PROGRESS IN THE DEVELOPMENT OF MOSAIC RESISTANT SUGARCANE VIA TRANSGENESIS

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Introduction

The sugarcane disease mosaic causes severe economic losses in South Africa and other sugarcane-growing areas worldwide. The causative agent is the sugarcane mosaic virus (SCMV), which belongs to the *Potyvirus* genus. Currently, development and use of resistant cultivars is the most effective method of controlling mosaic in the South African sugar industry. However, susceptibility to mosaic has seen ~16% of the best varieties regularly eliminated from the breeding programme at a late stage of selection (Butterfield and Thomas, 1996). Recent advances in genetic engineering may provide an alternative approach for the development of mosaic resistant cultivars. This report describes the strategy employed for the conferment of resistance to mosaic in high sucrose yielding but susceptible cultivars by induction of post-transcriptional gene silencing (PTGS), an incompletely characterised but powerful inhibitory cellular mechanism. PTGS may be induced by the transcription of a viral gene introduced into the genome of the host plant using genetic engineering techniques. The products of transcription (RNA) result in the degradation of the corresponding viral genes during the life cycle of the pathogen. This type of induced molecular resistance has been developed successfully in many crops including sugarcane (Ingelbrecht *et al.*, 1999). In the case described here, a viral coat protein gene isolated from a strain of SCMV prevalent in the Midlands (Huckett and Botha, 1996; Goodman *et al.*, 1998) was used to construct transformation (genetic engineering) vectors for delivery to the sugarcane genome of two susceptible South African varieties.

Experimental Approach

Plasmid pUBI510 (ECACC deposit reference number 00042603) was used in the development of transformation vectors pAMC510 and pUMC510. The Cauliflower Mosaic Virus (CaMV) 35S and maize ubiquitin promoter regions incorporated in tandem in pUBI510 have been shown to confer high levels of expression on the inserted gene of interest in sugarcane (Groenewald *et al.*, 2000). Plasmid vector pAMC510 was constructed with the full-length virus coat protein (CP) gene in the antisense orientation, while vector pUMC510 was designed to contain a slightly truncated and untranslatable form of the CP gene in the sense orientation.

Plasmids pAMC510 and pUMC510 were delivered individually and in combination to the sugarcane genome. Although both types of transgene, antisense and untranslatable sense CP genes, are known to be capable of inducing virus inhibition by PTGS, the combination of the two has been shown to be particularly powerful (Waterhouse *et al*., 1998). Microprojectile bombardment was the gene transfer system of choice. The selection plasmid pEmuKN, containing the *nptII* gene, was co-delivered with the CP constructs as it confers resistance to geneticin, thus enabling the development of transformed plant material on medium containing the antibiotic. Both leafroll discs (1.5 weeks old) and embryogenic callus (8-12 weeks old) developed from leafroll of the sugarcane cultivar N19 and the unreleased genotype 75E0247 were used as target materials in bombardment experiments. Putatively transformed plants were regenerated via indirect somatic embryogenesis.

Outcomes to Date

Confirmation of CP gene insertion and orientation in the newly constructed transformation vectors pAMC510 and pUMC510 was demonstrated by restriction analysis (Figure 1) in conjunction with PCR and DNA sequencing (Figure 2). To date, only products of transformation with pAMC510 have been obtained. One line of 75E0247 and nine lines of N19 survived geneticin selection. Specific polymerase chain reaction (PCR) amplification of part of the transgene from the genomic DNA of these lines indicated that only the 75E0247 transformant was positive for SCMV CP (Figure 3A). This was confirmed by Southern blotting analysis of the genomic DNA using the CP gene as probe (Figure 3B).

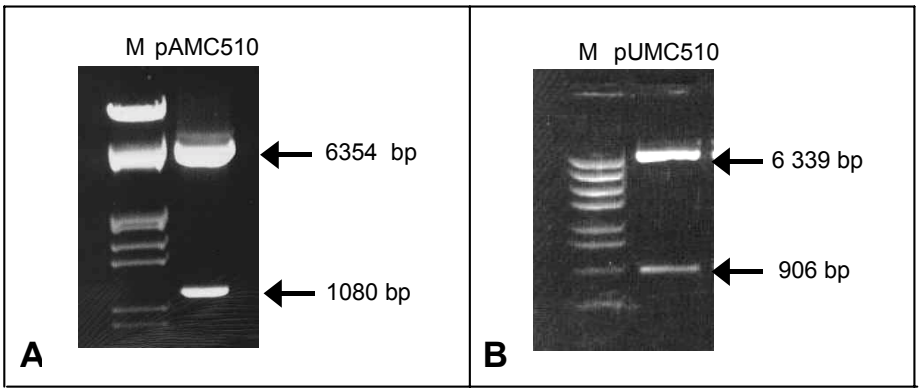

Figure 1. Restriction digestion analysis of transformation vectors. Excision of gene insert from (A) pAMC510 and (B) pUMC510 with restriction endonucleases BamHI (A) and BamHI/EcoRI (B) which target the insertion sites (see Figure 2).

Results indicate the presence of the full-length (~1080 bp) and truncated (~906 bp) CP sequences, respectively. M: molecular eight markers: EcoRI/HindIII-digested λ DNA (A) and EL markers (BAM Potier) (B). Arrows indicate position and expected sizes of fragments after digestion, compatible with size markers.

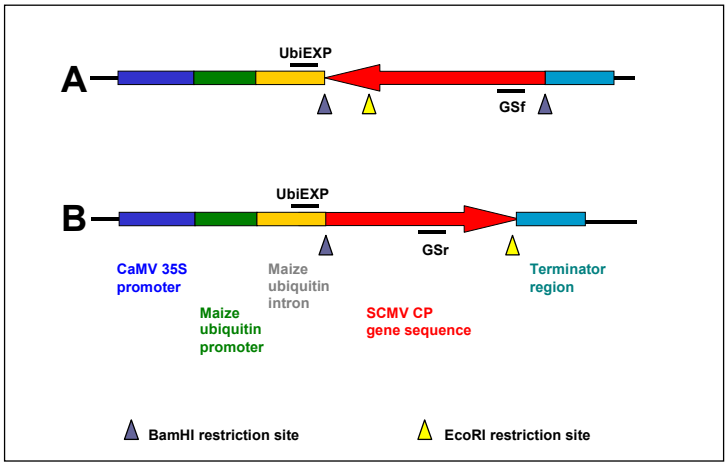

Figure 2. Structure of transformation vectors pamc510 (a) and pumc510 (b) showing essential gene expression cassettes in linear format.

Diagrams show the positions of Bamhi and Ecori restriction sites and the positions of the primer pairs used to establish the orientation of the SCMV CP gene insert in each case. Forward primer UBIEXP binds in the vector just upstream of the insert. Forward and reverse primers GSF and GSR bind within the SCMV CP gene insert itself. The ubiexp-GSF primer combination would generate an amplification product only if the insert was in antisense, while the UBIEXP-GSR pair would amplify only from an insert in the sense orientation. DNA sequencing analysis using UBIEXP as primer confirmed insert orientation and SCMV CP gene identity was demonstrated by comparison of sequence data with SCMV accessions in the international Genbank database using BLASTN (basic local alignment search tool, national centre for biotechnology information).

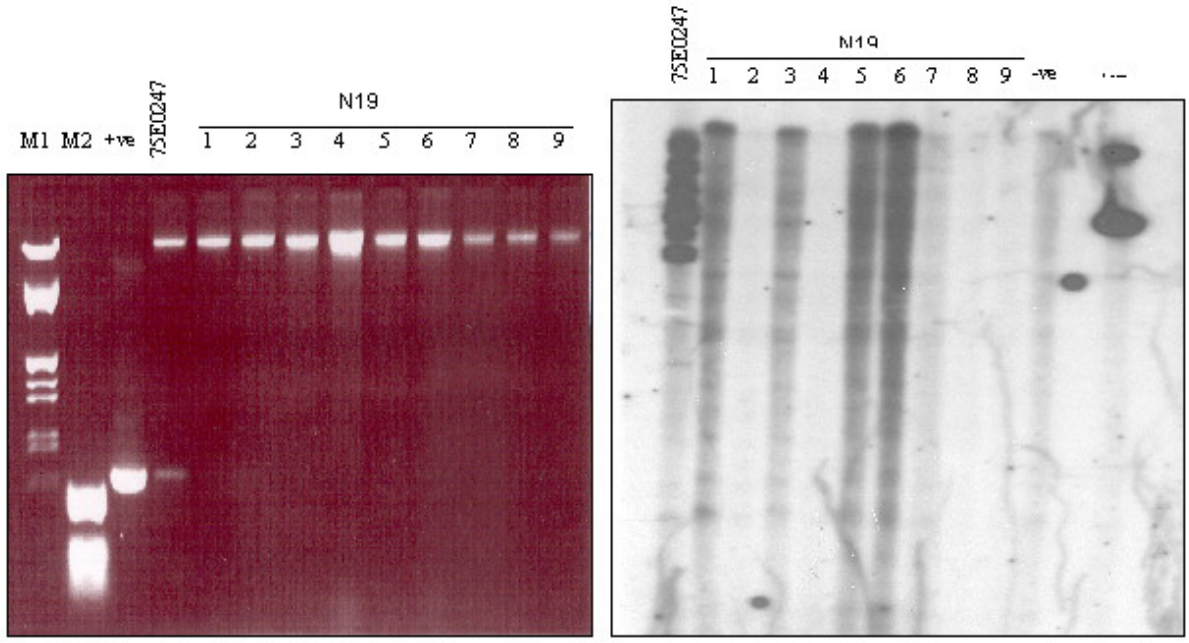

Figure 3. Molecular analysis of putative transformants. A. PCR amplification of the CP transgene from extracted DNA using the UBIexp and GSf primers. The insert was detected only in the positive control (plasmid vector; +ve) and the 75E0247 line. M: molecular weight markers: M1, EcoRI/HindIII-digested λ DNA; M2, HaeII-digested pBR322 DNA. B. Southern hybridisation analysis of genomic DNA digested with the restriction enzyme HindIII and probed with radiolabelled CP sequence.

Results show unique bands indicating multiple gene copy insertions in the 75E0247 line, thus confirming its transgenic status. N19 lines show variable levels of hybridisation due to uneven DNA loading but all patterns of banding are similar to those in the negative control (untransformed N19 genomic DNA) (-ve) suggesting non-specific binding and negative CP gene insertion. Positive control (+ve): plasmid pAMC510 DNA.

Conclusions and future work

It has been established through molecular analysis that one 75E0247 plant has been transformed with the full-length SCMV antisense CP gene. This line will undergo phenotypic testing by infection with a local strain of SCMV, and subsequent observation of symptoms under controlled conditions, in order to determine its mosaic resistance characteristics during the course of development. Production of further transformant lines is ongoing. The combined use of plasmids pAMC510 and pUMC510 for transformation is now routine and NCo310 has been added to the repertoire of recipient cultivars.

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