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COMPLETE GENOME SEQUENCE OF INDIAN SUGARCANE MOSAIC VIRUS AND ITS GENETIC DIVERSITY WITH OTHER COUNTRY ISOLATES

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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 (Viswanathan et al. 2007). As a vegetatively propagated crop, sugarcane is prone to viral infection via seed cane. It is reported that SCMV and Sorghum mosaic virus (SrMV) cause mosaic in sugarcane under natural conditions (Grisham 2000). However, in India, there is no molecular evidence for the occurrence of SrMV in sugarcane. In addition to SCMV association of SCSMV was reported (Hema et al. 1999). Percentage of yield loss in sugarcane to mosaic depends highly on the variety, environmental factors and the area where the sugarcane crop is grown. Also mosaic is an important quarantine disease during germplasm exchange. In sugarcane older phenotypic exhibition of the mosaic symptom is high in some varieties and older leaves show symptom recovery based on host susceptibility in young growing leaves. As the leaves get older partial or no recovery will be seen in some varieties based on susceptibility and infecting virus strain. Hence both host as well as viral strain influences the degree

of symptom expression and severity. Plants once recovered from infection may be prone to reinfection by the same strain or from other strains.

Single and mixed infection of sugarcane viruses cause synergistic interaction among them leading to decrease in the yield through varietal degeneration. Due to this impact, mosaic is a serious concern that resulted in near extinction of elite cultivars from cultivation as witnessed recently to the erstwhile ruling varieties like Co 740 and CoC 671 in the tropical region in the country (Viswanathan and Balamuralikrishnan 2005).

Sugarcane mosaic was investigated in detail in the past and prevalence of many strains was reported. Molecular varieties in SCMV strain occurring in India was established based on variation in coat protein genome and this revealed unique grouping of Indian SCMV isolates as compared to the prevailing strains worldwide (Viswanathan et al. 2009). Based on molecular variations in coat protein genome molecular diagnostics were optimized in the institute. However, complete genome of the virus is required to completely characterize the virus from India. Hence detailed studies were conducted to establish complete genome of the virus.

Furhter study of full genome sequence on SCMV will give a good source of information to identify the genetic diversity among closely related strains or isolates. The correct and complete genome sequence generated will always be good historical information for the future evolving organisms. (Claire et al. 2002).

For the first time we determined complete nucleotide sequence of SCMV-IND from infecting popular variety CoC 671 from India. From the same variety complete genome sequence of SCSMV has already been reported by Parameswari et al. (2013) which is also the first study in India. Nine overlapping primer pairs were designed from the reference sequences collected from the genbank covering the whole genome of SCMV. cDNA was synthesized from the total RNA extracted from the variety CoC 671 and PCR conditions were optimized for each primer sets. The products were purified using GenElute Gel Extraction kit and the resulting fragments were cloned into the pTZ57R/T vector After the recombinant DNA was introduced into E. coli DH5α by transformation, positive clones were identified by colony PCR and two clones from each of the nine amplified fragments were sequenced in both directions to eliminate potential sequence heterogeneity introduced by Taq DNA polymerase. The nt and amino acid sequences of other known SCMV isolates were retrieved from the GenBank and used for comparison The overlapping sequences of the nine fragments were assembled using the BioEdit software.The linearly assembled single strand positive sense RNA genome of SCMV-IND was about 9573 nucleotides in length excluding the poly (A) tail. The genome encodes a polyprotein of about 3064 amino acid residues which is processed to form ten mature proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, VPg, NIa-Pro, NIb, and CP). Based on this whole genome study, the Indian isolate showed

close identity to the Australian isolate Brisbane of about 98.2% and 94.9% at the protein and nucleotide level, respectively. The Indian isolate shares the least identity with the American isolate Ohio of about 88.2% and 78.9% at the protein and nucleotide sequences respectively. This study supports the work done by Viswanathan et al. (2009) on SCMV coat protein gene analysis and its genetic variability where the Indian isolates shared a very close similarity with the Australian isolate sharing 99% nucleotide identity unlike other country isolates. Further, phylogenetic tree drawn using the software Bioedit through Neighbor joining method with 1000 bootstrap replications clearly depicted its close similarity with Australian and American isolate sharing a common progenitor whereas the other country isolates diverged to a different cluster. Within the monocot specific potyvirus, the Indian SCMV isolate shares close identity with Sorghum mosaic virus with 69.1% identity followed by Maize dwarf virus with 67.9% identity at the nucleotide level. Further study on recombination event among the SCMV complete genome sequences is in progress which will provide a good insight on SCMV evolutionary biology.

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