SANTE/11813/2017 guidelines to provide evidence that a method is fit for the intended purpose. Sample extracts are analysed using suitable chromatographic techniques like gas chromatography (GC), high performance liquid chromatography (HPLC), gas chromatography – mass spectrometry (GC-MS) and liquid chromatography – mass spectrometry (LC-MS/MS).

IV-SO24

FIRST REPORT OF *MAIZE YELLOW MOSAIC VIRUS* (MAYMV) INFECTING SUGARCANE AND MAIZE IN INDIA AND ITS MOLECULAR CHARACTERIZATION

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Maize yellow mosaic virus (MaYMV), a new novel member of the genus *Polerovirus, Luteoviridae* was first reported in the field grown maize crop showing mosaic and foliar yellowing symptoms in Yunnan provinces of China during the year 2016 (Chen *et al.*, 2016) by deep sequencing platform of small RNAs and Sanger sequencing of RT-PCR samples. The sequence comparisons and phylogenetic analyses of the entire 5642 nucleotides (nt) represented the new member of the genus *Polerovirus* in the family *Luteoviridae*. Since then, its widespread occurrence has been reported in other countries as 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) at Africa and Brazil (Gonçalves et al. 2017) and Ecuador (Bernreiter et al. 2017) in South America; and recently in *Panicum miliaceum*, and *Sorghum bicolor* in South Korea by high-throughput RNA sequencing (Lim et al. 2018a,b). In Asia, MaYMV occurrence has been reported on *Zea mays* only from China and South Korea and on *Saccharum* hybrids only 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). Recently, Goncalves et al. (2020) attempted mechanical and aphid transmission of the virus, in that, aphid transmission of the virus was confirmed from the symptomatic maize samples from different locations of Sao Paulo state, Brazil along with extracts of the corn leaf aphid, *Rhopalosiphum maidis.* In the study, the virus isolate obtained from

the aphid transmission caused the characteristic yellow mosaic foliar symptoms as single infection and caused severe interveinal necrosis symptoms as mixed infection with SCMV.

Based on the recent reports of widespread occurrence of MaYMV in many of the agriculturally important *Poaceae* crops at Asia, Africa and South America, we suspected the possible occurrence of the virus in India. Hence, during the year 2020, we collected about ten maize samples (var. Co 6) showing mosaic like symptoms grown nearby sugarcane fields in Coimbatore and Namakkal districts of Tamil Nadu and around 106 sugarcane samples from different varieties showing mosaic and yellow leaf symptoms and stored under -80^{o}C till further use. Total RNA was extracted from the collected maize and sugarcane leaf samples, using TRI Reagent (Sigma, USA) by following the manufacturer's protocol. MaYMV specific primers were designed from the consensus ORF 3 (P3-coat protein) and ORF 4 (P4- movement protein) regions retrieved from the Genbank using Primer blast with an expected amplicon length of 453 bp. These sequences covered the partial cds of ORF 3 and 4, coding the partial coat protein (CP) and the movement protein (MP). cDNA was synthesized from one µg of total RNA extracted from maize and sugarcane by following the standard protocols. The PCR programme was performed with initial denaturation at 94^{o}C for 5 min, followed by 35 cycles of denaturation at 94^{o}C for 1 min, annealing at 58^{o}C for 1 min, extension at 72^{o}C for 1min and final extension at 72^{o}C for 10 min. All the amplified products were run on 1.5% agarose gels stained with ethidium bromide (0.5 µg/ ml) and gel documented.

RT-PCR assay revealed that all the maize leaf samples and 14.15% (15/106) of the sugarcane leaf samples were found to be infected with MaYMV which was further confirmed by direct sequencing of the amplified products by Sanger method at both the ends. After ensuring the quality of the sequences (q=>30), they were aligned and subjected to Blastn analysis. Pair wise multiple sequence alignment of the nucleotide sequences obtained from this study and other representative MaYMV sequences retrieved from genbank (consensus sequences) was 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).

The pairwise multiple sequence alignment of all the 16 consensus sequences of this study from sugarcane and maize has clearly revealed the highest nucleotide similarities with 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 from China. The MaYMV isolate from *Saccharum* hybrid CP 81-1384 had shown the highest similarity of 100% with MaYMV of both the crops irrespective of the geographic regions whereas,

Saccharum hybrid LG 08478 isolate had shown 96.5% to 97.8% identity only with other MaYMV isolates.

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 source representatively taken from the reported countries) had clustered together in a single clade. However, host crop based sub clades were observed from different countries as most of the *Zea mays* MaYMV isolates sub clustered together irrespective of countries/ geographical regions and most of the MaYMV from sugarcane from this study as well as from China sub clustered separately.

Our research findings clearly showed that presence of MaYMV in India in maize and Sugarcane for the first time. Also, mixed infection of MaYMV with other sugarcane viruses predominantly infecting the crop was also recorded (data not shown). Similarly, natural infection of MaYMV was reported in sugarcane with mosaic type of disease symptoms at China (Sun et al., 2019). Recently, Luo et al. (2016) and Ahmad et al. (2019) reported that diverse viruses cause the mosaic symptoms as mixed infections hinder distinction of the viruses associated with this disease. However, further studies are needed to understand its symptomatology, vectors, epidemiology of the virus and its synergistic effect with other host poleroviruses, potyviruses and badnaviruses in order to devise effective management strategies. Since mixed infection of different viruses in sugarcane such as SCMV, SCSMV and SCYLV are a common phenomenon (Viswanathan and Karuppaiah, 2010) and SCYLV and MaYMV both are belonged to the *Polerovirus*, its mixed infection status and the kind of synergistic effect in symptom expression is to be studied further at large scale in future.

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IV-SO25

DEVELOPMENT OF A SIMPLE PCR-BASED ASSAY FOR DISCRIMINATING *MAT-1* AND *MAT-2* MATING TYPE HAPLOIDS OF *SPORISORIUM SCITAMINEUM*

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Sporisorium scitamineum (Syd.) is the causative agent of sugarcane smut, which is one of the most prevalent and devastating diseases in sugarcane producing countries worldwide (Sundar et al. 2012). The life cycle of *S. scitamineum* is related to that of the corn smut fungus *Ustilago maydis*, which has been well established as a model pathogenic fungus, and it involves transitions between three cell types; diploid teliospores, haploid sporidia and dikaryotic mycelia (Kamper et al. 2006). *S. scitamineum* possess a bipolar mating system with haploid sporidia representing two opposite mating types, "+" and "-", which is otherwise designated as *MAT-1* and *MAT-2* (Yan et al. 2016). In *U. maydis*, mating and pathogenic development are mediated by two unlinked mating type loci, *a* and *b*. Recognition of compatible mating types and haploid fusion events are triggered by the biallelic *a* locus that codes for a pheromone and a pheromone receptor system. The multiallelic *b* locus appears to control the formation of the dikaryotic filament and pathogenicity and, it encodes a pair of distinct