



THESIS APPROVAL
GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Agricultural Biotechnology)

DEGREE

Agricultural Biotechnology

FIELD

Interdisciplinary Graduate Program

PROGRAM

TITLE: Characterization, Genetic Variation and Diagnosis of *Sugarcane streak mosaic virus*, a New Poacevirus Infecting Sugarcane in Thailand

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Sugarcane leaves showing yellow streak mosaic symptoms were strikingly observed in farmers' fields in Kamphaeng Saen District, Nakhon Pathom Province, Thailand during disease surveys conducted in 2010. Diagnosis of symptomatic leaf samples by RT-PCR for *Sugarcane mosaic potyvirus* failed, but it revealed the presence of *Sugarcane streak mosaic virus* (SCSMV). In this study, SCSMV-infected sugarcane, designated as THA-NP3 isolate, was subjected to RNA extraction, followed by RT-PCR-based viral gene cloning and sequencing. The complete genome sequence of the isolate THA-NP3 contained 9,781 nucleotides, which encoded for a polyprotein of 3,130 amino acid residues. Protein sequence analysis indicated nine putative cleavage sites that yielded ten functional proteins namely P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPg, NIa-Pro, NIb and CP, and an additional frameshifted PIPO protein. Analysis by multiple sequence alignment revealed that THA-NP3 shared 97.84% nucleotide identity with JP2 from China and 81.39-97.78% identities to other recorded SCSMV sequences. Electron microscopy of purified virions revealed them to be flexuous rod shaped, and with length of 700-890 nm, which is characteristic of viruses in family *Potyviridae*. Molecular weight of the coat protein subunits as estimated by SDS-PAGE was 31 kilodaltons. To develop virus diagnosis tools, rabbit polyclonal antisera against SCSMV were produced, and specific virus detection was achieved by using the direct antigen coating enzyme linked immunosorbent assay (DAC-ELISA). In addition, the immunochromatographic strip (ICS) was successfully created for rapid virus diagnosis from a diseased leaf sap within 5 min. Surveys for streak mosaic disease incidence were conducted in natural sugarcane fields from 2010 to 2014 in five provinces of major sugarcane growing areas in Thailand, including Nakhon Pathom, Kanchanaburi, Nakhon Ratchasima, Khon Kaen and Udon Thani, and in two germplasm collection fields. Virus infected percentages obtained from SCSMV-positively diagnosed by DAC-ELISA and RT-PCR were 43.48-90.91% and 54.17-100% in collected farmers and germplasm fields, respectively. Genetic diversity based on complete coat protein (CP) coding sequences of the collective 58 SCSMV isolates showed 86.17-100% nucleotide identities among Thai isolates, and 85.70-99.29% identities to isolates from other countries. Phylogenetic analysis of CP sequences indicated two major clusters of virus variants, one in cropping fields and another in germplasm fields. Genetic variations of SCSMV isolates were consistently indicated according to the potential recombination events detected in CP gene regions. These findings represent essential knowledge and should be utilized to improve the SCSMV resistance of sugarcane varieties.

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