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ORIGINAL PAPER

First report of Maize yellow mosaic virus (MaYMV) infecting sugarcane in India and its molecular characterization

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Abstract

Maize yellow mosaic virus (MaYMV) was first reported in the field-grown maize crop showing mosaic and foliar yellowing symptoms in Yunnan provinces of China during the year 2016. Since then, its widespread occurrence has been reported in many countries of Africa, China, and South America in maize crop and other related hosts, sugarcane, sorghum, and *Panicum* sp. In India, we studied the prevalence of the virus in sugarcane during 2019–2020 by RT-PCR assays using the MaYMV specific primers designed from the consensus ORF 3 and ORF 4 regions. Our studies confirmed MaYMV infection in 14.15% of the sugarcane leaf samples in RT-PCR assays followed by sequencing. The pairwise multiple sequence alignment of all the 15 consensus sequences of this study from sugarcane had shown the highest nucleotide similarities with 97.4% to 100% among themselves as well as to other MaYMV sequences of maize from Africa and China and mainly, all the *Saccharum* isolates of China. In phylogenetic analysis, all the isolate sequences from this study and others retrieved from GenBank representing all the reported countries clustered together in a single clade. To the best of our knowledge, this is the first and new report of MaYMV infecting sugarcane in India, which necessitates more focused research on mixed infections of sugarcane viral diseases in perspectives of management.

Keywords MaYMV · Sugarcane · RT-PCR · India

Introduction

Sugarcane is an important commercial crop grown in more than a hundred countries, and it meets ~80% of the sugar needs of the world. Apart from sugar, it is considered the most viable alternative for renewable bioenergy production in some sugarcane-producing countries (Matsuoka et al. 2014). India is the world's second-largest sugarcane producer after Brazil. Viral diseases can be considered as universal production constraints to sugarcane cultivation because of their increased incidences, new reports of emerging viruses/strains and severe losses incurred by them in terms of cane yield and quality in most sugarcane growing countries, including India. Mosaic, leaf fleck, yellow leaf, fiji disease, and red leaf mottle are the major sugarcane viral diseases reported in different countries to date (Filloux et al. 2018), of which mosaic, leaf fleck, and yellow leaf caused by Sugarcane mosaic virus (SCMV) and Sugarcane streak mosaic virus (SCSMV), Sugarcane bacilliform virus (SCBV) and Sugarcane yellow leaf virus (ScYLV), respectively are the major constraints in India (Viswanathan et al. 2018). The viral diseases, especially mosaic and YL, cause severe yield losses of more than 50%, with 38.9–42.3% reduction in plant growth traits and 34.15% reduction in juice yield in the susceptible varieties under field conditions (Bagyalakhsmi et al. 2019, Viswanathan et al. 2014). Prevalence of the SCBV in India in all the *Saccharum* species viz. *S. officinarum*, *S. barberi*, *S. robustum, S. spontaneum,* and *S. sinense* is reported as a serious hindrance to exchange the germplasm materials (Viswanathan et al. 1996; Viswanathan and Premachandran 1998).

Recently, Maize yellow mosaic virus (MaYMV) was reported in the field-grown maize crop showing mosaic and foliar yellowing symptoms in Yunnan provinces of China during 2016 by deep sequencing platform of small RNAs and Sanger sequencing of RT-PCR samples (Chen et al. 2016). The sequence comparison and phylogenetic analyses of the entire 5642 nucleotides (nt) identified the virus as a new novel member of the genus *Polerovirus* in the

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family *Luteoviridae*. Since then, its widespread occurrence was reported in other countries as a potentially emerging virus and extended to other plants in the family Poaceae viz. sugarcane (*Saccharum* spp.) and itch grass (*Rottboellia cochinchinensis*) in Nigeria (Yahaya et al. 2017), maize in Burkina Faso (Palanga et al. 2017), Ethiopia (Guadie et al. 2018), and Tanzania (Read et al. 2019) in Africa; Brazil (Goncalves et al. 2017) and Ecuador (Bernreiter et al. 2017) in South America; and recently in *Panicum miliaceum*, and *Sorghum bicolor* in South Korea (Lim et al. 2018a, b). In Asia, MaYMV occurrence has been reported on *Zea mays* from China and South Korea and *Saccharum* hybrids from China. MaYMV reports from other countries on Poaceae crops suggest that it can be a new constraint to maize production worldwide along with other maize infecting viruses such as Sugarcane mosaic virus (SCMV) and Maize chlorotic mottle virus (MCMV) as mixed infections (Wang et al. 2017; Redinbaugh and Stewart 2018). Sugarcane being a vegetatively propagated crop, dissemination of the viruses occurs through stem cuttings (setts) used for commercial planting and germplasm exchange. Hence, there is a need for continuous monitoring of new viruses or new strains or genotypes of the viruses in India, the second major sugar-producing country. Earlier, we reported new viruses SCBV and ScYLV and characterized SCSMV as a new genus from India (Viswanathan, 2002; Viswanathan et al. 1996, 2008). Further studies were conducted to identify and characterize new viruses infecting sugarcane, and we report the occurrence of MaYMV from Indian sugarcane varieties for the first time based on RT-PCR assays and sequence analysis.

Materials and methods

Based on the recent reports of the widespread occurrence of MaYMV in many of the agriculturally important Poaceae crops in Asia, Africa, and South America, we suspected the possible occurrence of the virus in India. Hence, during 2019–2020, we have initiated our investigation with 106 sugarcane samples with mosaic, yellow leaf, and mixed symptoms of both the diseases in commercially cultivated varieties, foreign hybrids, and germplasm collections at ICAR-Sugarcane Breeding Institute, Coimbatore (11.006° N, 76.92° E) and its research Centre, Agali, Kerala (11°16'N, 76°68'E). First leaves in each of the clones were collected and stored at -80 °C until further processing.

Total RNA was extracted from the collected leaf samples (100 mg), ground in liquid nitrogen, and resuspended in 1 mL TRI Reagent (Sigma, USA) by following the manufacturer's protocol. The pellet was dissolved in a final volume of 30 µL RNase-free water and stored at -80 °C. The total RNA was treated with DNase I (1U/ µL) (Thermo Fischer scientific, USA) along with 10 × reaction buffer with $MgCl_2$ and RNase free water to make up the final volume 10 µL, kept at 37 °C for 2 h incubation in water bath and were enzyme de-activated by adding 1 µL of 50 mM EDTA and continued incubation at 60–65 °C for 10 min. The integrity, concentration, and purity of RNA were assessed on 1% EtBr-stained agarose gel and a Nanodrop™ 2000 Spectrophotometer (Thermo Scientific, USA), and stored at -80 °C. MaYMV specific primers, MaYMV-FP: 5' CGCGCTCGCAATAAT AACCG 3' and MaYMV-RP: 5' TTCTGATGAGTCGCG CCAAA3' were designed from the consensus ORF 3 and ORF 4 regions retrieved from the GenBank using Primer blast with an expected amplicon length of 453 bp. These sequences covered the partial cds of ORF of 3 and 4, coding the partial coat protein (CP) and the movement protein (MP).

Fig. 2 Phylogenetic tree constructed by the maximum likelihood method using Tamura-Nei model with nearest neighbour joining interchange tree options (MEGA X v.10.1.6) with 1000 bootstrap replications (Kumar et al., 2018), showing the closest genetic relationship of MaYMV sugarcane isolates from this study and other MaYMV isolates sequences of *Zea mays* retrieved from GenBank database reported from Africa, South America, and China and *Saccharum* sp from China. Bootstrap values are expressed as percentage of 1000 replications, and branch lengths are proportional to the number of substitutions. ScYLV was used as an outgroup

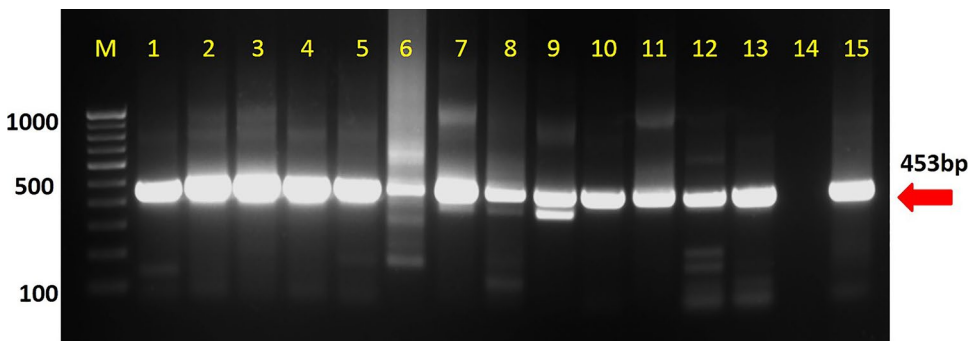
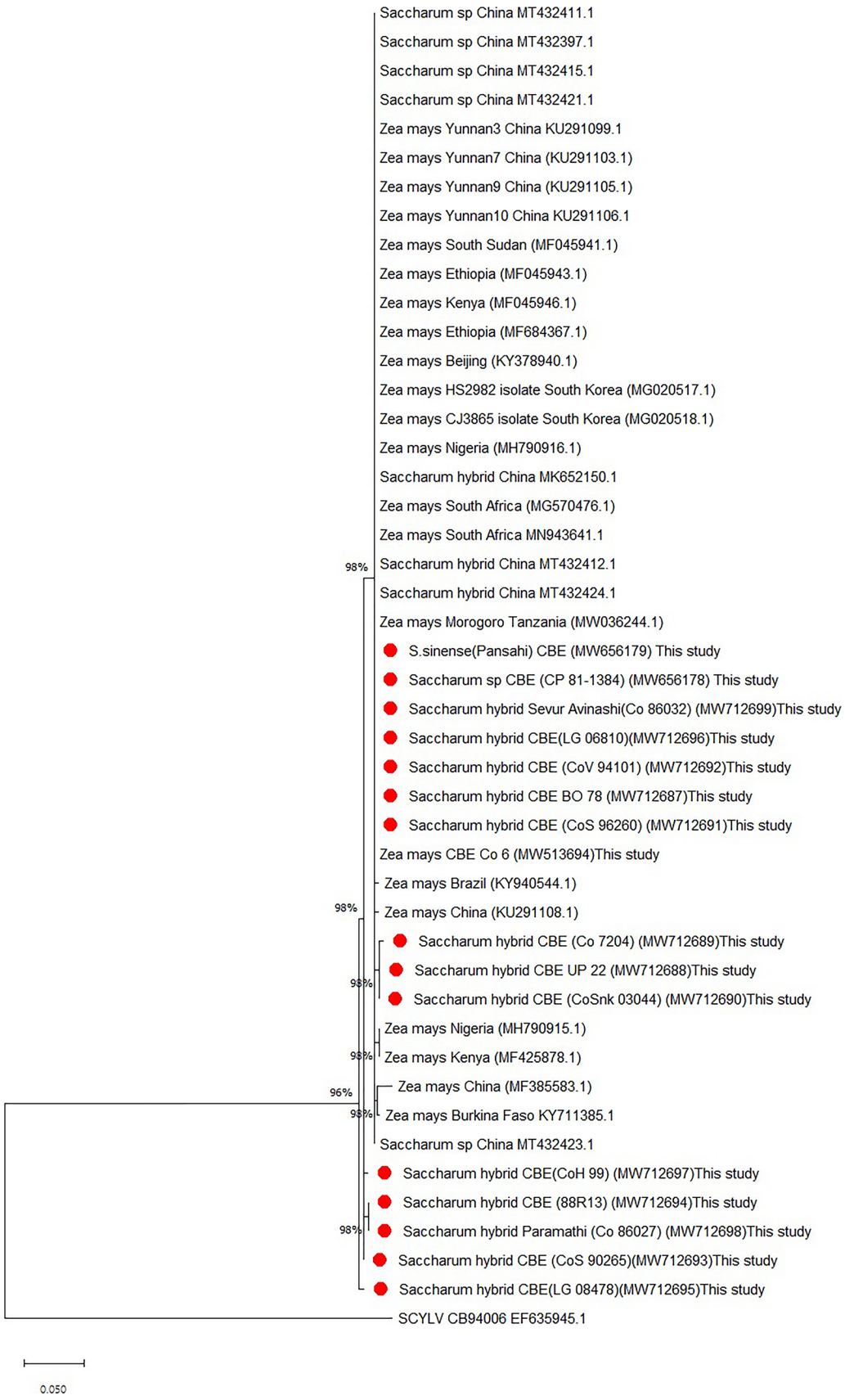


Fig. 1 MaYMV amplification from *Saccharum* hybrid leaf samples. Lane M: 100 bp marker (StepUp 100 bp DNA ladder, GeNei), Lanes 1–15: BO 78, UP 22, Co 7204, CoSnk 03044, CoS 96260, CoV 94101, CoS 90265, 88R13, LG 08478, LG 06810, Co 86032, CP 81–1384, Pansahi (*S. sinense*), Negative control and MaYMV positive from *Zea mays*


Saccharum sp China MT432411.1
Saccharum sp China MT432397.1
Saccharum sp China MT432415.1
Saccharum sp China MT432421.1
Zea mays Yunnan3 China KU291099.1
Zea mays Yunnan7 China (KU291103.1)
Zea mays Yunnan9 China (KU291105.1)
Zea mays Yunnan10 China KU291106.1
Zea mays South Sudan (MF045941.1)
Zea mays Ethiopia (MF045943.1)
Zea mays Kenya (MF045946.1)
Zea mays Ethiopia (MF684367.1)
Zea mays Beijing (KY378940.1)
Zea mays HS2982 isolate South Korea (MG020517.1)
Zea mays CJ3865 isolate South Korea (MG020518.1)
Zea mays Nigeria (MH790916.1)
Saccharum hybrid China MK652150.1
Zea mays South Africa (MG570476.1)
Zea mays South Africa MN943641.1
98%
Saccharum hybrid China MT432412.1
Saccharum hybrid China MT432424.1
Zea mays Morogoro Tanzania (MW036244.1)
S.sinense(Pansahi) CBE (MW656179) This study
Saccharum sp CBE (CP 81-1384) (MW656178) This study
Saccharum hybrid Sevur Avinashi(Co 86032) (MW712699)This study
Saccharum hybrid CBE(LG 06810)(MW712696)This study
Saccharum hybrid CBE (CoV 94101) (MW712692)This study
Saccharum hybrid CBE BO 78 (MW712687)This study
Saccharum hybrid CBE (CoS 96260) (MW712691)This study
Zea mays CBE Co 6 (MW513694)This study
Zea mays Brazil (KY940544.1)
98%
Zea mays China (KU291108.1)
Saccharum hybrid CBE (Co 7204) (MW712689)This study
Saccharum hybrid CBE UP 22 (MW712688)This study
98%
Saccharum hybrid CBE (CoSnk 03044) (MW712690)This study
Zea mays Nigeria (MH790915.1)
98%
Zea mays Kenya (MF425878.1)
96%
Zea mays China (MF385583.1)
98%
Zea mays Burkina Faso KY711385.1
Saccharum sp China MT432423.1
Saccharum hybrid CBE(CoH 99) (MW712697)This study
Saccharum hybrid CBE (88R13) (MW712694)This study
98%
Saccharum hybrid Paramathi (Co 86027) (MW712698)This study
Saccharum hybrid CBE (CoS 90265)(MW712693)This study
Saccharum hybrid CBE(LG 08478)(MW712695)This study
SCYLV CB94006 EF635945.1
0.050

One µg of total RNA extracted from the samples was reverse transcribed using RevertAid H Minus First Strand cDNA Synthesis Kit (MBI Fermentas, USA), primed with 50 pmol MaYMV-R453 by following the manufacturer's protocol in a thermal cycler (C1000, Bio-Rad, USA). The PCR reaction was performed in a total volume of 25 µl containing 2.5 µl 10X Taq buffer with 15 mM $MgCl_2$, 0.5 µl of 10 mM dNTP mix, 10 µM of each forward and reverse primers, 1.25 units of Taq polymerase (Origin, Kerala, India), 2 µl of cDNA and sterile MilliQ water to make up the final volume. The PCR program was performed with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. All the amplified products were run on 1.5% agarose gels stained with ethidium bromide (0.5 µg/ ml) and documented in gel documentation system (GBox: Syngene, UK).

The amplicons from the RT-PCR assay were sequenced by direct sequencing by Sanger method at both ends (Eurofins, Bengaluru, India). The nucleotide sequences obtained from this study were subjected to sequence analyses by pairwise multiple sequence alignment with other representative MaYMV sequences retrieved from GenBank (consensus sequences) and performed through Bio edit v7.1.9 (Hall 1999). Maximum likelihood Tamura-Nei model based phylogenetic tree analysis was performed with nearest neighbour interchange (NNI) tree options (MEGA X v.10.1.6) with 1000 bootstrap replications (Kumar et al. 2018).

Results

RT-PCR assay revealed specific amplification of 453 bp amplicon in 15 of the 106 sugarcane samples (14.15%) tested and suggested MaYMV infections (Fig. 1). This was further confirmed by direct sequencing of the amplified products by Sanger method at both ends. After ensuring the quality of the sequences (q = > 30), they were aligned and subjected to Blastn analysis. All the sugarcane MaYMV sequences were submitted to GenBank under the accession numbers of MW712687 to MW712699 and MW656178.

The pairwise multiple sequence alignment of all the 15 consensus sequences of this study from sugarcane has clearly shown the highest nucleotide similarities of 97.4% to 100% among themselves as well as to other MaYMV sequences of maize from Africa (Tanzania, South Africa, Ethiopia, Kenya, South Sudan, and Burkino Faso) and China and all the *Saccharum* isolates of China. The MaYMV isolate from *Saccharum* hybrid CP 81–1384 had shown the highest similarity 100% with MaYMV of maize and sugarcane irrespective of the geographic regions whereas *Saccharum* hybrid LG 08478 isolate had shown 96.5% to 97.8% identity only with other MaYMV isolates (Supplementary table 1).

Fig. 3 MaYMV infected sugarcane cv CoH 99 exhibiting prominent mosaic symptoms in the young leaves in the canopy. The plants also exhibit mid rib yellowing associated with yellow leaf

In phylogenetic analysis, all the 45 isolates sequences (15 from sugarcane varieties and one from maize in this study and the remaining 29 retrieved from GenBank representing isolates from other countries) clustered together in a single clade. However, a few *Saccharum* hybrids isolates from this study viz. CoH 99, 88R13, Co 86027, CoS 90265, and LG 08478 formed a subclade. ScYLV, a distinct member of the *Polerovirus* genus used as an outgroup, clustered separately (Fig. 2).

Discussion

Our research findings clearly showed infection of MaYMV in sugarcane from India for the first time. Similarly, natural infection of MaYMV was reported in sugarcane with the mosaic type of disease symptoms in China (Sun et al. 2019). Luo et al. (2016) and Ahmad et al. (2019) reported that diverse viruses cause mosaic symptoms as mixed infections hindering the distinction of the viruses associated with this disease. In our study, we found MaYMV positive sugarcane clones with varying intensities of mosaic symptoms and yellow leaf (Fig. 3). Mixed infection of MaYMV with other sugarcane viruses predominantly infecting sugarcane was also analyzed (data not shown). However, further studies are needed to understand its symptomatology, vectors, epidemiology of the virus and synergistic effect, and impact with other host poleroviruses, potyviruses, and badnaviruses in order to devise effective management strategies. Under Indian conditions, mixed infection of different viruses in sugarcane such as SCMV, SCSMV and ScYLV is common (Viswanathan and Karuppaiah 2010; Viswanathan et al. 2010). Since both the viruses ScYLV and MaYMV belong to the genus *Polerovirus*, it will be interesting to study their mixed infection, sieve elements colonization, and synergistic effect in symptom expression. Recently, Gonçalves et al. (2020) attempted mechanical, and aphid transmission of MaYMV, and aphid transmission of the virus was confirmed from the symptomatic maize samples from different locations of Sao Paulo state, Brazil and from the corn leaf aphid, *Rhopalosiphum maidis* extracts. In the study, the virus isolate obtained from the aphid transmission caused the characteristic yellow mosaic foliar symptoms as a single infection and caused severe interveinal necrosis symptoms as a mixed infection with SCMV.

Despite the wide occurrence of reports of MaYMV in different countries such as Nigeria, Burkina Faso, Ethiopia, and Tanzania in Africa; Brazil and Ecuador in South America and South Korea and China in Asia during the last five years (Sun et al. 2021), the disease incidence, impact on crop growth and yield losses, symptomatology of the virus, host cell localization through EM analysis, the viral titer threshold limit for symptom expression through qPCR assays, etc. have not been studied in detail, to date. Furthermore, all these countries´ reports were mostly from *Zea mays* host, and only China has reported it in both *Zea mays* and commercial cultivars of *Saccharum* spp in Asia. To the best of our knowledge, this is the first and new report of MaYMV infecting commercial cultivars of *Saccharum* hybrids and *S. sinense* in India, which necessitates more focused research including mixed infections with other sugarcane viruses and synergistic effects in perspectives of effective management to minimize the losses and sustain the sugarcane production.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1007/s13313-021-00809-w.

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