

The Effect of Hosts on Evolution of Sugarcane Mosaic Virus in China

Xiaoya Jing[&], Liuqing Sun[&], Wei Chen^{*}

School of Life Science, Shanxi Normal University, Linfen, 041000, Shanxi

[&] These authors contributed equally to this work and should be considered co-first authors

^{*} Corresponding author

Keywords: SCMV; selection pressure; host; amino acid.

Abstract: Sugarcane mosaic virus (SCMV) is a great threat to the yield of sugarcane and maize. It is necessary to understand the evolutionary mechanism of SCMV. The study aimed to reveal the effect of host on evolution of SCMV. The leaf samples of maize with typical symptom were collected from Shanxi province and tested by DAS-ELISA and RT-PCR. Compared with online databases, one new SCMV isolate was found and further studied. The complete genome of the isolate is 9594 nt long, and it encodes a 3064 amino acid poly-protein. It was pairwise compared and phylogenetic analyzed with 20 SCMV isolates from genebank. The results showed that the 21 isolates could be divided into two divergent evolutionary groups (Group I and II) based on the complete genome, which was associated with the host species. Each gene of the 21 isolates were also conducted with pairwise comparison and phylogenetic analysis, similar results were obtained for six genes (6K2, CP, HC-Pro, NIa-Vpg, P1 and P3), among which six amino acid (AGGVFI) of 6k2 from 27 to 32 sites seemed to be conservative among SCMV sugarcane isolates, while SAGVFT were conservative among SCMV maize isolates. The results confirmed that the host produced the selection pressure on the evolution of SCMV, and the six amino acid in 6K2 may respond to this selection. The finding might be helpful for understand the host effect on the evolution of SCMV. In addition, it may be useful for the design of long-term and sustainable management strategies for controlling SCMV.

1. Introduction

Sugarcane mosaic virus (SCMV) is a member of Potyvirus within Potyviridae, which induces systemic symptoms to maize including mosaic, dwarf and/or distortion (Xia et al. 2016). The genome of SCMV, a positive single-strand RNA (Chauhan et al. 2015), is approximately 10,000 nucleotides (Gell et al. 2014). The genome encodes a poly-protein which is subsequently cleaved into ten mature proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-Vpg, NIa-Pro, NIb, CP) by three self-encoded proteinases (Urcuqui-Inchima et al. 2001). SCMV can be transmitted by aphids and mechanical inoculation to maize, wheat, sugarcane and so on (Tao et al. 2013). In these hosts, SCMV frequently occurs in combination with other virus such as Maize dwarf mosaic virus (MDMV) (Gell et al. 2014). This co-infection causes the decrease in yield quality and quantity (Achon et al. 2007). Due to the lack of proofreading activity of RNA virus polymerases, short generation time, and large population size, SCMV mutate at the maximum error rate (Elena and Sanjuan 2005; Li et al. 2013). As a result, SCMV exists as numerous strains (Padhi and Ramu 2011; Wu et al. 2012). Host is also one of the most important selection pressures to promote the evaluation of SCMV (Xu et al. 2008). It is not only an important aspect of evolutionary biology but also an issue for SCMV control to understand the whole genome information and genetic structure.

2. Materials and methods

2.1 Materials

In this study, maize leaf samples with typical symptom were collected from Shanxi province and studied. All the samples were transported to the laboratory within 24 hours in ice boxes and marked

the location and collection time.

2.2 RNA extraction and RT-PCR

Total RNA was extracted from these samples using the Universal Plant Total RNA Extraction Kit (BioTeke, China). cDNA was synthesized using PrimeScript RT reagent Kit (TaKaRa, Japan) according to the manufacturer's instructions. Five complete genome sequences of SCMV isolates (GenBank ID: AJ297628.1, AY042184.1, AJ310102.1, AM110759.1 and KP860936.1) were obtained from NCBI and analyzed by the DNAMAN program. Based on the conserved domains, ten primers were designed for detecting SCMV by Primer Premier 5.0 (Chen et al. 2015). PCR were carried out in the DNAEngine Peltier Thermal Cyclers (Bio-Rad, Hercules, CA). For 3'- and 5'-terminal sequence, 3'RACE and 5'RACE reaction was conducted using the RACE system (TaKaRa, Japan).

2.3 Sequencing Genome assembly

The PCR products were purified by Gel Extraction Kit (BioTeke, Beijing, China). The purified DNA fragments were ligated into the pMD19-T simple vector (TaKaRa, Japan), and cloned into *Escherichia coli* JM109 according to the manufacturer's instruction. At least three clones for each fragment were sequenced. If there was any difference or error at any position of the sequences, at least four more clones were sequenced to obtain the consensus sequence.

2.4 Phylogenetic Analysis

These sequences were analyzed by Clustal X v2.1 and Mega 5.2, and generated the genome of SCMV (Tamura et al. 2016).

3. Results

3.1 SCMV genome analysis

The whole genome of SCMV-Shanxi is 9594 nt including the 3' and 5' tail encoding a polyprotein with 3064 amino acid. The SCMV isolate was deposited into GenBank database with accession KT736022. The whole genome contains a single large open reading frame (ORF) (Figure 1B). The putative ORF starts at AUG (nt 148-150) in the sequence and encodes a polyprotein of 3,064 amino acids (aa) with an estimated molecular weight of 346.13 kDa (Figure 1B). The large polyprotein is cut into 10 proteins (P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-Vpg, NIa-Pro, NIb and CP) (Figure 1B). The putative cleavage sites of each protein were identified by comparison to other potyviruses (Figure 1B).

3.2 Genetic diversity of ACLSV

SCMV-Shanxi isolate was compared with 20 other species of the family Potyviridae using DNAMAN (Lynnon Biosoft, Quebec, Canada) (Hu et al. 2011). The results showed that the sequence homologies of SCMV was ranged from 57.71% (Brome streak mosaic virus) to 71.20% (Sorghum mosaic virus) and from 20.57% (Sweet potato mild mottle virus) to 52.61% (Maize dwarf mosaic virus) at the nt and aa level, respectively. Phylogenetic analysis on the genome sequences of SCMV and other species in the genus Potyvirus was carried out by Mega 5.2. The SCMV-Shanxi was clustered tightly with MDMV and Sorghum mosaic virus. These results indicated that SCMV-Shanxi was a member of a subgroup in Potyvirus.

Genome pairwise comparisons of the SCMV isolate with those of 20 other SCMV isolates showed that SCMV shared identities from 87.22% (AJ278405.1) to 99.37% (AJ297628.1) at nt level, from 87.59% (JX237862.1) to 99.11% (AY042184.1) at aa level. For the ten putative genes, the identities were different among these isolates. The CP identity was highest, followed by NIa-Pro, NIa-Vpg, 6K2, NIb, P3, CI, 6K1 and HC-Pro, P1 was the lowest at nt level (Table 1). While at aa level, CI was highest, followed by HC-Pro, NIa-Pro, P3, 6K1, CP, 6K2, NIa-Vpg and NIb, P1 was the lowest (Table 1). These results showed the different degrees of conservation for the different genes of SCMV. These differences may be due to the different functions of the various genes (Le et

al. 1999). P1 corresponds to the N-terminal of SCMV, which enhances amplification and movement of Potyvirus (Ryan and Flint, 1997). Deletion and mutational analysis had shown that P1 was not strictly required for virus infection (Verchot and Carrington 1995). The highest polymorphism degree of P1 gene was therefore detected in SCMV, which was also found in Potato virus Y (PVY) and Zucchini yellow mosaic virus (ZYMV) isolates (Tordo et al. 1995).

4. Discussion

Phylogenetic analysis was also introduced into explore the relationship among SCMV isolates. 21 SCMV isolates were clustered into two main groups based on the phylogenetic analysis of complete genome sequences (Figure 2A). 16 isolates from maize were formed Group I, the rest 5 isolates from sugarcane were formed Group II. In addition, both Group I and II included isolates from China. These results confirmed the genetic diversity of SCMV isolates was closely associated with host species instead of geography.

In order to analyze the possible reason for the relation between the genetic diversity and host species of SCMV isolates, ten genes were used for further analysis. The phylogenetic trees were generated based on the ten genes. When compared with the phylogenetic trees based on the genome, the same or similar results were got based on six genes (6K2, CP, HC-Pro, NIa-Vpg, P1 and P3). These results indicated that these genes may take part in the interaction between SCMV and host. Some special amino acid sequences were found among these genes. For 6K2, AGGVFI from 27 to 32 were seem to be conservative among SCMV sugarcane isolates, while SAGVFT among SCMV maize isolates (Figure 2B). We hypothesized that the mutative direction of these sites in 6K2 gene may determine the isolate was sugarcane or maize on these facts. In order to test the hypothesis, more online sequences covered these sites were analyzed by BLAST. And the hypothesis was also confirmed. In other words, host produced the selection pressure on the SCMV isolates, and these sites may respond to this selection. It is our hypothesis based on the results of bioinformatics, but experiments in the lab will be needed to confirm the hypothesis.

5. Conclusion

This study demonstrated that the host may be the important evolutionary factors shaping the genetic structure of SCMV isolates and produced the selection pressure on the evolution of SCMV. The response of different genes to selection pressure were different, the amino acid sites from 27 to 32 of 6K2 gene may be relative with host range of SCMV. Our findings will provide a foundation for understanding the evolution mechanism of SCMV.

Acknowledgments

The study was supported by the Shanxi Key Laboratory of Germplasm Improvement and Utilization in Pomology and the Project from the Education Ministry of China (No.B07049).

This work was supported by the Scientific and Technological Innovation Projects of Colleges and Universities in Shanxi Province (20171116) and Innovation and Entrepreneurship Training Program for College Students in Shanxi Province (2017578).

References

- [1] Achon MA, Serrano L, Alonso-Duenas N, Porta C (2007) Complete genome sequences of maize dwarf mosaic and sugarcane mosaic virus isolates coinfecting maize in Spain. *Archives of Virology* 152(11):2073-8
- [2] Chauhan RP, Rajakaruna P, Verchot J (2015) Complete genome sequence of nine isolates of canna yellow streak virus reveals its relationship to the sugarcane mosaic virus (SCMV) subgroup of potyviruses. *Archives of Virology* 160(3):837-44

- [3] Chen W, Luo ZP, Liu WT, Liu H, Ma DF, Snyder JH, Cheng JL, Wu YF (2015) Assessing the genetic diversity and resistance to cucumber mosaic virus, and tobacco etch virus, of tobacco cultivars in china. *Australasian Plant Pathology* 44(4): 365-374
- [4] Elena SF, Sanjuan R (2005) Adaptive value of high mutation rates of rna viruses: separating causes from consequences. *Journal of Virology* 79(18):11555-8
- [5] Gell G, Sebestyen E, Balázs E (2014) Recombination analysis of maize dwarf mosaic virus (MDMV) in the sugarcane mosaic virus (SCMV) subgroup of potyviruses. *Virus Genes* 50(1):79-86
- [6] Le RFO, Candresse T, Maule AJ, Le GO (1999) New advances in understanding the molecular biology of plant/potyvirus interactions. *Molecular Plant Microbe Interactions* 12(5):367-376
- [7] Li Y, Liu R, Zhou T, Fan Z (2013) Genetic diversity and population structure of sugarcane mosaic virus. *Virus Research* 171(1):242-6
- [8] Padhi A, and Ramu K (2010) Genomic evidence of intraspecific recombination in sugarcane mosaic virus. *Virus Genes* 42(2):282-5
- [9] Ryan MD, Flint M (1997) Virus-encoded proteinases of the picornavirus super-group. *Journal of General Virology* 78:699–723
- [10] Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular biology and evolution* 24:1596-1599
- [11] Tao Y, Lu J, Liu Q, Yan Z, Rui Z, Ingvaridsen CR (2013) Combined linkage and association mapping reveals candidates for scmv1, a major locus involved in resistance to sugarcane mosaic virus (SCMV) in maize. *BMC Plant Biology* 13(17):4868-4874
- [12] Tordo VMJ, Chachulska AM, Fakhfakh H, Le RM, Robaglia C, Astiermagnificier S (1995) Sequence polymorphism in the 5' NTR and in the P1 coding region of potato virus Y genomic RNA. *Journal of General Virology* 76:939-949
- [13] Urcuqui-Inchima S, Haenni AL, Bernardi F (2001) Potyvirus proteins: a wealth of functions. *Virus Research* 74(1-2):157-75
- [14] Verchot J, Carrington JC (1995) Debilitation of plant potyvirus infectivity by P1 proteinase-inactivating mutations and restoration by second-site modifications. *Journal of Virology* 69:1582–1590
- [15] Wu L, Zu X, Wang S, Chen Y (2013) Sugarcane mosaic virus– long history but still a threat to industry. *Crop Protection* 42:74-78
- [16] Xia Z, Zhao Z, Chen L, Li M, Zhou T, Deng C (2016) Synergistic infection of two viruses MCMV and SCMV increases the accumulations of both MCMV and MCMV- derived sirnas in maize. *Scientific Reports* 6:20520
- [17] Xie X, Chen W, Fu Q, Zhang P, An T, Cui A (2016) Molecular variability and distribution of sugarcane mosaic virus in shanxi, China. *Plos One* 11(3):e0151549
- [18] Xu DL, Park JW, Mirkov TE, Zhou GH (2008) Viruses causing mosaic disease in sugarcane and their genetic diversity in southern China. *Archives of Virology* 153(6):1031-9