi and Lockhart 2000) the frequency of its detection is very high. van Antwerpen (2006) reported that the release of imported varieties from quarantine had been seriously hampered by the frequent presence of the SCYLV. Chatenet *et al*. (2001) detected SCYLV in the CIRAD sugarcane quarantine at Montpellier by reverse transcription-polymerase chain reaction and/or tissue-blot immunoassay (TBIA) in varieties from Barbados, Brazil, Cuba, Florida, Guadeloupe, Indonesia, Malaysia, Mauritius, Philippines, Puerto Rico, Réunion and Taiwan suggesting a worldwide distribution of the pathogen. Parmessur *et al*. (2002) also detected this virus in a number of varieties imported from different countries and undergoing quarantine in Mauritius. Occurrence of at least four genotypes of the SCYLV (Abu-Ahmad *et al*. 2006a) justifies the precautions taken against this disease by several quarantine facilities.

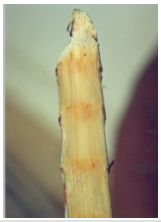
Detection is a critical step in the containment of sugarcane pathogens. Molecular diagnostics have been instrumental in achieving this, in addition to the understanding of their epidemiology and diversity.

DETECTION OF SUGARCANE DISEASES USING MOLECULAR TOOLS

Traditionally the detection and characterization of plant pathogens are based on symptoms expressed by the host plant. For some bacterial and fungal pathogens, isolation of the microorganism and microscopic observations are used. For viruses and phytoplasmas, recourse to electron microscope or transmission to indicator plants is required. In the 1970s with the advent of serological techniques such as enzyme linked immunosorbent assay (ELISA) and DNA recombinant technology, diagnostics of plant pathogens have been greatly improved. Over the years, further development of these technologies have resulted in improved techniques such as polymerase chain reaction (PCR), reverse transcriptase-PCR (RT-PCR), immuno-capture-PCR, Real-Time PCR and microarrays.

Molecular diagnostics are available for most of the sugarcane pathogens of bacterial, viral, phytoplasmal, and fungal origin. Xie *et al*. (2009) reported the simultaneous detection of four viruses using one step RT-PCR. Efficient PCR methods have been developed for the quantification and detection of major bacterial pathogens of sugarcane (Rott and Davis 2004), while molecular tools have been instrumental in improving detection of phytoplasmas (Dookun-Saumtally *et al*. 2008). Prospects for the future include microarray or DNA chips and processors that will be made available to characterize unknown DNA samples to enable identification of sugarcane pathogens.

Table 1 Major sugarcane diseases that may be introduced through importation of cuttings, their causal organism and distribution.

Diseases	Pathogen	Distribution according to Rott *et al*. (2000b)
Bacterial		
Gumming	*Xanthomonas axonopodis* pv. *vasculorum*	Antigua, Argentina, Belize, Brazil, Colombia, Cuba, Dominica, Dominican Republic, Fiji, Ghana, India, Madagascar, Madeira, Malawi, Mauritius, Mozambique, Panama, Puerto Rico, Réunion, St Kitts and Nevis, St Lucia, St Vincent, South Africa, Swaziland, Zimbabwe
Leaf scald 	*Xanthomonas albilineans*	Argentina, Australia, Barbados, Belize, Benin, Brazil, Burkina Faso, Burundi, Cameroon, Chad, China, Colombia, Congo, Côte D'Ivoire, Cuba, Democratic Republic of the Congo, Dominica, Dominican Republic, Ecuador, Fiji, Ghana, Grenada, Guadeloupe, Guatemala, Guyana, Hawaii, India, Indonesia, Iraq, Jamaica, Japan, Kenya, Madagascar, Malawi, Malaysia, Martinique, Mauritius, Mexico, Morocco, Mozambique, Myanmar, Nigeria, Pakistan, Panama, Papua New Guinea, Philippines, Puerto Rico, Réunion, St Kitts and Nevis, St Lucia, St Vincent, South Africa, Sri Lanka, Surinam, Swaziland, Tahiti, Tanzania, Taiwan, Thailand, Trinidad, Uruguay, USA, Venezuela, Vietnam, Zambia, Zimbabwe
Ratoon stunt 	*Leifsonia xyli* subsp. *xyli*	Antigua, Argentina, Australia, Bangladesh, Barbados, Belize, Bolivia, Brazil, Burkina Faso, Cameroon, China, Colombia, Congo, Costa Rica, Côte D'Ivoire, Cuba, Democratic Republic of the Congo, Dominican Republic, Ecuador, Egypt, El Salvador, Ethiopia, Fiji, Guadeloupe, Guyana, Hawaii, India, Indonesia, Japan, Kenya, Madagascar, Malawi, Malaysia, Mali, Martinique, Mauritius, Mexico, Mozambique, Myanmar, Nicaragua, Nigeria, Pakistan, Panama, Papua New Guinea, Peru, Philippines, Puerto Rico, Réunion, St Kitts and Nevis, South Africa, Sri Lanka, Sudan, Swaziland, Taiwan, Tanzania, Thailand, Trinidad, Uganda, Uruguay, USA, Venezuela, Zambia, Zimbabwe
Fungal		
Downy mildew	*Peronosclerospora sacchari*	Indonesia, India, Fiji, Japan, Papua New Guinea, Philippines, Taiwan, Thailand
Red rot 	*Colletotricum tucumanensis*	Afghanistan, Angola, Antigua, Argentina, Australia, Bangladesh, Barbados, Belize, Benin, Bolivia, Brazil, Burkina Faso, Burundi, Cambodia, Central African Republic, Chad, China, Colombia, Côte D'Ivoire, Cuba, Democratic Republic of the Congo, Dominican Republic, Egypt, El Salvador, Fiji, Gabon, Ghana, Guadeloupe, Guam, Guatemala, Guyana, Haiti, Hawaii, India, Indonesia, Iraq, Jamaica, Japan, Kenya, Madagascar, Madeira, Malawi, Malaysia, Mauritius, Mexico, Morocco, Mozambique, Myanmar, Nepal, Nicaragua, Niger, Nigeria, Pakistan, Panama, Papua New Guinea, Peru, Philippines, Puerto Rico, Réunion, St Kitts and Nevis, St Lucia, Samoa, Solomon Islands, South Africa, Sri Lanka, Swaziland, Taiwan, Tanzania, Thailand, Togo, Trinidad, Uganda, Uruguay, USA, Vanuatu, Venezuela, Vietnam, Zimbabwe
Smut 	*Ustilago scitaminea*	Afghanistan, Antigua, Argentina, Australia, Bangladesh, Barbados, Belize, Bolivia, Brazil, Burkina Faso, Burundi, Cambodia, Cameroon, Chad, China, Colombia, Congo, Costa Rica, Côte D'Ivoire, Cuba, Democratic Republic of the Congo, Dominica, Dominican Republic, Egypt, El Salvador, Ethiopia, Fiji, Gabon, Guadeloupe, Guatemala, Guyana, Haiti, Hawaii, Honduras, India, Indonesia, Iran, Iraq, Jamaica, Japan, Kenya, Madagascar, Malawi, Malaysia, Mali, Martinique, Mauritius, Mexico, Morocco, Mozambique, Myanmar, Nepal, Nicaragua, Niger, Nigeria, Pakistan, Panama, Paraguay, Peru?, Philippines, Portugal, Puerto Rico, Réunion, St Kitts and Nevis, Senegal, Somalia, South Africa, Sri Lanka, Sudan, Swaziland, Taiwan, Tanzania, Thailand, Trinidad, Uganda, Uruguay, USA, Venezuela, Vietnam, Zambia, Zimbabwe
Phytoplasma		
Grassy shoot – GS	GS phytoplasma	Bangladesh, India, Iran, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, Sudan, Thailand
White leaf – WL 	WL phytoplasma	Japan, Pakistan, Sri Lanka, Taiwan, Thailand
Viral		
Fiji leaf gall	*Fiji disease virus*	Australia, Fiji, Indonesia, Madagascar (not detected since 1971), Malaysia, New Caledonia, Papua New Guinea, Philippines, Samoa, Solomon Islands, Thailand, Vanuatu

Mosaic 	*Sugarcane mosaic virus* *Sorghum mosaic virus*	Andaman Islands, Angola, Argentina, Australia, Bangladesh, Belize, Bolivia, Brazil, Burundi, Cambodia, Cameroon, Cape Verde, China, Colombia, Congo, Costa Rica, Côte D'Ivoire, Cuba, Democratic Republic of the Congo, Dominican Republic, Ecuador, Egypt, El Salvador, Ethiopia, Fiji, Gabon, Ghana, Guatemala, Haiti, Hawaii, Honduras, India, Indonesia, Iran, Iraq, Italy, Jamaica, Japan, Kenya, Laos, Malawi, Malaysia, Mexico, Morocco, Myanmar, Nepal, Nicaragua, Nigeria, Pakistan, Panama, Papua New Guinea, Paraguay, Peru, Philippines, Puerto Rico, Réunion, St Kitts and Nevis, St Thomas, Sierra Leone, South Africa, Spain, Sri Lanka, Surinam, Swaziland, Tanzania, Taiwan, Thailand, Trinidad, Turkey, Uganda, Uruguay, USA, Venezuela, Vietnam, Zambia, Zimbabwe
Streak	*Sugarcane streak virus*	Benin, Cape Verde, Côte D'Ivoire, Egypt, India, Kenya, Madeira, Malawi, Mauritius, Mozambique, Pakistan, Réunion, South Africa, Sudan, Uganda, Zimbabwe
Streak mosaic	*Sugarcane streak mosaic virus*	Bangladesh, India, Pakistan, Sri Lanka, Thailand, USA (Chatenet *et al*. 2005)
Yellow leaf 	*Sugarcane yellow leaf virus*	Argentina, Australia, Barbados, Brazil, Colombia, Cuba, Dominican Republic, El Salvador, Guadeloupe, Guatemala, Hawaii, India, Iran, Jamaica, Kenya, Malawi, Martinique, Mauritius, Mexico, Morocco, Mozambique, Nicaragua, Papua New Guinea, Peru, Réunion, Senegal, South Africa, Swaziland, Thailand, Uganda, USA, Venezuela, Zambia, Zimbabwe
Chlorotic streak	unknown	Argentina, Australia, Brazil, Cambodia, China, Colombia, Côte D'Ivoire, Cuba, Dominican Republic, Fiji, Grenada, Guadeloupe, Guyana, Hawaii, Honduras, Indonesia, Jamaica, Madagascar, Mali, Martinique, Mauritius, Mexico, Mozambique, Nicaragua, Pakistan, Panama, Papua New Guinea, Philippines, Puerto Rico, Réunion, St Lucia, Samoa, South Africa, Surinam, Taiwan, Thailand, Trinidad, Turkey, USA, Venezuela

Source of Photographs: MSIRI

VIRUS DISEASES

Sugarcane yellow leaf virus

Sugarcane yellow leaf virus, the causal agent of yellow leaf of sugarcane is present in all major sugarcane producing countries of the world (Lockhart and Cronjé 2000) and is characterized by intense yellowing of the midrib and necrosis of leaves. However, similar symptoms may occur as a result of stress conditions or in the presence of a phytoplasma (Cronjé *et al*. 1998; Aljanabi *et al*. 2001). But it is also known that infected plants can remain symptomless. Using SCYLV specific primers YLS462 and YLS111 (M. Irey, unpublished), Comstock *et al*. (1998) developed a specific RT-PCR assay for sensitive detection of the virus.

When this virus was first described, it proved to be a major obstacle in the international movement of germplasm especially due to its ubiquitous nature and because of the inability of hot water treatment to eliminate this pathogen. Subsequently, tissue culture techniques (Chatenet *et al*. 2001; Parmessur *et al*. 2002) have been successfully applied to clean SCYLV infected sugarcane plants. The RT-PCR test developed by Comstock *et al*. (1998) is extremely important in confirmation of virus elimination, considering that one is dealing with plantlets with very low virus titre.

Improvement over the conventional RT-PCR has been proposed, in the form of a real-time Taqman PCR with 100-fold increase in sensitivity (Korimbocus *et al*. 2002). A major advantage of this protocol is the inclusion of an internal positive control which increases the reliability of the test by eliminating false negatives. Another sensitive SCYLV diagnostic, that detects even up to 100 fg of purified virus, uses molecular beacons for real-time detection of amplified PCR products (Gonçalves *et al*. 2002).

Molecular studies have been useful in studying genetic diversity of SCYLV. Moonan and Mirkov (2002) reported geographical grouping of strains collected from North, South and Central America. Diversity, both between and within strains, collected from various worldwide geographic locations was shown by Borg *et al*. (2001). Abu-Ahmad *et al*. (2006a, 2006b) identified four genotypes of SCYLV of different geographical origins (genotypes BRA from Brazil, PER from Peru, CUB from Cuba and REU from Réunion). Three specific RT-PCR tests have been developed for the genotypes REU, CUB and BRA-PER, the latter clustering groups BRA and PER, due to limitation of the primers to differentiate between them. Variations in infection capacity and virulence have also been reported among the different genotypes (Abu-Ahmad *et al*. 2007). Recently, two new genotypes have been also reported (Viswanathan *et al*. 2008b; Wang and Zhou 2010).

Sugarcane mosaic virus

Sugarcane mosaic virus (SCMV) and *Sorghum mosaic virus* (SrMV), two viruses causing mosaic disease of sugarcane, are known to consist of several strains based on symptoms on differential host plants. The RT-PCR assay of Smith and Van de Velde (1994) allowed sensitive detection of SCMV, but strain differentiation was not possible. Restriction fragment length polymorphism (RFLP) analysis following RT-PCR allowed the discrimination of strains of SCMV and SrMV (Yang and Mirkov 1997). PCR-RFLP has been useful in the study of genetic diversity of viruses producing mosaic symptoms in Louisiana (Grisham and Pan 2007) and Tucumán, Argentina (Perera *et al*. 2009). Using these sets of primers and RFLP analysis, Grisham and Pan (2007) studied the genetic diversity of viruses resulting in mosaic symptoms in Louisiana. New variants of SCMV were reported in India (Viswanathan *et al*. 2009) following use of SCMV coat protein specific primers and sequencing of the amplified products. As a means to simplify the study of new variants, Gomez *et al*. (2009) used a crude method of RNA purification (submerging of leaf in extraction buffer followed by incubation at 95°C). PCR products were then sequenced directly using primers similar to those used in RT-PCR.

Sugarcane streak mosaic virus

The occurrence of new sugarcane diseases, *Sugarcane streak mosaic virus* (SCSMV) for instance, highlights the importance and need for effective detection methods at quarantine level. This disease was originally thought to be a member of the mosaic group based on symptoms, but failed to react with antisera produced against SCMV (Hall *et al*. 1998; Hema *et al*. 1999). Subsequent molecular characterization has established that the virus belongs to a new virus, *Susmovirus* in *Potyviridae* (Viswanathan *et al*. 2008a). Initially confined to the Indo-Pak subcontinent, this virus has since been detected using RT-PCR in germplasm collection in China (Xie *et al*. 2009) and could possibly be more common in Asia than SCMV.

Sugarcane striate mosaic associated virus

Based on the complete sequence and genome organization analysis, *Sugarcane striate mosaic associated virus* (ScSMaV), Thompson and Randles (2001) concluded that it does not belong to any of the common viruses and represent a new taxon. An RT-PCR assay was used to amplify a 820-bp fragment specific to SCSMaV (Choi *et al*. 1999).

Sugarcane streak virus

Three viruses are known to cause streak symptoms in sugarcane; SSV: *Sugarcane streak virus-Natal*, SSMV: *Sugarcane streak Mauritius virus*, and SSEV: *Sugarcane streak Egypt virus* (Bigarré *et al*. 1999). For the latter, a PCR-ELISA protocol combining the specificity of molecular tools and ease of colourimetric methods was shown to be 10-100 fold more sensitive than electrophoretic determination of PCR products (Shamloul *et al*. 2001). Recently, a non-PCR based amplification strategy, using phi29 DNA polymerase was developed (Owor *et al*. 2007). This technique was useful for the study of diversity of African streak viruses (Shepherd *et al*. 2008; Varsani *et al*. 2008).

Sugarcane Fiji disease virus

The Fiji leaf gall disease caused by *Sugarcane Fiji disease virus* (SCFDV) is an important quarantine disease in many sugarcane growing areas of the world. Detection based on symptoms is time-consuming and difficult in some cases. Lab-based techniques, both serological and molecular, have been devised for the sensitive detection of SCFDV. cDNA probes were proposed as an improvement in sensitivity over ELISA (Skotnicki *et al*. 1986; Smith *et al*. 1994). Detection sensitivity using the northern blot was up to 0.5 pg of virus (Smith *et al*. 1994). The SCFDV has a double stranded RNA genome and Smith *et al*. (1992) utilized a primer/template boil-quench strategy for specific amplification of a fragment of the virus using RT-PCR. A sensitivity level of 100 ag was observed, being 10^4-fold more sensitive than detection using probes. New primer pairs for SCFDV were designed from various regions of the segmented region of the virus genome and RT-PCR protocol was optimized by Smith and Van de Velde (1994). These authors reported enhanced sensitivity by performing hybridization with biotinylated probes after the RT-PCR assay. The virus was detected in a $1{:}10^{-7}$ dilution nucleic acids extracted from 250 mg of infected leaves.

Sugarcane bacilliform virus

A *Sugarcane bacilliform virus* (SCBV) specific primer pair SCBVF5/R5, amplifying a 221-bp product from the virus, was utilized and showed widespread occurrence of the virus in *Saccharum* and related germplasm (Braithwaite *et al*. 1995).

BACTERIAL DISEASES

Implementation of accurate diagnostic techniques is essential in the control of sugarcane bacterial diseases on several fronts; use of clean planting material, quarantine diagnostics and evaluation of varietal resistance. Ratoon stunt (caused by *Leifsonia xyli* subsp. *xyli*) and leaf scald (caused by *Xanthomonas albilineans*) are two important bacterial diseases of sugarcane. Gumming disease caused by *Xanthomonas axonopodis* pv *vasculorum* is also important in some parts of the world. Due to limitations in traditional detection methods, DNA-based methods have been increasingly proposed as more effective alternatives.

Ratoon stunt

Sensitive PCR protocols based on the ITS (internal transcribed spacer) region of the 16S-23S rDNA have been developed for the detection of *Leifsonia xyli* subsp. *xyli*- *Lxx* (Fegan *et al*. 1998; Pan *et al*. 1998). These assays permitted amplification of the *Lxx* specific fragment directly from infected sugarcane vascular sap through the inclusion of PCR additives such as polyvinylpyrrolidone (PVP) and ficoll, which limited the negative effects of PCR inhibitors. Taylor *et al*. (2003) developed *Lxx* specific primers based on amplification products of RAPD primers OPC2 and OPC11. Since these PCR assays use vascular sap, the sampling technique utilized is extremely important, and limitations include number of stools to be sampled as well as time of sampling during the season. Even though infection due to *Lxx* is systemic in nature, the application of PCR techniques using leaf tissues were not as reliable as expected, most probably due to the low titre and occurrence of PCR inhibitors. Recently, Grisham *et al*. (2007) proposed a real-time PCR for the detection of *Lxx* in sugarcane leaf tissue at all stages of growth. It was shown that at older stages of plant growth, conventional PCR is less effective than real-time PCR for the detection of *Lxx*, due to increased levels of inhibitors. One of the advantages of using real-time PCR is the possibility of quantifying infection level occurring in tissues at a given time. The proposed real-time assay for *Lxx* compared favourably with tissue blot immunoassay in ranking cultivar susceptibility to the disease, even though sampling was performed at an earlier stage of plant growth; 3-4 months for real-time assay as compared to 7-10 months for tissue blot assay.

Xanthomonas albilineans

Pan *et al*. (1997) designed primer pair Ala4/L1 which amplified a 360 bp fragment of the 16S/23S ITS region from *Xanthomonas albilineans* (*Xa*) worldwide isolates (including serovars I, II and III). However, the concurrent amplification of non-specific fragments (280, 420, and 460 bp) from three unidentified saprophytic bacteria was a major problem and visualization of PCR products required complicated modifications at electrophoresis level or even by Southern hybridization. An improved primer pair from the same region was further designed by Pan *et al*. (1999) resulting in a sensitive assay (up to 1 pg of *Xa* genomic DNA) targeting a specific 288 bp fragment from different strains.

An unknown fragment from *Xa* type A isolate was cloned and sequenced and information thus obtained was used to design *Xa* specific primers, XAF1/XAR1 (Wang *et al*. 1999). Conventional PCR with these primers and vascular sap as template had a detection limit of 2×10^4 CFU/ml, whilst Bio-PCR (pre-enrichment of bacteria on artificial media followed by PCR) and isolation on semi-selective medium were 100x more sensitive for detection of *Xa*.

Using subtractive hybridization of common sequences between *X. albilineans* (serovar I) and *Xanthomonas axonopodis* pv. *vasculorum*, Jaufeerally-Fakim *et al*. (2000) cloned a fragment from an unspecified region of and eventually designed primers S83A/S83B. These produced a 300 bp fragment from genomic DNA of 38 isolates of *Xa* but not

from other related isolates.

Gumming disease

The gumming disease bacterium (*Xanthomonas axonopodis* pv. *vasculorum*) is of economic importance in Mauritius and Réunion Island. Diversity among strains has been proved using biochemical and physiological properties, serology as well as fatty acids and lipopolysacchararide profiles (Dookun 1993; Saumtally 1996). DNA-based assays, including RAPD (Random amplification of polymorphic DNA) primers and ERIC-PCR (enterobacterial repetitive intergenic consensus primers) have also been useful in classification of strains (Saumtally 1996).

DISEASES CAUSED BY PHYTOPLASMAS

Phytoplasmas cannot be cultured *in vitro* and molecular tools, especially notably nested PCR are widely used for their detection. The known phytoplasma diseases infecting sugarcane include Sugarcane white leaf (SCWL), Sugarcane grassy shoot (SCGS) and sugarcane yellows phytoplasma (SCYP) (Cronjé *et al*. 1998; Aljanabi *et al*. 2001; Arocha *et al*. 2005). Nested PCR based on the 16S rRNA gene followed by RFLP is a common method for identification and classification of phytoplasmas (Lee *et al*. 1993; Schneider *et al*. 1993). A universal Taqman based assay, developed by Hodgetts *et al*. (2009), was similar to nested PCR in terms of sensitivity for the detection of 29 phytoplasma isolates, including all known 16S r RNA groups.

FUNGAL DISEASES

Smut

Prompt diagnosis of sugarcane fungal diseases is essential for implementation of disease management practices. A sensitive PCR assay was devised to detect *Sporisorium scitamineum* (syn. *Ustilago scitaminea*) DNA in smut-infected sugarcane plants (Albert and Schenck 1996). Using primers for the *U. maidis* mating-type gene, these authors amplified the gene from *S. scitamineum*. Subsequently, primer pair bE4/bE8 was designed to amplify specifically a 459 bp product from the (+) mating type of the smut pathogen. This technique was proven to be more sensitive and rapid than microscopy in detecting the pathogen from tissue culture plantlets (Albert and Schenck 1996; Singh *et al*. 2004). Whilst, this method proved to be effective in quarantine and diagnostic, it was not possible to assess the degree of smut resistance under natural infection.

Brown rust and orange rust

Molecular methods are extremely useful for accurate identification of rust diseases of sugarcane, namely brown rust (*Puccinia melanocephala*) and orange rust (*Puccinia kuehnii*). Although these two diseases are not transmitted by cuttings, the latter, especially, is of quarantine importance in many parts of the world. Orange rust, originally believed to be confined to Asia and Australia (Ryan and Egan 1989), was recently reported in Florida, USA (Comstock *et al*. 2008), Central and Latin America (Ovalle *et al*. 2008; Chavarria *et al*. 2009; Flores *et al*. 2009; Barbasso *et al*. 2010) and in Africa (Saumtally *et al*. 2011). Sequence diversity in the ribosomal DNA region is commonly used for development of detection methods for fungal pathogens. Based on partial ITS1, ITS2 and complete 5.8S sequences of worldwide isolates of *P. melanocephala* and *P. kuehnii*, Glynn *et al*. (2010a) developed specific primers for use in conventional PCR and real-time PCR allowing sensitive detection of both pathogens (up to 0.19 pg). For *P. kuehnii*, sequence analysis of the ITS1 region revealed a SNP – single nucleotide polymorphism (A substituted for G) and a primer-introduced restriction analysis-PCR (PIRA-PCR) strategy was devised with nested primers. Whilst the 183-A allele was observed in all samples, the 183-G allele was specific to samples from Asia and Australia.

Red rot

Red rot of sugarcane (*Collelotrichum falcatum*) is an important fungal disease of sugarcane in the Indian subcontinent resulting in yield losses (Natarajan *et al*. 1998; Hussnain and Afghan 2006). Breeding for resistance against the pathogen remains the major management strategy. However, traditional methods of identification of resistant cultivars are time and labour consuming. Molecular markers provide an attractive alternative. PCR-RFLP analysis of the ITS ribosomal DNA revealed the occurrence of two groups from a collection of six isolates from Andhra Pradesh, Orissa and Karnataka in India (Mishra and Behera 2009). RAPD grouping of *C. falcatum* isolates has been shown to correlate with pathogenicity on host cultivars (Mohanraj *et al.* 2002). Kumar *et al*. (2010) studied a total of 25 *C. falcatum* isolates from northeastern states of India using three marker types; RAPD, inter simple sequence repeats – ISSRs and universal rice primers – URPs (Kang *et al*. 2002). URPs are repeat sequences from Korean weedy rice which are used for fingerprinting plants, animal and microbial genomes (Kang *et al*. 2002). In molecular analysis and phylogenetic analysis of 580 bp region of 5.8s rDNA-ITS genome of nine major pathotypes used for screening red rot resistance in India clearly showed divergence of two groups in *C. falcatum*. Vegetative compatibility grouping (VCG) test was standardized based on successful heterokaryon formation among *nit* mutants. These mutants were grouped into five major groups based on their compatibility and this approach was able to distinguish *C. falcatum* from other species and also it recognizes different isolates of the same pathotype. Although pathogenicity, VCG and ITS studies have slight variation in grouping the pathotypes, all the results distinguished two major groups with the incompatibility of Cf1148 and Cf7717 which is congruent with those groups established earlier on RAPD, differentials pathogenicity and serological approach (Malathi *et al*. 2010).

DEVELOPMENT AND APPLICATION OF MOLECULAR MARKERS FOR DISEASE RESISTANCE

The most efficient way to control diseases in sugarcane is through conventional breeding and use of resistant cultivars. However, conventional breeding faces a number of difficulties in producing superior cultivars with pathogen resistance. Traditionally, resistance to diseases is evaluated in the field by exposing test varieties to the pathogen and assessing their reaction. Such trials are often influenced by a number of factors such as low or high disease pressure, physiological age, plant architecture, climatic conditions, soil factors and growth condition of the plant. The numerous factors pose difficulties in accurately assessing the behaviour of a genotype to pathogens. Although a number of methods have been described for bacterial and fungal testing (Saumtally *et al*. 2000), evaluation to viruses and phytoplasmas, which involves insect vectors remains to be difficult to assess.

DNA markers associated with disease resistant traits can now provide valuable information in the breeding and selection of sugarcane. Progress in genetic mapping of sugarcane has identified quantitative trait loci for a number of disease resistance traits namely brown rust, smut, and yellow spot (*Mycovellosiella koepkei*). The first major gene for sugarcane to be mapped was that for brown rust resistance (*Bru1* gene) in a segregating population from a selfed cultivar R 570 which revealed a 3:1 segregation ratio indicating a monogenic gene (Daugrois *et al*. 1996). This work has led to evaluate the potential of map-based cloning in sugarcane (Tomkins *et al*. 1999) as well as fine mapping of the rust resistance gene (Asnaghi *et al*. 2000). Later, Raboin *et al*. (2006) identified another potential major gene for rust resistance in cultivar MQ 76-53. This gene differs from

Bru1 gene and belongs to a different haplotype. PCR-based markers to identify sugarcane *Bru1* resistance are available (D'Hont, pers comm.) and can be used to screen for resistant cultivars carrying this gene.

In 2007, Aljanabi *et al*. (2007) identified markers linked to QTLs for yellow spot disease resistance gene. A major QTL was found linked at 14 cM to an AFLP marker on the M 134/75 genetic map constructed using 557 single-dose polymorphic markers. Sorghum EST-markers are being exploited to further saturate the region with the major QTL (Parmessur *et al*. 2010). For smut, quantitative trait locus mapping in a population from a cross involving cultivar R 570 x MQ 76/53, generated many markers with little effect, showing a complex determinism for smut resistance (Raboin *et al*. 2003).

Microsatellite markers associated with SCYLV resistance in progeny of a cross of Green German (susceptible) by IND 81-146 (resistant) has been reported (Comstock *et al*. 2004). Furthermore the authors predicted multiple gene mechanisms involved in SCYLV resistance.

Expressed sequence tags (ESTs) resources developed through the Brazilian Sugarcane EST (SUCEST) project have also been useful in the identification of resistance gene analogs-RGAs (Rossi *et al*. 2003). The authors identified 88 RGAs with sequence similarity of the three major typical groups of resistance genes; nucleotide-binding sites – leucine-rich repeat (NBS-LRR), LRR and serine-threonine kinase (S/T kinase) domains. A number of the RGAs were successfully mapped on the sugarcane reference genetic map developed by Grivet *et al*. (1996) and Hoarau *et al*. (2001) with the objective to investigate the genomic distribution of the RGAs with respect to disease resistance loci located in sugarcane. In addition to this work, You-Xiong *et al*. (2010) reported the cloning of six NBS-LRR type RGAs from cDNA of sugarcane variety NCo376. One of the RGAs expression profile was found to be influenced by smut infection to some extent.

RECENT ADVANCES IN SUGARCANE GENOMICS

In 2003, sugarcane DNA sequence information became available through the release of some 238,000 expressed sequence tags (ESTs) of sugarcane assembled under the Sugarcane EST project (SUCEST) (http://sucest.lad.ic.unicamp.br.en) (Vettore *et al*. 2003). This work highly complemented a small collection ESTs produced by South African and Australian researchers (Carson and Botha 2000; Casu *et al*. 2001; Carson and Botha 2002; Carson *et al*. 2002). EST sequencing has significantly contributed to gene discovery and expression studies to associate functions to sugarcane genes (Menossi *et al*. 2008). Through the transcriptome information genes involved in biotic and abiotic stress response, disease resistance and sucrose accumulation have been identified (Nishiyama *et al*. 2010). Mining of the sugarcane ESTs from the SUCEST project by Kuramae *et al*. (2002) managed to identify EST clusters similar to plant-signaling molecules including pathogenicity related proteins.

With part sequences of the sugarcane genome complemented with the genome sequences of pathogens, candidate genes with potential applications in sugarcane protection should be available soon. The complete genomes of the two sugarcane bacteria, namely *Leifsonia xyli* subsp. *xyli* and *Xanthomonas albilineans* have been sequenced and the sequences are useful information for identification of pathogenicity genes (Monteiro-Vitorello *et al*. 2004; Pieretti *et al*. 2009). The genome of *Xa* has been found not to possess *hrp* genes that encode a type III secretion system, usually found in most gram-negative plant pathogenic bacteria (Pierreti *et al*. 2009).

As for the gram-positive coryneform bacterium *Leifsonia xyli* subsp. *xyli*, the sequence revealed a 2584158 bp genome in length with a high content of G and C bases (Monteiro-Vitorello *et al*. 2004) and 307 pseudogenes; if functional would likely be associated in the degradation of plant heterosaccharides (Pierreti *et al*. 2009). It is also known that limited number of pathogenicity genes is present in *Lxx*, and this could explain the discrete symptoms of the disease even at high bacterial titre (Monteiro-Vitorello *et al*. 2004). Furthermore the desaturase pathogenicity gene identified is likely involved in the synthesis of abscisic acid, a hormone that arrests growth and hence responsible for the stunting appearance of infected plants with *Lxx* (Pierreti *et al*. 2009). Thus the sequence of *Lxx* has allowed a number of questions to be answered on the biology of the pathogen and this information are of immediate and future value for sugarcane breeding programmes aiming at developing pathogen resistant cultivars.

GENETIC TRANSFORMATION OF SUGARCANE TO INCREASE DISEASE RESISTANCE

Diseases represent a major constraint to high yield and sugar productivity and therefore the release of resistant or tolerant sugarcane varieties is a very important aspect of sugarcane breeding. Although traditional breeding methods have so far proved effective in controlling major sugarcane diseases, they are however time consuming. Consequently, biotechnological applications have a major role to play to speed up development of disease resistant varieties.

Genetic transformation of sugarcane together with tissue culture techniques has allowed the development of transgenic sugarcane. This technique, which in contrast to conventional breeding allows the combination of thousands of genes, provides a mean to transfer a specific gene which can be isolated from either a close or distantly related species. Although in 2009 some 134 million ha were exploited worldwide with transgenic cotton, maize or soybeans, so far there is no commercially grown transgenic sugarcane (James 2009).

Following the successful production of the first transgenic sugarcane plant in Australia expressing an antibiotic resistance (Bower and Birch 1992) a number of genes have been introduced in sugarcane. The first transgenic cane, for bacterial resistance, was developed by Birch (Zhang and Birch 1997), where a method to reduce or inhibit development of leaf scald in sugarcane stalk was reported. The pathogen *Xanthomonas albilineans* is a systemic, xylem invading bacterium and losses to the disease have been reported in a number of countries, but particularly in Australia, Guyana, Mauritius and South Africa (Ricaud and Ryan 1989). The bacterium produces a number of phytotoxins and albicidin being the major one that blocks DNA replication and plastid development, and hence promotes chlorosis in disease systemic infection (Zhang and Birch 1997; Birch 2001). Method to detoxify albicidin in susceptible sugarcane cultivars has been realized by the introduction and expression of the *albD* gene, isolated from another bacterium *Pantoea dispersa*, into the plant (Zhang and Birch 1997). Thereafter, little work has been done for bacterial resistance improvement in sugarcane via transgenesis, except for *Lxx* causing ratoon stunt (Avellaneda *et al*. 2008).

More attention has been given in developing transgenic canes against viral pathogens. Sugarcane lines transformed for resistance to SCMV have been reported and the same was evaluated in the field. SCMV is one of the most important viral pathogens of sugarcane worldwide. SCMV disease is caused by different strains of the virus and distinct potyviruses have been reported (Hardley *et al*. 2001). It comprises of a collection of four or five different potyviruses that include strains of SCMV; A, B, D and E, *Sorghum mosaic virus* (SrMV), strains SCH, SCI and SCM, *Johnson grass mosaic virus* and *Maize dwarf mosaic virus*. In the 1920s, a worldwide epidemic of the disease severely affected major sugar industries in Argentina, Brazil and Louisiana (Ingelbrecht *et al*. 1999). Although conventional breeding has been able to produce tolerant varieties, breakdown of resistance has been another problem as new strains appear. In 2005, the Florida sugarcane industry estimated a potential damage by SCMV to a tune of 1 billion US dollars (Gilbert *et al*. 2005). In order to increase SCMV

resistance in susceptible cultivars, recourse to genetic transformation has recently been investigated. A number of authors have reported progress towards the development of mosaic resistant sugarcane cultivars using the gene transfer technology. Joyce *at al*. (1998) produced SCMV resistant lines following microprojectile bombardment with the virus coat protein gene. Ingelbrecht *et al*. (1999) produced transgenic sugarcane resistant to SrMV-SCH strain after expressing an untranslatable region for of the coat protein (CP) gene of SrMV-SCH by inducing post-transcriptional gene silencing (PTGS). Sooknandan *et al*. (2003) also reported successful production of SCMV resistant sugarcane via transgenesis, again by induction of PTGS. Furthermore, transgenic sugarcane lines resistant to SCMV, strain E have been produced by Gilbert *et al*. (2005). From this study one hundred independent resistant lines have been evaluated in the field and a large variability was noted in both yield and disease resistance. However, the study allowed the identification of a number of transgenic lines with improved SCMV resistance when compared to commercial controls.

Another viral disease of sugarcane that has retained much attention in recent years is yellow leaf. SCYLV can cause yield reduction in susceptible cultivars in the range of 10 to 40% (Vega *et al*. 1997; Lockhart and Cronjé 2000; Rassaby *et al*. 2003; Lehrer *et al*. 2010). Resistance level varies from country to country. In Hawaii, resistance to SCYLV exists in several varieties (Schenck 2001), while in Mauritius 18 out of 20 commercial varieties checked for the presence of the virus were positive (Khoodoo *et al*. 2010) and only one variety was found free from the virus. But in general inadequate sources of SCYLV resistance limit the traditional breeding for resistance to the disease (Glynn *et al*. 2010b) hence once again genetic transformation technology is an appropriate approach to increase resistance. Resistance to the virus has been successful through coat-protein gene-based silencing and resistance levels were increased in transgenic lines compared to untransformed plants (Zhu *et al*. 2007). Inheritance of the transgenes in conventional breeding has been evaluated by using two transgenic SCYLV lines as parents in twelve crosses (Glynn 2010). The study demonstrated that 50% of the progeny inherited the SCYLV resistant transgene, thus showing the potential use of the transgenic resistant lines in conventional breeding.

Fiji leaf gall virus is another important disease of sugarcane that has caused devastating epidemics in Queensland, Australia. A plant hopper is the vector responsible for the transmission of the virus which belongs to the *Reoviridae* family and is a ds RNA virus. Much effort has been devoted by the Queensland sugar industry to develop resistant cultivars, which has been successful. However during the implementation of such programme, 80% of the germplasm was rejected for use in the breeding programme due to the virus susceptibility (Smith and Harding 2001). Cultivar Q 124, with average resistance to Fiji leaf gall virus was genetically transformed with a transgene encoding the translatable version of the segment 9 ORF 1 under the control of the *Ubi* promoter (McQualter *et al*. 2004). After glasshouse trials of various lines tested, one transgenic line with significantly enhanced resistance to the disease was obtained.

CLEAN SEED PRODUCTION

One of the methods to manage dissemination of seed-borne systemic sugarcane pathogens is through the provision of clean planting material from nurseries. Eradication of some sugarcane pathogens is possible through the application of tissue culture techniques. Tissue culture was first applied to sugarcane in the late 1960s and henceforth has been used to eliminate systemic pathogens. Viral diseases namely sugarcane mosaic, Fiji leaf gall, and yellow leaf have successfully been eliminated through meristem tip, axillary bud culture and callus culture (Leu 1972; Wagih *et al*. 1995; Chatenet *et al*. 2001; Fitch *et al*. 2001; Parmessur *et al*. 2002).

The elimination of sugarcane yellows phytoplasma (SCYP) causing leaf yellows disease has also been successful after regenerating plantlets from callus derived from young leaf rolls (Parmessur *et al*. 2002). After a number of subcultures of callus cells, Parmessur *et al*. (2002) managed to eliminate both SCYLV and SCYP from infected material. As these techniques are being developed and adopted, demand for exchange of germplasm in the form of tissue culture plants amongst sugarcane research institutions is becoming important in the safe transfer of planting material from one country to another.

CONCLUSION

Sugarcane is a highly productive plant and in recent years its interest as a biofuel source has attracted additional attention. Many countries in Asia, South America and Africa are expanding their sugarcane cultivation areas. As new varieties are released and grown in new areas, together with climate change, vulnerability to biotic stresses, it is expected that new problems might arise including new diseases and strains of pathogens. Proper disease management supported with an array of biotechnological progress, the understanding of genomes of the pathogens affecting sugarcane as well as the sugarcane genome and its related Graminaceous crops such as sorghum and rice will allow a more efficient production system. The integration of biotechnological tools in the classical sugarcane improvement programme will no doubt improve the quality of varieties and tailor-made varieties could in the long term be possible with the progress made in genomics, understanding the sugarcane genome as genetic transformation technology. In the next decade it is expected to see major advances in the diagnosis of sugarcane pathogens diagnostics as more sequence information would be made available. The release of transgenic sugarcane improved for disease tolerance is also very likely to take place in the next decade as progress continues along this field.

REFERENCES

Abu Ahmad Y, Rassaby L, Royer M, Borg Z, Braithwaite KS, Mirkov TE, Irey MS, Perrier X, Smith GR, Rott P (2006a) Yellow leaf of sugarcane is caused by at least three different genotypes of sugarcane yellow leaf virus, one of which predominates on the Island of Réunion. *Archives of Virology* **151**, 1355-1371

Abu Ahmad Y, Royer M, Daugrois JH, Costet L, Lett JM, Victoria JI, Girard JC, Rott P (2006b) Geographical distribution of four *Sugarcane yellow leaf virus* genotypes. *Plant Disease* **90**, 1156-1160

Abu Ahmad Y, Costet L, Daugrois JH, Nibouche S, Letourmy P, Girard JC, Rott P (2007) Variation in infection capacity and in virulence exists between genotypes of *Sugarcane yellow leaf virus*. *Plant Disease* **91**, 253-259

Albert HH, Schenck S (1996) PCR amplification from a homolog of the bE mating-type gene as a sensitive assay for the presence of *Ustilago scitaminea* DNA. *Plant Disease* **80**, 1189-1192

Aljanabi S, Parmessur Y, Kross H, Dhayan S, Saumtally S, Ramdoyal K, Autrey LJC, Dookun-Saumtally A (2007) Identification of a major quantitative trait locus (QTL) for yellow spot (*Mycovellosiella koepkei*) disease resistance in sugarcane. *Molecular Breeding* **19**, 1-14

Aljanabi SM, Parmessur Y, Moutia Y, Saumtally S, Dookun A (2001) Further evidence of the association of a phytoplasma and a virus with yellow leaf syndrome in sugar cane. *Plant Pathology* **50**, 628-636

Arocha Y, Lopez M, Fernández M, Pinol B, Horta D, Peralta EL, Almeida R, Carvajal O, Picornell S, Wilson MR, Jones P (2005) Transmission of a sugarcane yellow leaf phytoplasma by the delphacid planthopper *Saccharisynde saccharivora*, a new vector of sugarcane yellow leaf syndrome. *Plant Pathology* **54**, 634-642

Asnaghi C, Paulet F, Kaye C, Grivet L, Deu M, Glaszmann JC, D'Hont A (2000) Application of synteny across Poaceae to determine the map location of sugarcane rust resistance gene. *Theoretical and Applied Genetics* **101**, 962-969

Avellaneda MC, Mirkov ET, Victoria JI (2008) Transgenic resistance to ratoon stuting and leaf scald diseases of sugarcane variety CC 85-92 using the 1SD gene. *ISSCT IX Plant Pathology and VI Molecular Biology Workshop*, 23-27 June 2008, Cali Colombia, p 6 (Abstract)

Bailey RA, Comstock JC, Croft BJ, Saumtally AS, Rott P (2000) Procedures for the safe movement of sugarcane germplasm. In: Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally AS (Eds) *A Guide to Sugarcane Diseases*, CIRAD, France, pp 311-316

Barbasso D, Jordão H, Maccheroni W, Boldini J, Bressiani J, Sanguino A (2010) First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in Brazil. *Plant Disease* **94 (9)**, 1170

Bigarré L, Salah M, Granier M, Frutos R, Thouvenel J-C, Peterschmitt M (1999) Nucleotide sequence evidence for three distinct sugarcane streak mastreviruses. *Archives of Virology* **144**, 2331-2344

Birch RG (2001) *Xanthomonas albilineans* and the antipathogenesis approach to disease control. *Molecular Plant Pathology* **2**, 1-11

Birch RG, Bower R, Elliott A, Hansom S, Basnayake S, Zhang L (2000) Regulation of transgene expression: Progress towards practical development in sugarcane, and implications for other plant species. In: Arencibia AD (Ed) *Plant Genetic Engineering: Towards the Third Millenium*, Elsevier, Amsterdam, pp 118-125

Borg Z, Moonan F, Braithwaite K, Mirkov TE, Smith G (2001) Characterising the genetic diversity of *Sugarcane yellow leaf virus*. *Proceedings of Internal Society of Sugarcane Technologists* **24**, 654-656

Bower R, Birch RG (1992) Transgenic sugarcane plants via microprojectile bombardment. *Plant Journal* **2**, 409-416

Braithwaite KS, Egeskov NM, Smith GR (1995) Detection of sugarcane bacilliform virus using the polymerase chain reaction. *Plant Disease* **79**, 792-796

Carson DL, Botha FC (2000) Preliminary analysis of expressed sequence tags for sugarcane. *Crop Science* **40**, 1769-1779

Carson DL, Botha FC (2002) Genes expressed in sugarcane maturing internodal tissue. *Plant Cell Reporter* **20**, 1075-1081

Carson DL, Huckett BI, Botha FC (2002) Sugarcane ESTs differentially expressed in immature and maturing internodal tissue. *Plant Science* **162**, 289-300

Casu R, Dimmock C, Thomas M, Bower N, Knight D, Grof C, McIntyre L, Jackson P, Jordan, D, Whan V, Drenth J, Toa Y, Manners J (2001) Genetic and expression profiling in sugarcane. *Proceedings of the International Society of Sugar Cane Technologists* **24**, 542-546

Chavarría E, Subirós F, Vega J, Ralda G, Glynn NC, Comstock JC, Castlebury LA (2009) First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Costa Rica and Nicaragua. *Plant Disease* **93 (4)**, 425

Chatenet M, Delage C, Ripolles M, Irey M, Lockhart B, Rott P (2001) Detection of *Sugarcane yellow leaf virus* in quarantine and production of virus-free sugarcane by apical meristem culture. *Plant Disease* **85**, 1177-1180

Chatenet M, Mazarin C, Girard JC, Fernandez E, Gargani D, Rao GP, Royer M, Rott P (2005) Detection of *Sugarcane streak mosaic virus* in sugarcane from several Asian countries. *Proceedings of the International Society of Sugarcane Technologists Congress, Guatemala City, Guatemala* **XXV**, 656-663

Cheavegatti-Gianotto A, Couto de Abreu HM, Arruda P, Bespalhok Filho JC, Burnquist WL, Creste S, Cierco L di, Ferro JA, Figueira AV de O, Filfueiras T de S, Grossi-de-sá M de F, Guzzo EC, Hoffmann HP, Landell MG de A, Macedo N, Matsuoka S, Reinach F de C, Romano E, da Silva WJ, Filha M de CS, Ulian EC (2011) Sugarcane (*Saccharum X officinarum*): A reference study for the regulations of genetically modified cultivars in Brazil. *Tropical Plant Biology* **4**, 62-89

Choi YG, Croft BJ, Randles JW (1999) Identification of *Sugarcane striate mosaic-associated virus* by partial characterization of its double-stranded RNA. *Phytopathology* **89 (10)**, 877-883

Comstock JC, Chapparro JX, Tai PYP, Edme S, McCorkle K, Miller JD (2004) The association of microsatellite markers with resistance to *Sugarcane yellow leaf virus*. *Plant and Animal Genomes XII conference*, Jan 10-14, 2004, Town and Country Convention Centre, San Diego, CA, International Consortium for Sugarcane Biotechnology Workshop, **W126**

Comstock JC, Irey MS, Lockhart BEL, Wang ZK (1998) Incidence of yellow leaf syndrome in CP cultivars based on polymerase chain reaction and serological techniques. *Sugar Cane* **4**, 21-24

Comstock JC, Sood SG, Glynn NC, Shine JM, McKemy JM, Castlebury LA (2008) First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in the United States and Western Hemisphere. *Plant Disease* **92 (1)**, 175

Cronjé CPR, Tymon AM, Jones P, Bailey RA (1998) Association of a phytoplasma with a yellow leaf syndrome in sugarcane in Africa. *Annals of Applied Biology* **133**, 177-186

Daugrois JH, Grivet L, Roques, D, Hoarau JY, Lombard H, Glaszmann JC, D'Hont A (1996) A putative major gene for rust resistance linked with a RFLP marker in sugarcane cultivar 'R570'. *Theoretical and Applied Genetics* **92**, 1059-1064

D'Hont A, Souza GM, Menossi M, Vincentz M, Van-Sluys MA, Glaszmann JC, Ulian E (2008) Sugarcane: A major source of sweetness, alcohol, and bio-energy. In: Moore PH, Ming R (Eds) *Genomics of Tropical Crop Plants*, Springer, New York, pp 483-513

Dookun A (1993) The production, characterization and use of monoclonal antibodies for race differentiation of *Xanthomonas campestris* pv *vasculorum*, causal agent of gumming disease of sugar cane. PhD thesis, University of Reading, UK, 212 pp

Dookun-Saumtally A, Saumtally S, Parmessur Y, Joomun N, Aljanabi S (2008) Phytoplamas infecting sugarcane. In: Harrison NA, Roa GP, Marcone C (Eds) *Characterization, Diagnosis and Management of Phytoplasmas*, Studium Press LLC, USA, pp 275-291

Fegan M, Croft BJ, Teakle DS, Hayward AC, Smith GR (1998) Sensitive and specific detection of *Clavibacter xyli* subsp. *xyli*, causal agent of ratoon stunting disease with a polymerase chain reaction-based assay. *Plant Pathology* **47**, 495-504

Fitch MMM, Lehrer AT, Komor E, Moore PH (2001) Elimination of *Sugarcane yellow leaf virus* from infected sugarcane plants by meristem tip culture visualized by tissue blot immunoassay. *Plant Pathology* **50**, 676-680

Flores RC, Loyo JR, Ojeda RA, Rangel OCA, Cerón FA, Márquez W, Guerra-Moreno AS, Hernandez-Ibarra HM, González RE, Castlebury LA, Dixon LJ, Glynn NC, Comstock JC, Flynn J, Amador J (2009) First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Mexico, El Salvador, and Panama. *Plant Disease* **93 (12)**, 1347

Frison EA, Putter CAJ (1993) *FAO/IBPGR Technical Guidelines for the Safe Movement of Sugarcane Germplasm*. Food and Agriculture Organization of the United Nations, Rome/International Board for the Plant Genetic Resources, Rome, 44 pp

Girard JC, Guinet I, Roumagnac P, Fernandez E, Rott P (2011) Visacane, an innovative quarantine tool for the exchange of pest and disease-free sugarcane germplasm. 10th ISSCT Sugarcane Breeding and Germplasm and 7th ISSCT Molecular Biology Workshop: Breaking breeding and biotechnology paradigms – towards a complementary approach in sugarcane research, May 15-20, 2011, Maceio, Brazil, p 29 (Abstract)

Glynn NC, Dixon LJ, Castlebury LA, SzaboLJ, Comstock JC (2010a) PCR assays for the sugarcane rust pathogens *Puccinia kuehnii* and *P. melanocephala* and detection of a SNP associated with geographical distribution in *P. kuehnii*. *Plant Pathology* **59**, 703-711

Glynn NC, Gilbert RA, Comstock JC (2010b) Transmission of a *Sugarcane yellow leaf virus* resistance transgene to sexual progeny and screening by kanamycin inoculation. *Field Crops Research* **119**, 308-313

Gómez M, Rago AM, Serino G (2009) Rapid identification of viruses causing sugarcane mosaic by direct sequencing of RT-PCR products from crude extracts: A method for large scale virus surveys. *Journal of Virological Methods* **157 (2)**, 188-194

Gonçalves MC, Klerks MM, Verbeek M, Vega J, Van den Heuvel JFJM (2002) The use of molecular beacons combined with NASBA for the sensitive detection of *Sugarcane yellow leaf virus*. *European Journal of Plant Pathology* **108**, 401-407

Grisham MP, Pan YB (2007) A genetic shift in the virus strains that cause mosaic in Louisiana sugarcane. *Plant Disease* **91**, 453-458

Grisham MP, Pan YB, Richard EP (2007) Early detection of *Leifsonia xyli* subsp. *xyli* in sugarcane leaves by Real-Time Polymerase Chain Reaction. *Plant Disease* **91 (4)**, 430-434

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in cultivated sugarcane (*Saccharum* spp.): Genome organization in a highly polyploidy and aneuploid interspecific hybrid. *Genetics* **142**, 987-1000

Hall J, Adams B, Parsons T, French R, Lane L, Jensen SG (1998) Molecular cloning, sequencing, and phylogenetic relationships of a new potyvirus: *Sugarcane streak mosaic virus*, and a reevaluation of the classification of the Potyviridae. *Molecular Phylogenetics and Evolution* **10 (3)**, 323-332

Handley JA, Smith GR, Harding RM (2001) Current concepts of the taxonomy and variation of sugarcane infecting potyviruses. In: Rao GP, Ford RE, Tosic M, Teakle DS (Eds) *Sugarcane Pathology, Volume II: Virus and Phytoplasma Diseases*, Science Publishers Inc., Enfield, USA, pp 75-81

Hema M, Joseph J, Gopinath K, Sreenivasulu P, Savithri H (1999) Molecular characterization and interviral relationships of a flexuous filamentous virus causing mosaic disease of sugarcane (*Saccharum officinarum* L.) in India. *Archives of Virology* **144 (3)**, 479-490

Hoarau JY, Offman B, D'Hont A, Risterucci AM, Roques D, Glaszman JC, Grivet L (2001) Genetic dissection of a modern sugarcane cultivar (*Saccharum* spp.) I. Genome mapping with AFLP markers. *Theoretical and Applied Genetics* **103**, 84-97

Hodgetts J, Boonham N, Mumford R, Dickinson M (2009) Panel of 23S rRNA gene-based real-time PCR assays for improved universal and group-specific detection of phytoplasma. *Applied and Environmental Microbiology* **75 (9)**, 2945-2950

Hussnain Z, Afghan S (2006) Impact of major cane diseases on sugarcane yield and sugar recovery. *Annual Report*, Shakarganj Sugar Research Institute, Jhang, Pakistan

James C (2009) Global status of commercialized/Biotech GM crops: 2009. *ISAAA Brief* **41**, ISAAA, Ithaca, New York, 290 pp

Jaufeerally-Fakim Y, Autrey JC, Daniels MJ, Dookun A (2000) Genetic polymorphism in *Xanthomonas albilineans* strains originating from 11 geographical locations, revealed by two DNA probes. *Letters of Applied Microbiology* **30**, 287-293

Joyce PA, McQualter RB, Bernard MJ, Smith GR (1998) Engineering for resistance to SCMV in sugarcane. *Acta Horticulturae* **461**, 385-391

Kang HW, Park DS, Park YJ, You CH, Lee BM, Eun MY, Go SJ (2002) Fingerprinting of diverse genomes using PCR with universal rice primers generated from repetitive sequence of Korean weedy rice. *Molecules and Cells* **13**, 281-287

Khoodoo MHR, Behary-Paray N, Ganeshan S, Saumtally AS (2010) Distri-

bution of *Sugarcane yellow leaf virus* in commercial cultivars in Mauritius and its potential impact on yield. *Proceedings of the International Society of Sugar Cane Technologists* **27**, 9 pp

Korimbocus J, Coates D, Barker I, Boonham N (2002) Improved detection of *Sugarcane yellow leaf virus* using a real-time fluorescent (TaqMan) RT-PCR assay. *Journal of Virological Methods* **103**, 109-120

Kumar N, Jhang T, Satyavir, Sharma TR (2011) Molecular and pathological characterization of *Colletotrichum falcatum* infecting subtropical Indian sugarcane. *Journal of Phytopathology* **159**, 260-267

Kuramae EE, Fenille RC, Rosa VE, Dias DR, Monteiro JP, Gimenes MA, Palmieri DA, Lima MM, Favaro RD (2002) Mining of sugarcane ESTs involved in the mechanisms against plant pathogen and environmental stress. *Summa Phytopatológica* **4**, 315-324

Lee I-M, Hammond RW, Davis RE, Gundersen DE (1993) Universal amplification and analysis of pathogen 16s rDNA for classification and identification of mycoplasma-like organisms. *Phytopathology* **83**, 834-842

Lehrer AT, Wu KK, Komor E (2010) Impact of *Sugarcane yellow leaf virus* on growth and sugar yield of sugarcane. *Journal of General Plant Pathology* **75**, 288-296

Leu LS (1972) Freeing sugarcane from mosaic virus by apical meristem and tissue culture. *Taiwan Sugar Experiment Station Report* **57**, 57-63

Lockhart BEL, Irey M, Comstock JC (1996) Sugarcane bacilliform virus, sugarcane mild mosaic virus and sugarcane yellow leaf syndrome. In: Croft BJ, Piggin CM, Wallis EM, Hogarth DM (Eds) *Sugarcane Germplasm Conservation and Exchange*. *ACIAR Proceedings* **67**, 108-112

Lockhart BEL, Cronjé PR (2000) Yellow leaf syndrome. In: Rott P, Bailey RA, Croft BJ, Comstock JC, Saumtally AS (Eds) *A Guide to Sugarcane Diseases*, La Librarie du CIRAD, Montpellier, France, pp 291-295

Magarey RC, Kuniata LS, Samson PR, Croft BJ, Chandler KJ, Irawan, Braithwaite KS, Allsopp PG, James AP, Rauka GR (2008) Research into exotic disease and pest threats to *Saccharum* germplasm in Australia and neighbouring countries. *Sugar Cane International* **26**, 21-25

Malathi P, Viswanathan R, Ramesh Sundar A, Prakasam N, Padmanaban P, Jothi R, Renuka Devi SR, Poongothai M (2010) Variability among *Colletotrichum falcatum* pathotypes used for screening red rot resistance in sugarcane. *Sugar Cane international* **28 (2)**, 47-52

McQualter RB, Dale JL, Harding RM, McMahon JA, Smith GR (2004) Production and evaluation of transgenic sugarcane containing Fiji disease virus (FDV) genome segment S9-derived synthetic resistant gene. *Australian Journal of Agricultural Research* **55**, 139-145

Menossi M, Silva-Filho MC, Vincentz M, Van-Sluys M-A, Souza GM (2008) Sugarcane functional genomics: Gene discovery for agronomic trait development. *International Plant Journal of Plant Genomics* **2008**, 1-11

Mishra MK, Behera B (2009) Pathogenic and molecular variability of *Colletotrichum falcatum* Went. isolates from sugarcane with red rot disease symptoms. *Journal of Crop Science and Biotechnology* **12 (1)**, 31-35

Mohandraj D, Kumaresan S, Sreenivasan TV (2002) Molecular characterisation of isolates of the sugarcane red rot pathogen. *Indian Phytopathology* **55 (2)**, 147-151

Monteiro-Vitorello CB, Camargo LE, Van Sluys MA, Kitajima JP, Truffi D, do Amaral AM, Harakava R, de Oliveira JC, Wood D, de Oliveira MC, Miyaki C, Takita MA, da Silva AC, Furlan LR, Carraro DM, Camarotte G, Almeida NF Jr, Carrer H, Coutinho LL, El-Dorry HA, Ferro MI, Gagliardi PR, Giglioti E, Goldman MH, Goldman GH, Kimura ET, Ferro ES, Kuramae EE, Lemos EG, Lemos MV, Mauro SM, Machado MA, Marino CL, Menck CF, Nunes LR, Oliveira RC, Pereira GG, Siqueira W, de Souza AA, Tsai SM, Zanca AS, Simpson AJ, Brumbley SM, Setúbal JC (2004) The genome sequence of the Gram-positive sugarcane pathogen *Leifsonia xyli* subsp. *xyli*. *Molecular Plant-Microbe Interactions* **17**, 827-836

Moonan F, Mirkov TE (2002) Analyses of the genotypic diversity among North, South, and Central American isolates of *Sugarcane yellow leaf virus*: Evidence for Colombian origins and for intraspecific spatial phylogenetic variation. *Journal of Virology* **76**, 1339-1348

Moonan F, Molina J, Mirkov TE (2000) *Sugarcane yellow leaf virus*: An emerging virus that has evolved by recombination between luteoviral and poleroviral ancestors. *Virology* **269**, 156-171

Natarajan US, Ramana Rao TS, Balasundaram N, Palanichamy K, Tripathi BK (1998) Red rot resistance in sugarcane – a critique. *Sugar Cane* **1**, 11-14

Nishiyama-Jr MY, Vicente FFR, Lembke CG, Sato PM, Dal-Bianco ML, Fandiño RA, Hotta CT, Souza, GM (2010) The SUCEST-FUN regulatory network database: Designing an energy cane. *Proceedings of the International Society of Sugar Cane Technologists* **27**, 10pp

Ovalle W, Comstock JC, Glynn NC, Castlebury LA (2008) First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in Guatemala. *Plant Disease* **92 (6)**, 973-973

Owor BE, Shepherd DN, Taylor NJ, Edema R, Monjane AL, Thomson JA, Martin DP, Varsani A (2007) Successful application of FTA classic card technology and use of bacteriophage *phi*29 DNA polymerase for large-scale field sampling and cloning of complete maize streak virus genomes. *Journal of Virological methods* **14**, 100-105

Pan Y-B, Grisham MP, Burner DM, Damann KE, Wei Q (1998) A polymerase chain reaction protocol for the detection of *Clavibacter xyli* subsp. *xyli*, the causal bacterium of sugarcane ratoon stunting disease. *Plant Disease* **82**, 285-290

Pan Y-B, Grisham MP, Burner DM (1997) A polymerase chain reaction protocol for the detection of *Xanthomonas albilineans*, the causal agent of sugarcane leaf scald disease. *Plant Disease* **81**, 189-194

Pan Y-B, Grisham MP, Burner DM, Legendre BL, Wei Q (1999) Development of Polymerase Reaction Chain primers highly specific for *Xanthomonas albilineans*, the causal bacterium of sugarcane leaf scald disease. *Plant Disease* **83**, 218-222

Parmessur Y, Aljanabi S, Saumtally S, Dookun-Saumtally A (2002) *Sugarcane yellow leaf virus* and sugarcane yellows phytoplasma: Elimination by tissue culture. *Plant Pathology* **51**, 561-566

Parmessur Y, Aljanabi S, Dookun-Saumtally A (2010) Mapping of sugarcane variety M 134/75 using EST-SSR markers. *Proceedings of the International Society of Sugar Cane Technologists* **27**, 10 pp

Perera MF, Filippone MP, Ramallo CJ, Cuenya MI, García ML, Ploper LD, Castagnaro AP (2009) Genetic diversity among viruses associated with sugarcane mosaic disease in Tucumán, Argentina. *Phytopathology* **99**, 38-49

Pieretti I, Royer M, Barbe V, Carrere S, Koebnik R, Cociancich S, Couloux A, Darrasse A, Gouzy J, Jacques M-A, Lauber E, Manceau C, Mangenot S, Poussier S, Suguren B, Szurek B, Verdier V, Arlat M, Rott P (2009) The complete genome sequence of *Xanthomonas albilneans* provides new insights into the reductive genome evolution of the xylem-limited *Xanthomonadaceae*. *BMC Genomics* **10**, 616-631

Raboin LM, Hoarau JY, Costet L, Telismart H, Glaszmann JC, D'Hont A (2003) Progress in genetic mapping of sugarcane smut resistance. *Proceedings of the South African Sugar Technologists' Association* **77**, 134-141

Raboin LM, Oliveira KM, Lecunff L, Telismart H, Roques D, Butterfield M, Hoarau JY, D'Hont A (2006) Genetic mapping in sugarcane, a high ploidy, using bi-parental progeny: Identification of a gene controlling stalk colour and a new rust resistance gene. *Theoretical and Applied Genetics* **112**, 1382-1391

Rassaby L, Girard J-C, Letourmy P, Chaume J, Irey MS, Lockhart BEL, Kodja H, Rott P (2003) Impact of *Sugarcane yellow leaf virus* on sugarcane yield and juice quality in Réunion Island. *European Journal of Plant Pathology* **109**, 459-466

Ricaud C, Ryan CC (1989) Leaf Scald. In: Ricaud C, Egan BT, Gillaspie AG Jr., Hughes CG (Eds) *Diseases of Sugarcane. Major Diseases*, Elsevier Science Publishers, Amsterdam, The Netherlands, pp 39-58

Rossi M, Araujo PG, Paulet F, Garsmeur O, Dias VM, Chen H, Van Sluys M-A, D'Hont A (2003) Genomic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. *Molecular Genetics and Genomics* **269**, 406-419

Rott P, Bousquet JF, Muller M, Chatenet M (1997) The CIRAD sugarcane quarantine unit in Montpellier. *Agriculture et Développement* **13**, 22-28

Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally S (2000 a) Common names of sugarcane diseases and their causal agent(s). In: Rott P, Bailey RA, Comstock JC, Croft, BJ, Saumtally, AS (Eds) *A Guide to Sugarcane Diseases*, CIRAD, Montpellier, France, pp 317-322

Rott P, Bailey RA, Comstock JC, Croft BJ, Saumtally S (Eds) (2000b) *A Guide to Sugarcane Diseases*, CIRAD, Montpellier, France, 339 pp

Rott P, Davis MJ (2004) Identification of sugarcane bacterial pathogens and assessing their genetic diversity using molecular techniques. In: Roa GP, Saumtally AS, Rott P (Eds) *Sugarcane Pathology (Vol III) Bacterial and Nematode Diseases*, Science Publishers Inc., Enfield, USA, pp 175-183

Ryan CC, Egan BT (1989) Rust. In: Ricaud C, Egan BT, Gillaspie AG, Hughes CG (Eds) *Diseases of Sugarcane, Major Diseases*, Elsevier, Amsterdam, pp 189-210

Saumtally S (1996) Genetic variability in *Xanthomonas campestris* pv *vasculorum* (Cobb) Dye, causal agent of gumming diseases of sugarcane. PhD thesis, University of Reading, UK, 192 pp

Saumtally S, Dookun-Saumtally A, Autrey LJC (2000) Sugarcane diseases and their management. In: Rott P, Bailey RA, Comstock JC, Croft, BJ, Saumtally AS (Eds) *A Guide to Sugarcane Diseases*, Montpellier, CIRAD, pp 407-424

Saumtally S, Dookun-Saumtally A, Autrey LJC (2005) Sugarcane diseases and their management. In: Solomon S, Grewal SS, Yang-rui Li, Magarey RC, Rao GP (Eds), *Sugar Cane Production Management and Agro-Industrial Imperatives*, International Book Distributing Co., India, pp 409-424

Saumtally AS, Viremouneix TR, Ahondokpê B, Girard, J-C, Castlebury LA, Dixon L, Glynn NC, Comstock JC (2011) First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Ivory Coast and Cameroon. *Plant Disease* **69**, 357

Scagliusi SM, Lockhart BEL (2000) Transmission, characterization, and serology of a luteovirus associated with yellow leaf syndrome of sugarcane. *Phytopathology* **90**, 120-124

Schenck S (2001) Sugarcane yellow leaf syndrome: History and current concepts. In: Roa GP, Ford RE, Tosic M, Teakle D (Eds) *Sugarcane Pathology (Vol II) Virus and Phytoplasma Diseases*, Science Publishers Inc, Plymouth, UK, pp 25-35

Schneider B, Ahrens U, Kirkpatrick BC, Seemuller E (1993) Classification

of plant-pathogenic mycoplasma-like organisms using restriction-site analysis of PCR amplified 16s rDNA. *Journal of General Microbiology* **139**, 519-527

Shamloul AM, Abdallah NA, Madkour MA, Hadidi A (2001) Sensitive detection of the Egyptian species of sugarcane streak virus by PCR-probe capture hybridization (PCR-ELISA) and its complete nucleotide sequence. *Journal of Virological Methods* **92**, 45-54

Shepherd DN, Varsani A, Windram PO, Lafeuvre P, Monjane AM, Owor BE, Martin DP (2008) Novel sugarcane streak and sugarcane streak Réunion mastreviruses from Southern Africa and La Réunion. *Archives of Virology* **153 (3)**, 605-609

Singh N, Somai BM, Pillay D (2004) Smut disease assessment by PCR and microscopy in inoculated tissue cultured sugarcane cultivars. *Plant Disease* **167**, 987-994

Smith GR, Harding RM (2001) Genetic engineering for virus resistance in sugarcane. In Rao GP, Ford RE, Tosic M, Teakle DS (Eds) *Sugarcane Pathology (Vol II) Virus and Phytoplasma Diseases*, Science Publishers Inc., Enfield, USA, pp 337-365

Skotnicki AH, Dale JL, Skotnicki ML (1986) Detection of Fiji disease virus in infected sugarcane by nucleic acid hybridization. *Journal of Virological Methods* **13 (1)**, 71-77

Smith GR, Clarke ML, Van de Velde R, Dale JL (1994) Chemiluminescent detection of Fiji disease virus in sugarcane with biotinylated DNA probes. *Archives of Virology* **136**, 325-334

Smith GR, Van de Velde R, Dale JL (1992) PCR amplification of a specific double-stranded RNA region of Fiji disease virus from diseased sugarcane. *Journal of Virological Methods* **39 (3)**, 237-246

Smith GR, Van de Velde R (1994) Detection of *Sugarcane mosaic virus* and *Fiji disease virus* in diseased sugarcane using the polymerase chain reaction. *Plant Disease* **78**, 557-561

Sooknandan S, Synman SJ, Potier BAM, Huckett BI (2003) Progress in the development of mosaic resistant sugarcane via transgenesis. *Proceedings of South African Sugar Technologists Association* **77**, 624-627

Taylor PWJ, Petrasovits LA, Van der Velde R, Birch RG, Croft BJ, Fegan M, Smith GR, Brumbley SM (2003) Development of PCR-based markers for detection of *Leifsonia xyli* subsp. *xyli* in fibrovascular fluid of infected sugarcane plants. *Australasian Plant Pathology* **32**, 367-375

Thompson N, Randles JW (2001) The genome organisation and taxonomy of *Sugarcane striate mosaic associated virus*. *Archives of Virology* **146 (8)**, 1441-1451

Tomkins JP, Yu Y, Miller-Smith H, Frisch DA, Woo SS, Wing R (1999) A bacterial artificial chromosome library for sugarcane. *Theoretical and Applied Genetics* **99**, 419-424

USDA (2009) Sugar: World Production Supply and Distribution, United States Department of Agriculture. May 2009. Available online: http://www.fas.usda.gov/htp/sugar/2009/May%20sugar%202009.pdf

van Antwerpen T, McFarlane K, Subramoney DS (2001) Recent improvements in procedures to improve security and efficiency of sugarcane quarantine in South Africa. *Proceedings of the Annual Congress - South African Sugar Technologists' Association* **75**, 255-256

van Antwerpen T, Bailey RA, Subramoney DS, Macfarlane K, Rutherford RS, Nuss KJ (2006) Eighty years of sugarcane quarantine in South Africa. *Sugarcane International* **24**, 9-12

Varsani A, Oluwafemi S, Windram OP, Shepherd DN, Monjane AM, Owor BE, Rybicki EP, Lefeuvre P, Martin DP (2008) Panicum streak virus diversity is similar to that observed for maize streak virus. *Archives of Virology* **153 (3)**, 601-604

Vega J, Scagliusi SMM, Ulian EC (1997) Sugarcane yellow leaf disease in Brazil: Evidence of association with a luteovirus. *Plant Disease* **81**, 21-26

Vettore AL, da Silva FR, Kemper EL, Souza GM, da Silva AM, Ferro MIT, Henrique-Silva F, Giglioti EA, Lemos MVF, Coutinho LL, Nobrega MP, Carrer H, Franca SC, Bacci Jr. M, Goldman MHS, Gomes SL, Nunes LR, Camargo LE A, Siqueira WJ, Van Sluys MA, Thiemann OH, Kuramae EE, Santelli RV, Marino CL, Targon MLP, Ferro JA, Silveira HCS, Marini DC, Lemos EGM, Monteiro-Vitorello CB, Tambor JHM, Carraro DM, Roberto PG, Martins VG, Goldman GH, de Oliveira GC, Truffi D, Colombo CA, Rossi M, de Araujo PG, Sculaccio SA, Angella A, Lima MMA, de Rosa Jr. VE, Siviero F, Coscrato VE, Machado MA, Grivet L, Di Mauro SMZ, Nobrega FG, Menck CFM, Braga MDV, Telles GP, Cara FAA, Pedrosa G, Meidanis J, Arruda P (2003) Analysis and functional annotation of an expressed sequence tag collection for tropical crop sugarcane. *Genome Research* **13**, 2725-2735

Viswanathan R, Balamuralikrishnan M, Karuppaiah R (2008a) Characterization and genetic diversity of sugarcane streak mosaic virus causing mosaic in sugarcane. *Virus Genes* **36**, 553-564

Viswanathan R, Balamuralikrishnan M, Karuppaiah R (2008b) Identification of three genotypes of sugarcane yellow leaf virus causing yellow leaf disease from India and their molecular characterization. *Virus Genes* **37(3)**, 368-379

Viswanathan R, Karuppaiah R, Balamuralikrishnan M (2009) Identification of new variants of SCMV causing sugarcane mosaic in India and assessing their genetic diversity in relation to SCMV type strains. *Virus Genes* **39 (3)**, 375-386

Wagih ME, Gordon GH, Ryan CC, Adkins SW (1995) Development of an axillary bud culture technique for Fiji disease virus elimination in sugar cane. *Australian Journal of Botany* **43**, 135-144

Wang M-Q, Zhou G-H (2010) A near-complete genome sequence of a distinct isolate of *Sugarcane yellow leaf virus* from China, representing a sixth new genotype. *Virus Genes* **41 (2)**, 268-272

Wang ZK, Comstock JC, Hatziloukas E, Schaad NW (1999) Comparison of PCR, Bio-PCR, DIA, ELISA and isolation on semiselective medium for detection of *Xanthomonas albilineans*, the causal agent of leaf scald of sugarcane. *Plant Pathology* **48**, 245-252

Xie Y, Wang M, Xu D, Li R, Zhou G (2009) Simultaneous detection and identification of four sugarcane viruses by one-step RT-PCR. *Journal of Virological Methods* **162**, 64-68

Yang ZN, Mirkov TE (1997) Sequence and relationships of Sugarcane mosaic and *Sorghum mosaic virus* strains and development of RTPCR-based RFLPs for strain discrimination. *Phytopathology* **87**, 932-939

Xiong QY, Wei LJ, Sen ZJ, Hong RM, Ping XL, Qing ZM (2008) Molecular cloning and characterization of a non-TIR-NBS-LRR type disease resistance gene analogue from sugarcane. *Sugar Tech* **10**, 71-73

Zhang L, Birch RG (1997) The gene for albicidin detoxification from *Pantoea dispersa* encodes an esterase and attenuates pathogenicity of *Xanthomonas albilineans* to sugarcane. *Proceedings of the National Academy of Sciences USA* **94**, 9984-9989

Zhu Y-J, McCafferty H, Osterman G, Agbayani R, Schenck S, Lehrer A, Komor E, Moore P (2007) Transgenic sugarcane with coat protein gene-based silencing increased resistance to sugarcane yellow leaf virus (ScYLV). *Proceedings of the International Society of Sugar Cane Technologists* **26**, 963-967