on the basis of their reaction to red rot on 0 9 scale (Srinivasan and Bhat, 1961). All the reactions of the twelve isolates were compared with the standard isolates of the red rot pathogen.

The results revealed that the sugarcane differentials/genotypes tested varied much in their reaction to different isolates of red rot pathogen by plug method of inoculation. Out of twelve isolates, six isolates viz., *Cf* 671, *Cf* 6304, *Cf* 86032, 93V297, *Cf* 91017 behaved similar to *Cf* 06 and the isolates *Cf* 90063, *Cf* 95045 were similar to *Cf* 10. The isolate Co 95020 was similar to *Cf* 04. Hence, the differential study conducted from 2005 2011 indicated the existence of only three pathotypes of red rot pathogen in Tamil Nadu and there is no introduction of new pathotypes.

Reference

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COMPARISON OF DAC-ELISA AND DUPLEX IC-RT-PCR FOR THE DIAGNOSIS OF *SUGARCANE STREAK MOSAIC VIRUS* AND *SUGARCANE MOSAIC VIRUS* IN SUGARCANE

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Mosaic is one of the most widespread diseases of sugarcane which is caused by *Sugarcane streak mosaic virus* (SCSMV) and *Sugarcane mosaic virus* (SCMV) either alone or in combination in India. The mosaic disease causes decrease in yield significantly through varietal degeneration. Also this is an important quarantine disease during germplasm exchange. Precise diagnosis is the most effective way in identifying the disease and it prevents introduction of the disease through germplasm exchange. Always the disease does not cause diagnostic symptoms in sugarcane. Symptoms caused by other viruses and certain nutritional deficiencies also mimic mosaic like symptoms. Hence, it is essential to develop a precise diagnostic technique to maintain an efficient monitoring program that can aid in disease management. Ideally, methods for the detection of plant virus should be rapid, specific, sensitive, inexpensive and amenable for large scale detection.

Currently, serological and nucleic acid procedures and/or combinations of both are in vogue. Serological techniques like direct antigen coating-enzyme linked immunosorbent assay (DAC-ELISA), dot blot immune assay (DBIA) and tissue blot immune assay (TBIA) used for plant virus diagnosis. Nucleic acid procedures such as reverse transcriptase polymerase chain reaction (RT-PCR), immunocapture reverse transcriptase-PCR (IC-RT-PCR), micro array and real time-PCR assays can be performed for the detection of plant viruses. DAC-ELISA is the most commonly preferred method because of its simplicity, reliability, cost effectiveness and large no of samples can be tested in a relatively short period of time. Although serological methods are commonly used, they have certain disadvantages in which the technique is based on the antigenic properties of the virus coat protein that represents only 10% of the total virus genome. However, this technique is unsuitable for detecting

mixed infections.

Obviously, nucleic acid-based diagnostic assays became the method of choice. RT-PCR is a popular technique for detection of plant viruses containing RNA as a genome. It is the "gold standard" molecular method used for the detection of plant viruses due to its high sensitivity and specificity. However, detection of several individual viruses separately by RT-PCR is expensive and time consuming. To reduce this, a duplex RT-PCR has been developed to detect SCMV and SCSMV together (Viswanathan *et al*., 2008).

A further refinement of PCR is IC-RT-PCR, wherein the virus particles are captured by immobilized antibodies for the detection of a specific virus. The detection of plant viruses by this method has gained popularity as it often improves the sensitivity and specificity of the assay, reduces the problems from PCR inhibitors in the sample and provides faster and cheaper method for preparing template for amplification. This method is especially used in concentrating virus particles where virus titer is low, or where compounds that inhibit PCR are present. Thus, IC-RT-PCR is used in alternative to RT-PCR for the detection of plant viruses. We have expressed SCSMV coat protein and polyclonal antisera were raised against the expressed protein (Viswanathan *et al*., 2011). Furthermore, studies were conducted to standardize IC-RT-PCR to detect SCSMV and SCMV with more specificity and sensitivity.

A total of 30 samples of different varieties of sugarcane which varried in symptoms from severe mosaic symptoms to asymptomatic were collected. The samples were first diagnosed by DAC-ELISA using recombinant SCSMV coat protein antiserum in which it gave both positive and negative values. Some of the samples with mild symptoms were found to be negative in the assay, hence it warranted further confirmation of negative samples employing molecular tools. In order to accomplish this, a duplex IC-RT-PCR was developed for a simultaneous diagnosis of both the viruses in a single reaction. Here, the samples were homogenized using phosphate buffer and the crude extracts from them were used as antigen to detect the samples. From DAC-ELISA results, 12 negative samples were diagnosed by duplex IC-RT-PCR using recombinant SCSMV-coat protein antiserum. Among them, eight samples were positive to SCSMV and eleven samples were positive to SCMV, when diagnosed by IC-RT-PCR which indicated that this technique is more sensitive than DAC-ELISA and the results are illustrated by specific bands to SCSMV (~690bp) and SCMV (~380bp) in a single reaction. The antiserum was found to trap both the viruses in IC-RT-PCR. Our assays very clearly indicated that five varieties were positive to SCSMV in both DAC-ELISA and IC-RT-PCR, which confirms that these samples are infected only by SCSMV and not by SCMV. Four varieties were found to be negative to both in DAC-ELISA and duplex IC-RT-PCR. Another four varieties showed mixed infections of the two viruses when diagnosed by duplex IC-RT-PCR.

Comparison of IC-RT-PCR with DAC-ELISA revealed that the samples which are negative in DAC-ELISA were positive in IC-RT-PCR and that establishes higher sensitivity of the former technique. Although DAC-ELISA was found to be simple and cost effective to diagnose the virus, in samples with very low titre, IC-RT-PCR would be a more sensitive technique to detect the virus(es). Our study very clearly establishes that IC-RT-PCR is more sensitive to detect the viruses present in low titre more specifically than DAC-ELISA. This is the first report on the use of duplex-IC-RT-PCR to detect SCSMV and SCMV in sugarcane. Outcome of the study is expected to have applications in

sugarcane quarantine and virus diagnosis to develop healthy nursery programmes in sugarcane.

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VALIDATION OF SPECIES SPECIFIC PRIMERS FOR DIAGNOSIS OF *COLLETOTRICHUM FALCATUM* IN SUGARCANE TISSUES AND SOIL

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Conventionally infection of red rot pathogen *Colletotrichum falcatum* in sugarcane is diagnosed visually based on symptom expression and tissue bioassay is applied to confirm the pathogen association. Direct microscopy and immunological methods such as enzyme linked immunosorbant assay (ELISA) (Viswanathan *et al*.,2000) have been developed and used to certain extent to detect the pathogen in the suspected cane tissue . However these methods have their own limitations such as requiring longer period of fungal development, inability to detect the pathogen in mixed state of infection or in the contaminated soil and they also have limitations of identifying variability in *C. falcatum* population. To overcome all these problems, attempts have been made to design specific primers based on conserved gene sequences which are capable of identifying *C. falcatum* under any situation. Earlier studies on comparative analysis on conserved gene sequences with other species of *Colletotrichum* indicated genetic divergence of *C. falcatum* from other species and it resolved *C. falcatum* specific primer sets from 5.8S-ITS, actin, calmodulin, chitin synthase 1A (CHS-1A) and Glyceraldehyde 3-phosphate dehydrogenase (GPDH) sequences. Among these primers, actin, calmodulin and CHS-1A based primers have been selected based on their specificity compared to other *Colletotrichum* species and accuracy for detection in sugarcane tissues (Malathi *et al*., 2008). Subsequently these primers have been validated with different screening methods and also standardized to detect the pathogen in the soil. Besides attempts have been made to develop probe based diagnosis based on specific nucleotide sequence.

Application of molecular diagnostics in various screening methods

To include molecular diagnostic techniques in red rot screening programme standardized location of tissue sampling, methods and intervals of sampling for various methods of inoculation. Standardization has been done with 16 varieties for different methods of inoculation. For nodal method of inoculation under field conditions, sampling at 6-8 days interval under symptomless condition helped to differentiate the host resistance by PCR based diagnostics, while in controlled condition testing method, 4-6 days interval is required to confirm the resistance. Invariably for controlled condition testing and field screening with nodal method the top portion of cane has to be split up