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MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF *SUGARCANE STREAK MOSAIC VIRUS* ISOLATES FROM INDIA

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Sugarcane is affected by several viral diseases, of which mosaic is most widely spread in different countries since its first report from India by Barber in 1921. The viruses causing mosaic are categorized under RNA viruses and are characterized by large population sizes, fast replication and high mutation rates, which results in a huge evolutionary potential. Worldwide, mosaic disease is caused by *Sugarcane mosaic virus* (SCMV), but in India and several other Asian countries, *Sugarcane streak mosaic virus* (SCSMV; family *Potyviridae*), a member of the genus *Poacevirus*, is also found as a major cause of the disease. SCSMV was first recorded in southern India in 1999 and since then its spread has been reported from different geographical locations. Further studies on characterization of mosaic causing viruses of sugarcane confirmed that SCSMV is a major virus of mosaic disease complex in sugarcane. Viswanathan *et al.* (2008) conducted a detailed study on the molecular characterization of SCSMV in India and compared nearly 63 sequences with the other members of the *Potyviridae* family. As a result, existence of more than one strain in a single variety and also a very high level of variation were detected among the population. The HC-Pro gene which is located upstream of the P1 region is the silencing suppressor protein among the *Potyviridae* family. It is known to be involved in the RNA silencing mechanism by interacting with host proteins yet to be studied. Twelve HC-Pro (Helper component proteinase) isolates of Indian SCSMV of 1410bp was characterized and compared with the available database isolates like Pakistan, Indonesia and Japan. The overall nucleotide and protein level identity was 75-95% and 88-100% respectively. High level of variation was observed among the isolates. The phylogenetic analysis clustered Indian and Pakistan isolates into one group, whereas Japanese and Indonesian in the other group. Potential recombination event detected in the isolates using non-parametric methods showed parental and the recombinant sequences. Sequence analyses and phylogenetic techniques in recent years have been proven to be extremely effective methods for detecting and characterizing recombination events among RNA viruses. Merops Peptidase database confirmed the characterized sequences belong to the cysteine peptidase family. This is the first study to report the evidence of recombination between Indian SCSMV isolates. However, the isolates lacked the conserved motifs associated with aphid transmission at its N-Terminal region and suppression of RNA silencing at the central region. Also the zinc finger motif responsible for RNA silencing activity was found in the P1 region and not in HC-Pro as like other viruses in the *Potyviridae* family, when the complete genome sequence of SCSMV was characterized (Bagyalakshmi *et al.*2012). Since SCSMV shares more close identity with *Tritimovirus,* it is hypothesized that like *Wheat streak mosaic virus* (WSMV), P1 must be the suppressor protein in the SCSMV. This result further motivated to have a detailed characterization of the P1 region.

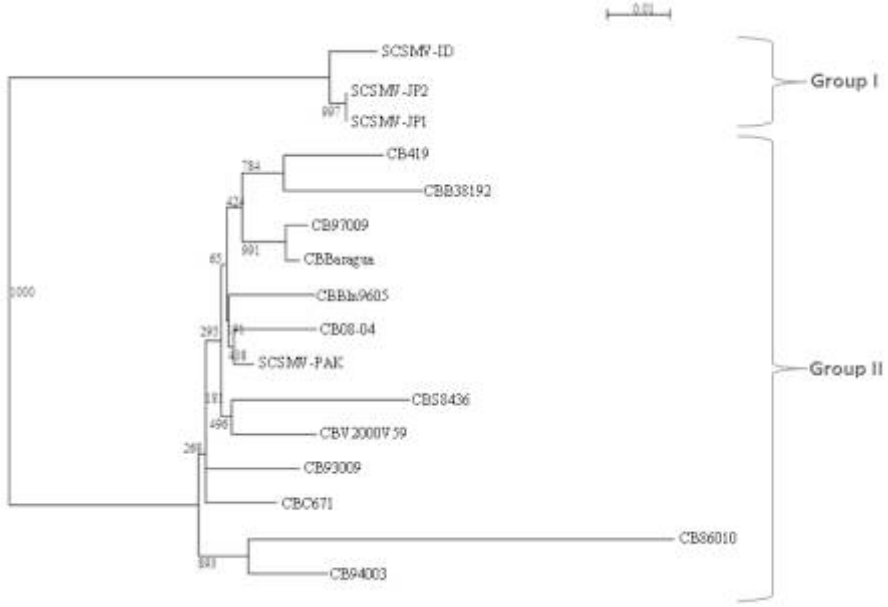

Fig. 1 Phylogenetic tree drawn using the software ClustalX with a bootstrap value of 1000 by comparing the amino acid sequences of HC-Pro of Indian sugarcane streak mosaic virus (SCSMV) isolates and isolates from other countries

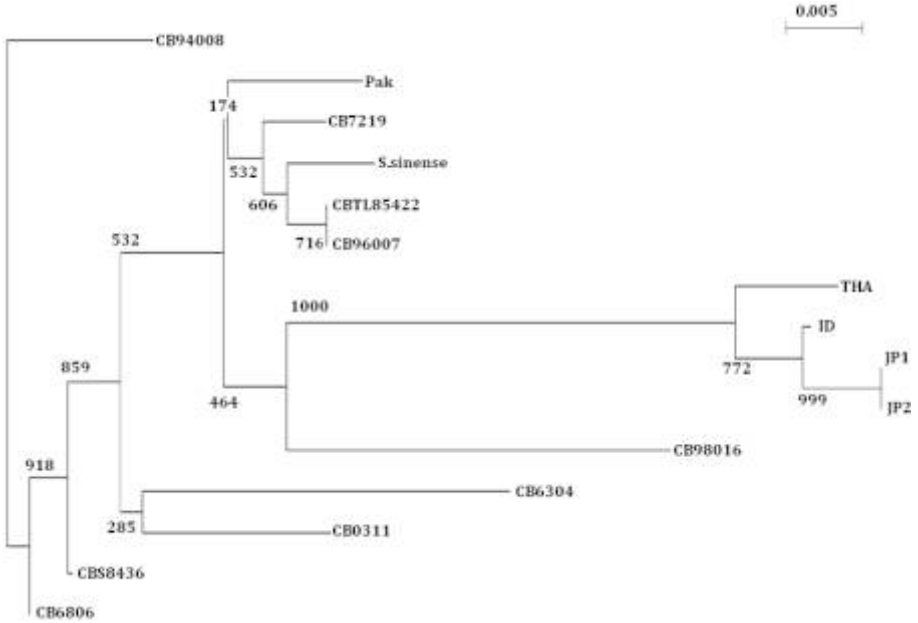

Fig.2 Phylogenetic tree drawn using the software ClustalX with a bootstrap value of 1000 by comparing the amino acid sequences of P1 of Indian sugarcane streak mosaic virus (SCSMV) isolates and isolates from other countries

In the present study, P1 gene of 10 Indian SCSMV isolates were sequenced and compared with five other isolates reported worldwide for their phylogenetic survey of recombination events revealed a similar pattern like H-Pro. The phylogenetic study was further supported with the SNPs (single nucleotide polymorphism), INDELs (insertion and deletion) and evolutionary distance analysis. The sequence lengths of the P1 genes of different SCSMV isolates are similar (1074 nucleotide) and each encoding a protein of 358 amino acids. Comparative sequence analysis of 10 Indian P1 isolates

revealed 86-98% nucleotide sequence identity among themselves and 83-91% identity to the other SCSMV isolates like Pakistan, Japan and Indonesia. Among the different isolates, those from Indian and Pakistani were closely related, with nucleotide and amino acid sequence identity of 87-91% and 96-99% respectively. In addition, sequence difference count matrix revealed 4155 nucleotide differences among all the Indian SCSMV isolates taken in this study. The ratio of non-synonymous to synonymous polymorphic sites is found to be negative in selection to purge the deleterious mutations in the coding sites. Merops peptidase database confirmed the characterized sequences belong to the Serine peptidase family a member of the P1 region. Since P1 is the first protein to be produced upon translation of the viral RNA, its further study will shed new lights to understand the mechanism of host viral interaction. Our studies are in progress to express the genome in RNA silencing vector to establish its silencing activity in model tobacco lines.

References

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GENETIC VARIABILITY AMONG INDIAN ISOLATES OF *SPORISORIUM SCITAMINEUM*-THE SUGARCANE SMUT FUNGI

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Sugarcane (*Saccharum officinarum*) -a life sustaining industrially important crop belonging to the grass family (*Poaceae*) is prone to several diseases caused by fungi, bacteria and viruses. Among the several diseases of sugarcane, smut caused by *Sporisorium scitamineum*-(Syn. *Ustilago scitaminea*)-a biotrophic basidomycete fungus was one of the earliest observable diseases, due to the emergence of conspicuous whip-like sori. This disease is accounted for significant tonnage loss and reduced juice quality and can devastate large areas cultivated with susceptible varieties. Though employing resistant cultivars can be an effective management strategy, it is often hindered by the existence of variability among the pathotypes (Xu *et al.,* 2000; Nzioki *et al*., 2010). Information on the prevalence and distribution pattern of races/pathotypes is vital for deployment of effective disease management strategies. Despite several reports on variability among sugarcane smut fungi, a detailed investigation among the Indian pathotypes remains to be explored. The use of differential hosts as reported by Gillaspie *et. al.* 1983 to identify variability among the isolates had been successful, however analyzing the molecular variability would aid significantly in understanding the varying virulence pattern. This study aims to investigate genetic diversity among the Indian isolates of *S. scitamineum* using Inter Simple Sequence Repeats (ISSR) (Menzies et al. 2003) and Simple Sequence