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Research Article

CHARACTERIZATION OF SUGARCANE MOSAIC VIRUS (SCMV) CAUSING MOSAIC DISEASE IN SUGARCANE

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Abstract: Sugarcane mosaic caused by Sugarcane Mosaic Virus (ScMV) is a serious problem in India's sugarcane production. ScMV was first reported in India from Pusa during 1921 in sugarcane variety D-99 and now it has been reported in every sugarcane growing areas across India due to its perpetuation through vegetative cuttings and regarded it as a potential threat to sugarcane industry. Our present study was focused on characterization of ScMV and use of PGPR strains to manage the disease. A survey was undertaken in sugarcane grown areas of Andhra Pradesh, India and were found the sugarcane aphid (*Melanophis sacchari*) and corn leaf aphid (*Rhopalosiphum maidis*) as potential vectors for ScMV. Vector transmission was confirmed using DAC-ELISA. Further, the ScMV was detected in diseased leaves through DAC-ELISA and RT-PCR during our survey. Scanning Electron Microscopy (SEM) was also used to detect ScMV from diseased leaf samples. The results showed that all the leaf samples collected were shown positive reaction to the presence of ScMV in RT-PCR with a band at around 0.98 kbp. Further, asymptomatic leaves were also have shown positive reaction with RT-PCR for the presence of ScMV. Whereas, SEM studies showed the presence of poty-virus filamentous particles related to ScMV.

Keywords: Sugarcane, Mosaic, Molecular Characterization, ELISA electron microscopy

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Introduction

Sugarcane is a pre-eminent commercial crop of India and is grown in tropical and subtropical regions. The crop is cultivated to a tune of 4.5 M ha in India with an annual production of 27.2 MMT [20]. Sugarcane productivity is hampered by several biotic stresses of which plant diseases cause a major havoc. Approximately, 55 diseases of sugarcane caused by fungi, bacteria, viruses and phytoplasmas and nematodes have been reported in India [9, 11]. Several disease epidemics in sugarcane have also been reported in India like red rot, smut, wilt, rust, leaf scald and viral diseases [19]. The extent of damage of sugarcane disease epidemics would depend on the nature of disease and its spread on the affected varieties. Despite continuous efforts in the areas of breeding for disease resistance, sugarcane is subjected to economic losses due to incidence of plant diseases. Sugarcane Mosaic disease caused by Sugarcane Mosaic Virus (ScMV) is hitherto a minor disease and is now considered a major one especially in states like Andhra Pradesh [22]. Sugarcane mosaic was first reported in India from Pusa, in 1921 on sugarcane variety D 99 and Sathi 131, an indigenous cane of Bihar [1]. In India, Sugarcane Mosaic disease oflate is causing significant yield losses [2, 4, 8 15, 22]. Earlier, in India, sugarcane mosaic disease was perceived to be caused by different strains of ScMV [7, 5, 2]. However, researchers have later established new strains of mosaic disease in sugarcane in India. For example, [12] Hema et al. reported Sugarcane streak mosaic virus (ScSMV) as the new casual virus of the mosaic disease in tropical India. This confirms that the mosaic disease on sugarcane in India is not only caused by the strains of ScMV subgroup but also by the newly described ScSMV. Both ScMV and ScSMV have been studied more extensively in India. Research results have indicated that ScSMV is the most widely spread and major cause of mosaic disease complex in India on sugarcane [16, 19, 21]. However, both the SCMV and SCSMV cause significant yield losses in promising cultivars of sugarcane throughout India [8, 12, 15]. Mixed infection of SCMV and SCSMV were also recorded on commercial crops of sugarcane all over India [18, 17].

Management of viral diseases in crop plants is an uphill task especially when the diseases assume an epidemic form. For timely control of viral diseases, and to reap a satisfactory harvest, understanding the disease progression over years is mandatory. For this, a comprehensive survey in determining the prevalence of Sugarcane Mosaic Disease, the extent of losses it causes and farmers' practices in overcoming the disease is essential. Sugarcane, in Coastal Andhra Pradesh is grown approximately to a tune of 65,000 ha and viral diseases such as Mosaic and Yellow Leaf Disease cause sizeable losses. Further, cane and jaggery quality is also deteriorated with the incidence of these viral diseases [19]. Sugarcane Mosaic disease incidence in Andhra Pradesh, India is also assuming a major biotic stress and several popularly grown cultivars are showing increased susceptibility over years. It is precisely at this juncture, understanding the exact susceptibility of ruling cultivars in a particular area over time to mosaic disease is essential. This is very important since it facilitates in advocating effective management strategies to sugarcane farmers for timely interventions. Our present research therefore attempted to understand the prevalence of sugarcane mosaic disease, its prevalence, determining the risk and sensitive areas in Coastal Andhra Pradesh since 2010. Further, comprehensive understanding on varietal susceptibility, vector transmission, serological studies and ultra structural studies using Scanning Electron Microscopy for confirming viral diseases in Coastal Andhra Pradesh was carried out. Our long term goal is to manage Sugarcane Mosaic Disease and through healthy seed material.

Material and Methods

Survey for incidence of Sugarcane Mosaic Disease and identification of hot spot areas

A survey was undertaken in Coastal Andhra Pradesh in selected districts such as Visakhapatnam, Vizianagaram, Srikakulam, and East Godavari districts from 2010-'11 to 2016-'17. Surveys were conducted thrice in a crop year in the selected districts.

A total of 10 mandals were selected in each district and three villages from each mandal. Mosaic incidence was recorded from 10 selected plots in each village and the data were pooled to arrive at a mean Mosaic disease incidence. Same villages were visited every year and proper care was ensured to visit the same farmers' fields every year from 2010-'11 through to 2016-'17. The per cent mosaic incidence was calculated and the mandals were categorized as mosaic incidence per cents as <10%; 11-16%; 17-23%; 24-37%; and 38-65% and above. Areas with mosaic incidence of 38-65% and above were categorized as High risk and sensitive areas and these areas were mapped using Global Positioning Systems duly recording the coordinates.

Cultivar susceptibility to Sugarcane Mosaic Virus

In screening trials for incidence of viral diseases at Regional Agricultural Research Station, Anakapalle, the mean disease incidence (%) of Sugarcane Mosaic Disease was enumerated based on visual observations annually. The cultivars that were selected for the present study were 87A298, 2003V46 and Co86032, and these cultivars are the popularly grown cultivars in Coastal Andhra Pradesh. Data on % Mosaic incidence on these cultivars were recorded from 2010-'11 to 2016-'17.

Confirmation of Mosaic Disease using serological, molecular and scanning electron microscopy

Serological Assays (DAC-ELISA)

Direct Antigen Coating Enzyme Linked Immunosorbent Assay (DAC-ELISA) was carried out using the kit obtained from ScMV specific antibodies obtained from Bioreba, Germany by following the standard protocol as detailed herewith. The plates were first coated with coating antibody supplied with the kit (diluted with coating buffer in 1:10 dilution) @ 100 µl per well, covered tightly and incubated for 4hrs at Room temperature i.e. 21-240C (RT) in a humid box. In the second step, wells are coated with 100 µl of the diseased leaf extract/aphid vectors, duly preparing the loading diagram along with positive and negative control. The plate was incubated at 4C overnight at for the binding of the antigen on the plate walls in a humid box. The enzyme conjugate was prepared just before use by diluting 1000 times in conjugate buffer and coated to the wells @ 100 µL each and incubated at RT for 2.5 h. In the final step PNP substrate was added to the wells 100 µl each and incubated at RT in dark for 30 to 60 minutes and observed for colour development. After each step, the wells are emptied and washed thoroughly with PBST washing buffer for 4-6 times. Observations were taken visually and also photometrically at 405 nm using Thermo Fisher Scientific Multiskan - X, ELISA reader and the readings are documented.

Molecular Studies (RT-PCR)

For RT-PCR, the protocols adopted by [13] Chatenet et al were used with slight modifications. Total RNA from sugarcane leaves showing mosaic symptoms by using standard protocols. Total RNA was eluted in a final volume of 40 µL of diethylpyrocarbonate-treated (DEPC) water and stored at -20C. RT-PCR assays to detect SCMV with primer pairs as detailed below were used according the protocol suggested by [10]. RT-PCR assays to amplify fragments specific to potyviruses of Poaceae with primer pair oligo 1 n-oligo2n were performed according to [6]. The RT-PCR program was 50C for 30 min, 95C for 15 min, 30 cycles at 94C for 1 min, 50C for 1 min and 72C for 1 min with a final 72C extension for 5 min. A 10 µL aliquot of each amplified product was analyzed by electrophoresis through a 1.2% agarose gel.

Primer Code	Primer Sequence	Location	Expected Amplicon Size
SCMV-F3 (24 mer)	5'-TTT YCA CCA AGC TGG AA-3'	CP	0.98 kbp
SCMV-R3 (24 mer)	5'-AGC TGT GTG TCT GTC TGT ATT CTC-3'	CP	0.98 kbp

Scanning Electron Microscopy (SEM)

Sugarcane Mosaic Virus-infected leaf material from sugarcane plants were collected from surveyed areas. Later, partially-purified leaf extracts were prepared from one gram of leaves according to the protocol described by [3]. Preparations were later used for observation by Scanning Electron Microscopy using standard leaf-dip protocols.

Results

Survey for incidence of Sugarcane Mosaic Disease and Identification of Hot spot Areas

Our survey results indicated that the incidence of mosaic disease steadily increased over years (2010-'11 through to 2016-'17) in the surveyed areas. The incidence was least during 2010-'11 (2%) and progressed steadily and reached peak during 2016-'17 (41%). In general, the higher incidence of mosaic disease was observed since 2013-'14 (>20%) [Fig-1]. Higher incidence of sugarcane mosaic disease in Coastal AP is attributed to increased susceptibility of all cultivars.

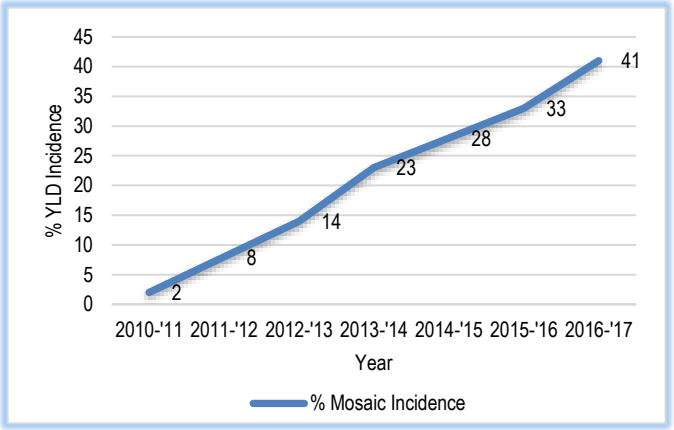

Fig-1 Incidence of Mosaic disease on sugarcane in Coastal Andhra Pradesh, India during 2010-'17.

Our survey results have also indicated that in the surveyed areas, mosaic disease incidence was least (11-16%) in Narsipatnam, Etikoppaka, Devarapally mandals (Visakhapatnam); Rajam, Salur, Jami, Ramabhadrapuram, Terlam, Bobbili, Merakamudi, Gajapathinagaram, Parvathipuram and Nemalam mandals (Vizianagaram); Sankili, Santhakaviti and Mandasa mandals (Srikakulam). High risk and sensitive areas (38-65%) in these districts include Munagapaka, Atchutapuram, Kasimkota and Anakapalle mandals (Visakhapatnam) [Fig-2].

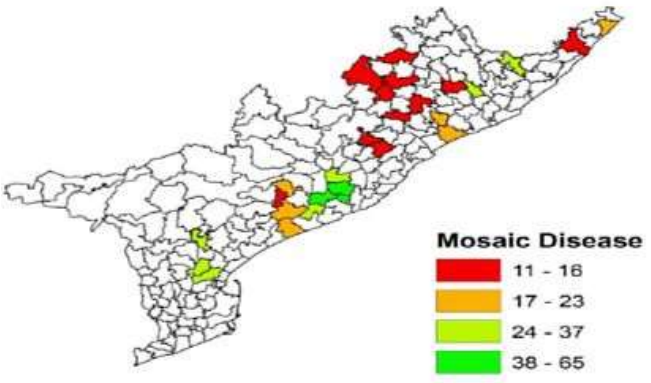

Fig-2 Mean Mosaic disease incidence on sugarcane in different mandals of Visakhapatnam, Vizianagaram, Srikakulam and East Godavari districts of Andhra Pradesh, India during 2010-'16.

Cultivar Susceptibility to Sugarcane Mosaic Virus

Further, our studies at experimental fields of Regional Agricultural Research Station, Anakapalle indicated that all the popularly grown sugarcane cultivars such as 87A298, 2003V46 and Co86032 have shown increased susceptibility in general over years from 2010-'11 through to 2016-''17. As a slight exception to this, marginal decrease in mosaic incidence was noticed on the cultivar, 87A298 in 2012-'13 (10%) when compared to during 2011-'12 (12%) [Fig-3]. Highest incidence of mosaic disease (36% in 87A298; 38% in 2003V46; and 46% in Co86032) was recorded on all the three cultivars during 2016-'17. Overall, our results suggest that all the three sugarcane cultivars under study were found susceptible to mosaic disease over due course [Fig-3].

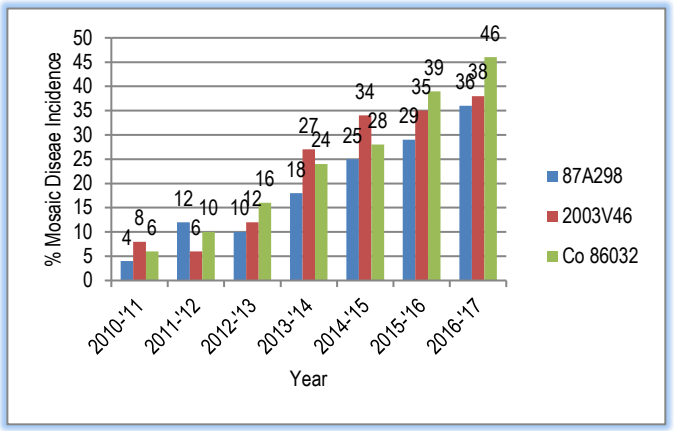

Fig-3 Mean per cent Mosaic disease incidence in popularly grown sugarcane cultivars of Coastal Andhra Pradesh, India during 2010 to 2017.

Confirmation of Mosaic Disease using serological, molecular and scanning electron microscopy

Serological and Molecular Studies

Further, the ScMV was detected in diseased leaves through DAC-ELISA and RT-PCR. The samples collected during survey and the tissue culture seedlings were tested for the presence or absence of the virus using DAC- ELISA and the absorbance values were recorded at OD 405 nm. In case of + ve reaction the OD values ranged from 2.66 to 2.515, but in case of – ve reaction the values are 0.254 to 0.212. Based on these results presence or absence of the virus was detected. Two types of aphid samples were observed from the mosaic infected sugarcane plants surveyed during the study period. The identification carried out by using aphid species identification keys. The two aphid species collected during the survey were identified as sugarcane aphid- *Melanaphis sacchari* (Zehntner) and corn leaf aphid- *Rhopalosiphum maidis* (Fitch).

RT- PCR

The samples collected during survey were tested for the presence or absence of the virus using RT-PCR. Even though two of the samples didn't show any symptoms at field level, all the samples showed positive reaction for the virus in RT-PCR test with presence of a band at around 0.98kbp length confirming the presence of ScMV in all samples [Fig-4].

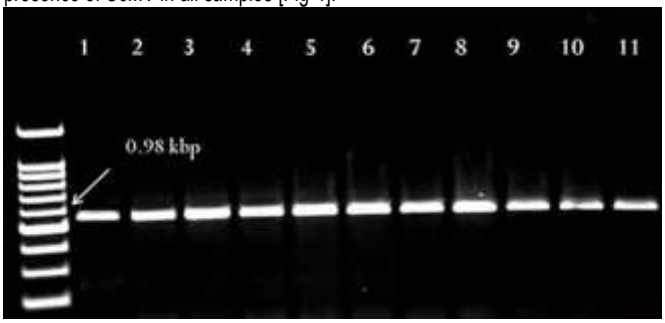

Fig-4 Agarose gel 1.2% showing the RT-PCR amplification product obtained from using the sugarcane mosaic specific primers (SCMV- F3 and SCMV- R3) Lane showing positive PCR amplification.

Scanning Electron Microscopy

Scanning Electron Microscopy (SEM) results have indicated the SEM studies showed the presence of non-enveloped, flexuous filaments characteristic of the poty-viridae family. The amount and size of particles varied among different diseased samples. The mean particle size was measured up to 800 nm length and 15 nm in width in SEM [Fig-5].

Discussion and conclusion

Our studies indicated the prevalence of Sugarcane Mosaic Disease in Coastal Andhra Pradesh. Further, popularly grown cultivars of Coastal Andhra Pradesh are being prone over time to mosaic, thus indicating the need to act swiftly in devising plant protection tactics comprehensively to this disease. In the surveyed districts, there was an increase in mosaic disease over time and the hot spot areas in each of the surveyed districts are of concern [Fig-2].

Fig-5 Scanning Electron Microscopy studies of diseased sugarcane leaf samples infected with mosaic disease showing sugarcane mosaic virus (ScMV).

Steady increase in mosaic disease from 2010-'11 to 2016-'17 over years [Fig-3] is majorly attributed to poor vector management and rationing of mosaic diseased crop. Earlier reports also established the relationship between high mosaic disease with use of diseased seed material, monocropping, and increased number of rationing and poor vector management [21]. In particular, aphids play a significant role in spread of virus diseases of sugarcane, thereby causing huge economic losses [14]. Proper care hence must be taken to educate the farmers on disease progression through various factors and on the ambient climatic conditions that prevail for taking up the prophylactic measures to overcome the same. In our studies, all the popularly grown cultivars have shown susceptibility over time from 2010-'11 to 2016-'17 to mosaic incidence [Fig-3]. Increased susceptibility of CVs: 87A298, 2003V46 and Co86032 over time to mosaic disease is also majorly attributed to increased number of rationings, use of diseased seed material and poor vector management. Our vector transmission studies have established the presence of virus particles in aphids collected from diseased plants/fields. Previously, researchers have established that proper vector management in conjunction with other virus management strategies can significantly bring down mosaic and other viral disease incidences in sugarcane [14].

Application of research: It is precisely at this juncture, the role of Integrated Disease Management of viral diseases assumes significance.

Research Category: Plant Virology

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