VISWANATHAN, R. (1996). Immunological techniques to detect sugarcane virus and MLO diseases. d) Ratoon stunting disease. **Annual Report of Sugarcane Breeding Institute 1995-96,** Coimbatore, pp.52-53.

VISWANATHAN, R., ALEXANDER, K.C. and GARG, I.D. (1996). Detection of sugarcane bacilliform virus in sugarcane germplasm. **Acta Virologica 40**: 5-8.

WORLEY, J.F. and GILLASPIE, A.G., JR.(1975). Electron microscopy *in situ* of the bacterium associated with ratoon stunting disease in Sudangrass. **Phytopathology** 65 287-295.

(Received : September 1996 Revised : February 1997)

Madras Agric. J., 84(7): 377-380 July 1997
https://doi.org/10.29321/MAJ.10.A00892

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DETECTION OF SUGARCANE MOSAIC VIRUS

R. VSWANATHAN

Plant Pathology Section
Sugarcane Breeding Institute (ICAR)
Coimbatore 641 007

ABSTRACT

Sugarcane mosaic virus (SCMV) a member of Potyvirus group is an important disease of sugarcane. Currently, the virus is identified based on the foliar symptoms exhibitd by the virus on differential hosts. An indirect-ELISA was standardised to detect the presence of virus in the host very precisely. Antigen dilution of 1:500, antiserum dilution of 1:1000 and enzyme conjugate dilution of 1:8000 were found optimum for the detection of the suspected virus in the host. In highly susceptible varieties, SCMV presence was detected even at a dilution of 1×10^{-5} by ELISA test. Polyclonal antisera developed against SCMV strain-N was found suitable to detect suspected samples of other SCMV strains also. This technique will have greater applications in the routine quaratine work of this Institute.

KEY WORDS : Sugarcane mosaic virus, ELISA, detection, quarantine

Sugarcane mosaic virus (Potyvirus group) is one of the most potentially dangerous diseases of sugarcane and is widely distributed in the sugarcane growing countries. Importance of this disease was recognised in earlier decades after its inadvertent introduction into Argentina with PoJ canes brought in from Java (Artschwager and Brandes, 1958). Only Mauritius and Guyana are known to have SCMV free sugarcane crop (Agnihotri, 1983). Several strains of the SCMV were reported from different countries. In India, strains of A, B, C, D, E, F, H and N were reported to occur (Kondaiah and Nayudu, 1984). Yield reduction effected by SCMV was linked to the strain of SCMV in India (Rishi *et al.*, 1975).

Though much work has been done on strains of SCMV in India, work on detection of SCMV is still based on visual symptoms or symptom expression on differential hosts. Detection of SCMV by improved techniques like serological and nucleic acid probes have been standardised in other countries (Smith, 1996). These techniques are more reliable, less time consuming, efficient and able to detect very low concentrations of the virus present in the suspected samples. Precise detection of SCMV is essential as this virus is of quarantine importance in India and abroad. So an indirect ELISA technique was standardized for the detection of SCMV in sugarcane and the same is reported hereunder.

MATERIALS AND METHODS

Production of antiserum

SCMV strain N was selected for the production of antiserum. Infected sugarcane leaves macerated in 0.01 M phosphate buffer, pH 7.0 containing 0.2% 2-mercaptoethanol at the rate of 9 ml per gm leaf material and the virus was transferred to 10 to 15 days old sorghum seedlings (genotype Rio). Two weeks after inoculation infected leaf materials homogenized in 0.5 M Sodium borate buffer, pH 8.0 containing 0.01M EDTA and 0.2% thioglycollic acid. The homogenate was filtered through muslin cloth and the filtrate was mixed with chloroform and carbon tetra chloride (1:1) mixture to 10% and Triton X-100 to 5%. The mixture was stirred for about 30 min. and centrifuged at 8200 rpm for 10 min. The

Table 1. Standardisation of detection of SCMV by Indirect ELISA using different concentrations of antigen*

Variety	Antigen dilution					
	5:1	5×10^{-1}	5×10^{-2}	5×10^{-3}	5×10^{-4}	5×10^{-5}
CoC 671	0.961	1.314	1.166	1.077	0.871	0.777
Co 8021	0.997	1.134	0.936	0.732	0.495	0.270
Healthy	0.200	0.187	0.191	0.168	0.142	0.094

* Mean of 6 values; Absorbance at 405 nm

supernatant was centrifuged at 27,000 rpm for 120 min at 4°C. The obtained pellet was resuspended in 0.05M borate buffer, pH 8.0 and centrifuged at 8200 rpm for 10 min. The supernantant was overlaid on 10-40 % linear sucrose gradient columns and centrifuged at 26,000 rpm for 120 min. The light scattering zone was collected and centrifuged at 35,000 rpm for 120 min. The pellet dissolved in 0.05M borate buffer, pH 8.0 and the purified virus suspension was used for immunization in rabbits. Polyclonal antiserum was produced and IgG was purified from the serum. The method followed for virus purification is a modified procedure followed by Moghul and Francki (1976).

ELISA

Indirect-direct antigen coating ELISA test was performed as followed for the detection of sugarcane bacilliform virus (Viswanathan *et al.*, 1996). To standardize optimum concentration of antigen, SCMV infected leaf materials from varieties CoC 671, Co 8021 and SCMV free clone were extracted in carbonate buffer (pH 8.0). Different concentrations of antigen extracts were coated onto the plates (200 µl) and incubated for 18 h at 4°C. Followed by washings in PBS-Tween (PBST) antiserum diluted to 1:1000 in PBST containing 2% polyvinyl pyrrolidone (PVP) and 0.2% ovalbumin (PBSTPO) was coated and incubated at 37°C for 3 hrs. After subsequent washings anti-rabbit goat immunoglobulins conjugated with alkaline phosphatase (Sigma, USA) diluted in PBSTPO was coated to the wells (1:8000). After incubation and washings enzyme substrate *para* nitrophenyl phosphate in 10% diethanolamine buffer (1 mg/ml w/v) pH 9.8 was added to the wells and allowed for colour development at room temperature. Colour intensity was measured at 405 nm in a ELISA Reader (Model EL 311s, Biotech Instruments, USA). Leaf samples from different varieties were obtained and ELISA test was performed as followed earlier. Leaf sample from SCMV-free clone M1 maintained at the institute served as negative control throughout the study. While assessing the SCMV infection in different varieties by ELISA, values of known virus- free clone serve as negative control. When ELISA value of a suspected sample is more than 3 times of the negative control it was considered positive to the virus.

RESULTS AND DISCUSSION

Results showed that indirect ELISA was a reliable technique to detect SCMV in the suspected samples. In the first test antigen dilutions of 1:5 to 1:5,00,000 of both varieties showed higher values to healthy control. However, high concentration of 1:5 showed less colour development than those of 1:50 which indicates that viral antigen binding to the walls of wells was inhibited at the higher concentration. In general after 1:50 there was a gradual reduction in ELISA values upon dilution (Table 1). Antigen dilution of 1:500, antiserum dilution of 1:1000 and enzyme conjugate dilution of 1:8000 were found optimum for the detection of SCMV under Indirect-ELISA system. Based on this, leaf samples from different genotypes were screened for the virus positiveness. The results clearly established that all the clones showing mild to severe symptoms of the virus were positive to the virus. The leaf samples from the different varieties showed variation in their ELISA values indicating the differences in the antigen concentration among the genotypes (Table 2). Genotypes like 94069 and 94070 which were showing very mild or no clear mosaic symptoms recorded ELISA values more than 2 times to healthy control. All other samples recorded multiple differences in their values to the healthy control.

SCMV was recorded in India long back and several strains are reported in India (Kondaiah and Nayudu, 1984). Usually under field conditions

Table 2. Detection of SCMV in sugarcane genotypes by indirect ELISA

Genotype	Absorbance values 405 nm*
Co 419	0.630
Co 658	0.898
Co 740	0.538
Co 1148	0.384
Co 1158	0.306
Co 6914	0.795
Co 7717	1.089
Co 8014	0.628
Co 8150	0.748
Co 8370	1.409
Co 62101	1.064
CoC 671	0.630
CoC 86062	0.614
CoJ 64	0.418
CoS 770	0.776
94069	0.196
94070	0.202
Health control	0.083

* Mean of 6 values.

mixture of strains are infecting a sugarcane variety and this determines the manifestation of foliar symptoms. The present study showed a clear variation on ELISA values in different varieties (Table 2). Although the varieties are suspected to be infected by different strains, antiserum raised against Strain-N reliably detected the presence of SCMV in many varieties. Use of a common antiserum to detect poty viruses infecting sugarcane, sorghum, Johnson grass and maize was reported by Shukla *et al.* (1989). Though the virus is widely present in India its effect on yield depends on the strains (Rishi *et al.*,1975). During 1995-96 crop season total crop loss in zonal varietal clones at Mandya occurred (Viswanathan, Unpublished). Similarly, SCMV causing yield reduction in many sugar factory areas of coastal Andhra Pradesh was observed. Here disease severity was aggravated under the influence of abiotic stresses (M.V.Nayudu, Personal communication). Actual loss caused by the SCMV is not quantified in India and at present most of the varieties in the field are infected by the virus strains. The virus is transmitted through infected planting materials and different species of aphids are spreading the virus from plant to palnt in the field.

Sugarcane Breeding Institute maintains world sugarcane germplasm collections at its research centre, Cannanore, Kerala State. More than 3000 genotypes are housed there in a SCMV free condition. These germplasm lines are being sent to different sugarcane growing countries every year and very often new genotypes from other countries are added to germplasm collections. So, quarantining every material exchanged is essentially required. Unlike fungal infections, virus infection needs more precised techniques for the detection in the suspected clones. In this situation an improved technique like ELISA would be more appropriate and it may be an alternative technique to the use of differential hosts for identification and detection of SCMV. In this experiment antigen dilution of 5×10^{-5} gave positive to SCMV which indicates the high sensitivity of the technique. Use of polyclonal (Shukla *et al.*, 1989) and monoclonal (Lin and Chen, 1994) antisera in detecting the virus and differentiating the strains are reported. Recently a high titre polyclonal antiserum was raised against recombinant SCMV-A coat protein which recognise most of the strains of the virus (Smith, 1996). Dot immunobinding assay (DIBA) was also standardised and was found equally effective for virus indexing in canes (Viswanathan, 1996). However, this technique gives only the qualitative assay.

Recently presence of Sugarcane bacilliform virus was detected in sugarcane germplasm and techniques such as ELISA and immuno specific electron microscopy were standardized for the detection of the virus (Viswanathan *et al.*, 1996). Presence of yet another virus, sugarcane mild mosaic virus was also suspected in sugarcane (Alexander and Viswanathan, 1996). Presence of many viruses in vegetatively propagated crop like sugarcane necessitates more attention on quarantine and improved techniques for the virus detection. Techniques like indirect ELISA and DIBA were standardised for the detection of SCMV for effective quarantine in sugarcane. Techniques based on DNA probe and reverse transcriptase polymerase chain reaction (RT-PCR) were utilised recently to detect all the strains of the virus (Smith and Van de velde, 1994). In India research on SCMV was not given prominence as compared to other sugarcane diseases. SCMV detection through ELISA is one such aspect to revitalize work on SCMV at Sugarcane Breeding Institute and attempts are being made to develop probes based

on DNA probes and RT-PCR to enhance the specificity and precision in SCMV detection.

ACKNOWLEDGEMENTS

Author is thankful to the Director, Sugarcane Breeding Institute for providing facilities and to Dr.P.Sreenivasulu, Virology Department, S.V.University, Tirupathi for providing SCMV antiserum.

REFERENCES

AGNIHOTRI, V.P.(1983). **Diseases of Sugarcane.** Oxford and IBH Pub. Co. New Delhi. 363 pp.

ALEXANDER, K.C. and VISWANATHAN, R.(1996). Conservation of sugarcane germplasm in India given the occurrence of new viral diseases. **Sugarcane Germplasm Conservation and Exchange** (Croft, B.J., Piggin, C.M. Wallis E.S. and Hogarth, D.M. eds.) **ACIAR Proceeding** No.67, pp. 19-21

ARTSCHWAGER, E. and BRANDES, E.W.(1958). Sugarcane (*Saccharum officinarum* L.) **US Deptt. Agri. Handbook, 122**. 307 pp.

KONDAIAH, E. and NAYUDU, M.V.(1984). A key to the identification of sugarcane mosaic virus strains. **Sugarcane** 6: 3-8.

LIN, C.P. and CHEN, C.T.(1994). Application of biotechnology in detecting sugarcane diseases. In: **Current Trends in Sugarcane Pathology**. (Rao, G.P. Gillaspie, A.G. Jr., Upadhyaya, P.P. Bergamin, A., Agnihotri, V.P. and Chen C.T. eds.) IBPSS, Delhi, pp. 221-233.

MOGHAL, S.M. and FRANCKI, R.I.B.(1976). Towards a system for the identification and classification of potyviruses. I. Serology and aminoacid composition of 6 distinct potyviruses. **Virology 73**: 350- 362.

RISHI, N., BHARGAVA, K.S. and JOSHI, R.D.(1975). Effect of mosaic virus in sugarcane. **Int. Sugar J., 77**: 298-299

SHUKLA, D.D., JOSIC, M., JILKA, J., FORD, R.E., TOLER, R.W. and LANGHAM, M.A.C. (1989). Taxonomy of poty viruses infecting maize, sorghum and sugarcane in Australia and the United States as determined by reactivities of polyclonal antibodies directed towards virus-specific N-termini of coat proteins. **Phytopathology 79**: 223-229.

SMITH, G.R. (1996). Sugarcane mosaic and Fiji disease. In:**Sugarcane Germplasm Conservation and Exchange** (Croft, B.J. Piggin, C.M. Wallis, E.S. and Hogarth, D.M. eds). **ACIAR Proceedings No. 67**, pp. 120-122.

SMITH, G.R. and VAN DE VELDE, R. (1994). Detection of sugarcane mosaic virus and Fiji disease virus in diseased sugarcane using the polymerase chain reaction. **Plant Dis. 78**: 557-561.

VISWANATHAN, R. 1996. Immunological techniques for the detection of sugarcane virus and MLOs. **Annual Report of 1995-96, Sugarcane Breeding Institute**, Coimbatore.

VISWANATHAN, R., ALEXANDER, K.C and GARG, I.D. (1996). Detection of sugarcane bacilliform virus in sugarcane germplasm collections. **Acta virologica 40**: 5-8.

(Received : November 1996 Revised : February 1997).

Madras Agric. J., 84(7): 380-382 July 1997

RESEARCH NOTES

IMPACT OF RETTING WATER ON QUALITY OF COIRPITH

Coirpith is a non-disposable waste product of coir industry. Its use as a soil conditioner in tropical farming is well established (Nagarajan *et al.*, 1991). The compostion of coirpith depended on fertility status of the coconut garden, methods of coir extraction, disposal and other environmental factors (Savithri and Hameed Khan, 1994). The degree of acidity/basidity and salinity of coirpith is the major problem that limits its utilisation in agriculture. So, the present work was under taken to study the quality of coirpith in selected districts of Tamil Nadu.

The coirpith and water (used for retting) samples were collected from selected districts of Tamil Nadu in order to judge the quality of coirpith as influenced by ground water (Coimbatore, Periyar and Salem), river water (Trichy and Thanjavur), and Coastal backwater (South Arcot, Nagai-Quaide-E millath and Kanyakumari). Coirpith samples were analysed for pH, EC, cellulose, hemicellulose and lignin. Water samples were analysed for salinity and sodicity.

The analytical values of coirpith and the water (used for retting) samples are presented in Table 1. The coirpith obtained from various industries located in different regions did not show marked variations in the pH, though the general trend was acidic due to their composition. But slightly higher values were recorded in the samples collected from coastal districts of Kanyakumari, Nagai Quaid-E-Millath and South Arcot. This variations might be due to the variations in the EC of water used for retting in different location. A positive correlation (r=0.959**) established between the EC