

A SURVEY OF SOUTH AFRICAN SUGARCANE MOSAIC VIRUS (SCMV) STRAINS BASED ON COAT PROTEIN GENE SEQUENCE ANALYSIS

BS GOODMAN^{1,2}, D MACDONALD² AND BI HUCKETT¹

¹South African Sugar Association Experiment Station, Private Bag X02, Mount Edgecombe, 4300

²Biotechnology Department, Technikon Natal, PO Box 953, Durban, 4000

Abstract

Sugarcane mosaic potyvirus (SCMV) infection can severely reduce sucrose yield in susceptible sugarcane varieties in South Africa. Knowledge of prevalent South African strains of SCMV is poor but strategically important for the development of better diagnostic procedures and transgene induced resistance. Since most strain variation resides in the capsid structure, molecular analysis of relative homology in nucleotide and inferred amino acid sequences in the most variable (N-terminal) coat protein region is the method of choice for distinguishing strains. Total RNA was extracted from symptomatic leaves collected from various regions. Viral RNA was reverse transcribed and a large fragment at the 3' end of the SCMV genome amplified by PCR using previously designed primers. Fragments, both cloned and uncloned, were cycle sequenced using dye terminator chemistry. Analysis of the sequences was performed using an ABI Prism 310 Genetic Analyser and related computer software. Consensus sequences of the first 240-360 bases of the coat protein gene were obtained and compared against international databases. The results indicate that SCMV strains in South Africa belong to the SC, E and A cluster.

Introduction

The South African sugar industry has suffered substantial losses in revenue due to mosaic induced yield decline. Mosaic, caused by sugarcane mosaic potyvirus (SCMV), is one of the most prevalent diseases of sugarcane in the world (Koike and Gillaspie, 1989). In South Africa, under conditions of severe SCMV infection, reduction in sucrose yield has been reported to be as high as 42% in a susceptible variety (Bailey and Fox, 1987). Consequently, the breeding of resistant varieties has been pursued vigorously, with great success. However, significant losses still occur because over 40% of new germplasm is ultimately discarded due to insufficient resistance to SCMV infection.

SCMV is a single-stranded, positive sense RNA virus, which is polyadenylated at the 3' end. The RNA genome is surrounded by helically arranged capsid (coat) proteins to form an elongated virion structure, 750 nm x 13 nm. The gene encoding the coat protein is well defined and produces a polypeptide with a highly variable, surface-exposed amino-(N)-terminus, a highly conserved core region and a surface-exposed carboxyl-(C)-terminus (Shukla *et al.*, 1988). The

N-terminus is the most significant region in the virus that is unique to each viral type and is thus the region where the majority of strain variation occurs.

An accurate assessment of SCMV strains prevalent in the South African sugar industry is lacking, but strategically important for the development of improved diagnostic procedures and transgene-mediated resistance. Since most strain variations reside in the capsid structure, molecular analysis of the relative homology of the nucleotide and inferred amino acid sequences of the most variable (N-terminal) coat protein region is the method of choice for distinguishing between strains of SCMV.

In this study, symptomatic sugarcane leaf samples were collected from several widely differing regions within the sugar industry. From each sample, RNA was extracted and a large fragment of the viral coat protein gene, including the variable N-terminal region, isolated by reverse transcription followed by polymerase chain reaction (RT-PCR). Fragments were cycle sequenced and the sequences analysed using computer software to determine base differences indicative of strain variation.

Materials and experimental approach

Infected leaf samples were collected from regions of infection throughout the industry, when available. Figure 1 gives a list of the regions sampled as well as their locations within South Africa. Virus-specific oligonucleotide primers used in this work were as described previously (Huckett and Botha, 1996).

Total RNA was extracted from freshly sampled infected leaves using a commercial kit (RNeasy, Qiagen). RT-PCR amplification of an 884bp coat protein gene fragment was achieved using specific primers BHf and GSr (Huckett and Botha, 1996). Amplified fragments were column purified and either cycle sequenced directly or sequenced after cloning into a phagemid vector (pCR-Script SK+, Stratagene) using dye terminator chemistry. Sequences obtained were analysed using an ABI Prism 310 Genetic Analyser and manually edited using related computer software. Fragment sequences were compared to those in international databases, e.g. GenBank, in order to obtain confirmation of SCMV identity. Alignment of the sequences obtained from each region allowed consensus sequences to be computed for the portion of the fragment representing the first 240-360 bases (variable

Region	Variety	Cane age
1. Pongola	86F3396	5 months
	N19	4 months
	N25	8 months
2. Melmoth	N12	8 months
3. Glendale	N16	7 months
	NCo376	6 months
4. Tongaat	N12	8 months
	NCo376	8 months
5. Mount Edgecombe	NCo376	8 months
	N12	6 months
6. Eston	NCo376	6 months
	N12	7 months
7. Ixopo	NCo376	7 months
	NCo376	12 months

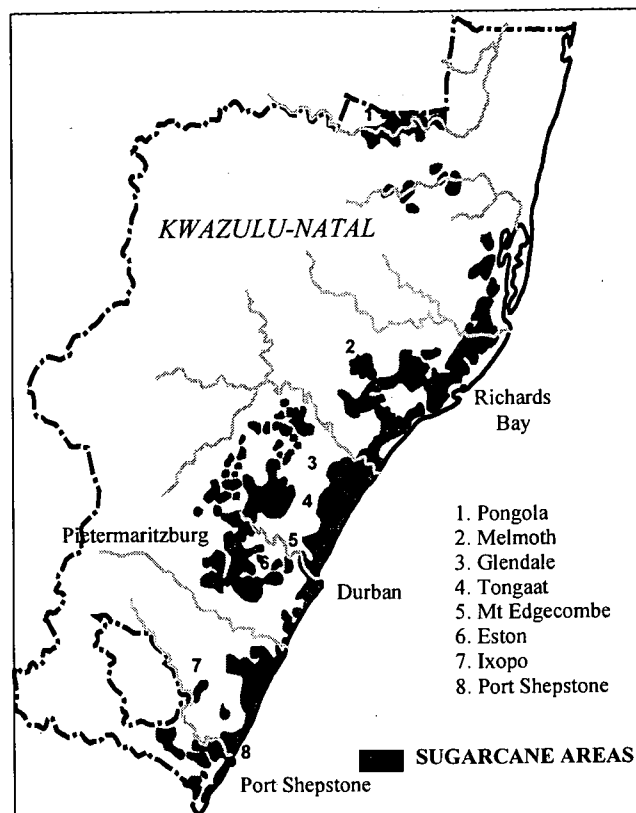


Figure 1. Geographic and varietal sources of SCMV-infected sugarcane sampled for viral genome analysis.

region) of the SCMV coat protein gene. These were compared with international databases for strain identification. Phylogenetic relationships between the SCMV isolates were determined using DNA analysis software (DNASIS, Hitachi Software).

Results and discussion

RNA yields of 4.5 µg or more were obtained for all 14 samples described in Figure 1. RNA stocks were stored at -80°C . RT-PCR of all samples resulted in the amplification of the expected 884bp coat protein gene fragment. Fragments were excised from gels, column purified (Gel Extraction Kit, Qiagen) and stored at -20°C until required for cloning. To date, coat protein fragments from eight varieties in five of the regions have been cloned successfully. Two positive clones were selected for variety 86F3396 from Pongola (far North) and two for variety NCo376 from Eston (Midlands) for preliminary sequence analysis. International database comparison of the sequences obtained confirmed SCMV identity. Consensus sequences of the first 248bp of the coat protein gene were computed. Computer-aided determination of phylogenetic relationships of SCMV from these sequences is shown in Figure 2. This preliminary analysis of partial sequences shows that great similarity exists between the isolates from each region as well as between those from the two regions. By incorporating published nucleotide sequences of the same coat protein region from known strains into the comparison, Pongola and Eston isolates were shown to be

closely related to the Australian strain SCMV-SC (Frenkel *et al.*, 1991). From the data obtained so far it can be inferred that South African strains belong to the SCMV-SC, E and A strain cluster (personal communication).

Continuation of this work will involve sequencing of all 14 samples (Figure 1) and final phylogenetic relationships and strain determination will be based on inferred amino acid sequences as well as nucleotide sequences.

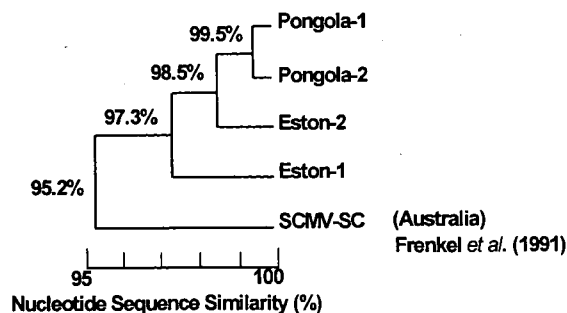


Figure 2. Phylogenetic relationships between SCMV coat protein gene nucleotide sequences (248bp) from two widely separated South African regions (Pongola and Eston) and the Australian SCMV sugarcane strain (SCMV-SC) determined using DNASIS computer software.

¹Erik Mirkov, Texas A&M Research Center, Weslaco, Texas, USA

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