BUREAU OF SUGAR EXPERIMENT STATIONS
QUEENSLAND, AUSTRALIA

FINAL REPORT
SRDC PROJECT BS10S

THE PRODUCTION AND EVALUATION OF GENE PROBES FOR DIAGNOSIS OF SUGARCANE MOSAIC VIRUS AND FIJI DISEASE VIRUS

by

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This Project was funded by the Sugar Research Council/Sugar Research and Development Corporation during the 1988/89, 1989/90 and 1990/91 financial years.

December 1991

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SUMMARY

Specific and sensitive cDNA probes have been developed for detection of both FDV and SCMV in diseased sugarcane plants. Results from glasshouse evaluation indicate that FDV probes are not sensitive enough to be used for the detection of the virus in asymptomatic diseased plants. Results are not yet available from the SCMV probe glasshouse trial.

OBJECTIVES

Develop sensitive and specific complementary DNA (cDNA) probes for the detection of sugarcane mosaic virus (SCMV) and Fiji disease virus (FDV).

Develop methodology for the routine screening of sugarcane plants for viral presence with these probes and compare the system with other diagnostic systems.

METHODOLOGY AND RESULTS

1. Development and evaluation of FDV-specific DNA probes

Fiji disease virus, the type species of the plant reovirus subgroup 2 (*Fiji virus*) has a segmented genome consisting of ten linear species of double-stranded RNA (dsRNA). FDV specific dsRNA was isolated from FDV infected sugarcane by CF11 cellulose column chromatography essentially as described by Skotnicki *et al* (1986). The dsRNA was cloned to complementary DNA (cDNA) by AMV reverse transcription of first strand DNA, followed by RNAse H/Klenow second strand synthesis (Dale and Bateson, 1987). The cDNA was then ligated into the commercially available plasmid pUC12, and transformed into the *E. coli* strain JM109 to produce a range of pFDV clones.

The pFDV clones were initially characterised for specificity to FDV dsRNA by hybridisation with ^{32}P labelled first strand FDV cDNA. Approximately 120 clones were selected for further study from these results. As the genetic material of FDV consists of ten linear species of dsRNA, the pFDV clones were evaluated for specificity to one band of the genome by alkaline northern blotting of purified dsRNA and hybridisation with the individual probes. This method was used because the type of ligation strategy employed (blunt end ligation) can lead to insertion of more than one cDNA fragment into the cloning site of the plasmid, leading to a loss of specificity. Four clones, pFDV7, pFDV29, pFDV55 and pFDV59 were selected as DNA probes for FDV.

Specificity. pFDV7, 29, 55 and 59 are specific for Fiji disease virus infected sugarcane. The cDNA insert does not have to be excised from the rest of the plasmid before probing, thus increasing the sensitivity of the probe as more biotin target is available.

Sensitivity. The probes are sensitive down to approximately 0.5 pg (0.5×10^{-12} g) of target dsRNA. This is approximately the same sensitivity as nick translated ^{32}P DNA probes.

2. Development of SCMV-specific probes

The original concept for the SCMV probe was to evaluate some of the SCMV clones from Project BS9S 'Production of genetic constructs for expression of sugarcane mosaic virus coat protein in sugarcane protoplasts' as probes. However, as the CSIRO SCMV-SC coat protein gene was available (Frenkel *et al*, 1990), it was decided to construct a probe recognising the central portion of the coat protein gene. This was achieved by subcloning the cDNA encoding this region into plasmid pUC18 to produce the clone pS400. The central region of the coat protein gene is highly conserved in all SCMV strains (-SC, -BC, JG, and Sabi) and is relatively well conserved in the potyvirus genus. Thus this probe should be capable of detecting any strains of SCMV or any other potyvirus infecting sugarcane (a somewhat unlikely event). pS400 is a 'defined probe' in that we know precisely the region of the pathogen that is targeted.

3. Development of technology for routine screening

The most versatile method for screening large numbers of samples is the dot or slot blot whereby 96 or 48 samples respectively are loaded onto a membrane via a manifold template. However with the type of system developed, false positives were evident in control samples. Changing the type of membrane, the sample loading buffer or post loading wash conditions did not resolve this problem. While alkaline northern blotting could be utilised to resolve this problem this technique is too slow for throughput of large numbers of samples. Handblotting has been tested and found to work very well, but can only be utilised for moderate numbers of samples due to the loading procedure. Handblotting was used to evaluate the samples from the glasshouse trials.

Evaluation of FDV-specific probes for detection of FDV in non-radioactive systems

The four pFDV clones were tested with the non-radioactive chemiluminescence detection system 'Photogene' (BRL). Photogene is a multistep detection system. Firstly the probe DNA is labelled with biotin molecules by the process of nick translation, then hybridised to the target RNA, which has already been denatured and fixed to a nylon membrane. After hybridisation, the membrane is washed and the biotin is detected by the protein streptavidin (SA) to which the enzyme alkaline phosphatase (AP) has been conjugated (SA-AP). After suitable washing the enzyme substrate AMPPD is added which the AP enzyme dephosphorylates, resulting in the production of photons (light) which are recorded on standard autoradiography film.

This method was used for all subsequent work.

Glasshouse trials

FDV. NCo310 (susceptible) and Q95 (moderately susceptible) were chosen as the two cultivars for this trial. Planthoppers (*Perkinsiella saccharicida* Kirk.), the only vector of FDV in Australia, were collected from the field at the BSES Pathology Farm, Eight Mile

Plains and caged on FDV infected plants. After two months planthoppers were collected from this cage and introduced onto individually caged NCo310 and Q95 plants. After a further six weeks, the insects were killed with insecticide and the test and control plants observed for signs of the characteristic FDV gall symptom. The cane was inspected, sampled and analysed for presence of FDV at regular intervals.

The conclusion from this trial is that the probe system does not have the sensitivity to detect FDV infected asymptomatic sugarcane, although the probes can confirm early observed symptoms as FDV infection. Samples did not reliably give positive confirmation when taken from symptomless leaves on infected plants, ie other leaves of the plants showed typical symptoms. Enhancing the sensitivity of these probes by PCR or other techniques should be evaluated to achieve this objective.

SCMV. Q95 (susceptible) and NCo310 (moderately susceptible) were chosen for this trial. Test plants were mechanically infected with inoculum prepared from SCMV-infected sugarcane by the 'scotch-brite' method, but very little infection occurred. The trial was re-established and SCMV inoculated into maize 'Iochief' as a source of inoculum, but again there were very low levels of infection in the maize. This trial is being established again with a new SCMV inoculum source, so results are not yet available.

4. Comparison of probe system with other diagnostic systems

The non-radioactive detection system with either FDV- or SCMV- specific probes has approximately the same sensitivity as probes labelled with ^{32}P by nick translation (Skotnicki *et al*, 1986). This is approximately the same level of sensitivity as ELISA (Enzyme Linked Immunosorbent Assay) (Rohozinski *et al*, 1981), although ELISA and DNA probe data are presented in different formats and are not directly comparable.

PROBLEMS EXPERIENCED DURING THE PROJECT

A serious limiting factor to the development and testing of DNA probes was the delay of more than one year in BSES obtaining a licence to possess and use radioactive substances, due to administrative inefficiencies within the Health and Medical Physics Division of the Queensland Department of Health. Consequently we investigated the potential of two non-radioactive detection systems (ECL and Photogene) for use with the DNA probes. Neither of these systems was originally designed for the detection of RNA - eventually only the Photogene system worked and all the data presented is from this system. The resignation of Marilyn Clarke from BSES in June 1990, also affected progress of the project, as Ms Clarke had been primarily responsible for characterisation and evaluation of the FDV probes.

SIGNIFICANCE OF PROJECT RESULT TO THE SUGAR INDUSTRY

The Queensland sugar industry now has reasonably sensitive and specific cDNA probes to detect and confirm the presence of FDV and SCMV in sugarcane. Unfortunately the sensitivity of these probes is not high enough to detect viral presence in asymptomatic sugarcane. BSES staff have also gained valuable expertise in the development and application of gene probes, and are in a position to develop and apply other probe systems. The experience gained in development of these probes will be invaluable in quickly enhancing probe sensitivity by PCR or other techniques.

RECOMMENDATION

Evaluate the polymerase chain reaction (PCR) to increase the level of sensitivity of the DNA probes.

This is already under way in SRDC Project BS48S. Initial results indicate that PCR increases the sensitivity of the DNA probes for detection of FDV by 10^4 fold. The expertise and experience gained in this project will be important for development and evaluation of DNA probes for detection of other sugarcane pathogens.

PUBLICATIONS

Smith, G R (1991) 'Detection of Fiji disease virus and sugarcane mosaic virus by biotinylated DNA probes (Abstr.). Proceedings of Third ISSCT Sugar Cane Pathology Workshop, Mauritius.

In preparation

Smith, G R, Clarke, M L, Van de Velde, R and Dale, J L. Development of a method for detection of Fiji disease virus with biotinylated DNA probes and chemiluminescence.

ACKNOWLEDGMENTS

We are extremely grateful of Prof James Dale (QUT) for his collaboration in developing and testing the DNA probes. We would also like to thank Marion Bateson, Vroni Futo, Jenny Berkman and Dave Sanders for their help and technical skills during this project. The support of BSES and SRDC during the project is gratefully acknowledged.

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