



The evolution of soybean mosaic virus: An updated analysis by obtaining 18 new genomic sequences of Chinese strains/isolates

Guang-Can Zhou^{a,1}, Zhu-Qing Shao^{a,1}, Fang-Fang Ma^a, Ping Wu^a, Xiao-Yi Wu^a,
Zhong-Yun Xie^a, De-Yue Yu^b, Hao Cheng^b, Zhi-Hua Liu^c, Zhen-Feng Jiang^d,
Qing-Shan Chen^d, Bin Wang^{a,*}, Jian-Qun Chen^{a,*}

^a Laboratory of Plant Genetics and Molecular Evolution, School of Life Sciences, Nanjing University, Nanjing 210023, China

^b National Center for Soybean Improvement, National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agriculture University, Nanjing 210095, China

^c College of Resources and Environment, Northeast Agriculture University, Harbin 150030, China

^d College of Agriculture, Northeast Agriculture University, Harbin 150030, China

ARTICLE INFO

Article history:

Received 14 May 2015

Received in revised form 12 June 2015

Accepted 13 June 2015

Available online 21 June 2015

Keywords:

Soybean mosaic virus

Recombination

Phylogenetic relationship

Population structure

Pathogenicity

ABSTRACT

Soybean mosaic virus (SMV) is widely recognized as a highly damaging pathogen of soybean, and various strains/isolates have been reported to date. However, the pathogenic differences and phylogenetic relationships of these SMV strains/isolates have not been extensively studied. In the present work, by first obtaining 18 new genomic sequences of Chinese SMV strains/isolates and further compiling these with available data, we have explored the evolution of SMV from multiple aspects. First, as in other potyviruses, recombination has occurred frequently during SMV evolution, and a total of 32 independent events were detected. Second, using a maximum-likelihood method and removing recombinant fragments, a phylogeny covering 83 SMV sequences sampled from all over the world was reconstructed and the results showed four separate SMV clades, with clade I and II recovered for the first time. Third, the population structure analysis of SMV revealed significant genetic differentiations between China and two other countries (Korea and U.S.A.). Fourth, certain SMV-encoded genes, such as *P1*, *HC-Pro* and *P3*, exhibited higher non-synonymous substitution rate (dN) than synonymous substitution rate (dS), indicating that positive selection has influenced these genes. Finally, four Chinese SMV strains/isolates were selected for inoculation of both USA and Chinese differential soybean cultivars, and their pathogenic phenotypes were significantly different from that of the American strains. Overall, these findings have further broadened our understanding on SMV evolution, which would assist researchers to better deal with this harmful virus.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Soybean mosaic virus (SMV, Family *Potyviridae*, genus *Potyvirus*) is widely known as a devastating pathogen of soybean [*Glycine max* (L.) Merr.], causing significant yield losses and seed quality deterioration in various soybean-growing regions around the world (Hill et al., 2007; Tolin and Lacy, 2004). To explore its evolution and mechanism of pathogenicity, researchers have put great efforts to collect SMV isolates from various regions and further to characterize them.

According to their disease reactions on differential soybean cultivars, collected isolates of SMV have been classified into various strains. In the United States, using two sensitive and six resistant soybean cultivars, Cho and Goodman (1979) successfully classified 98 SMV isolates into seven strains, namely G1–G7. The same differential system was also utilized in Korea, resulting in additional SMV strains such as G5H, G6H, and G7H identified (Cho and Chung, 1976, 1986; Kim and Lee, 1991; Kim et al., 2003; Seo et al., 2009a, 2009b, 2009d). In Japan and China, however, different sets of soybean cultivars were used as differentials, and isolates of SMV collected in these two countries were finally classified into five (A to E) and 21 (SC1 to SC21) strains, respectively (Guo et al., 2005; Li et al., 2010b; Takahashi et al., 1980; Wang et al., 2003, 2005; Zhan et al., 2006). Although it is understandable for researchers in different countries to establish their own differential systems, it inevitably causes confusions, leaving pathogenic phenotypes among groups of

* Corresponding authors. Tel.: +86 25 8968 1306; fax: +86 25 8663 3057.

E-mail addresses: binwang@nju.edu.cn (B. Wang), chenjq@nju.edu.cn (J.-Q. Chen).

¹ These authors are co-first authors.

SMV strains untested and their phylogenetic relationships largely unexplored.

Obtaining complete genome sequences from certain SMV strains has greatly helped researchers in exploring the relationships among these strains and their pathogenesis on soybean cultivars. Complete genome sequencing of strains G2 and G7 in 1992, for the first time, enabled researchers to recognize SMV genomic structures and to establish that strain G2 is distantly related to strain G7, because they showed a total number of 576 (6.0%) nucleotide and 99 (3.2%) amino acid differences (Jayaram et al., 1992). Further sequencing of closely related strains or isolates such as G7 and G7d, L and L-RB, and G5H and G7H, has identified some critical sites in SMV-encoded HC-Pro, P3, and CI proteins, which are virulent determinants of SMV on soybean cultivars 'PI96983', 'L29', and 'V94-5152', respectively (Chowda-Reddy et al., 2011a; Gagarinova et al., 2008a; Hajimorad et al., 2003, 2005, 2008; Seo et al., 2009c). Phylogenetically, a primary analysis including 13 SMV genomic sequences revealed two separate clades, with one consisting of seven strains/isolates (G2 and G2-N from USA, L and L-RB from Canada, G5 and G7H from Korea and HZ from China) and the other covering six (G7d, G7f, and G7x from USA, Aa and Aa15-M2 from Japan, and CN18 from Korea) (Gagarinova et al., 2008a). Further, by collecting many isolates in Korea and sequencing 30 SMV genomes, Seo et al. (2009d) conducted a comprehensive study on the evolution of SMV. Interestingly, the strains/isolates of Korea and those of North America exhibit a close relationship, with no significant genetic differences observed between the East Asian (mainly Korea) and North American populations of SMV (Seo et al., 2009d).

Recent studies have suggested that SMV, like soybean itself, originated in South and East Asia, particularly in China (Gai and Guo, 2001; Gai, 1997; Gibbs et al., 2008; Hymowitz, 1990, 2004), and a high level of genetic diversity of SMV populations has been reported in China (Li et al., 2010b). A special recombinant strain of SMV, SC7, that likely resulted from an interspecific recombination event between SMV and *Bean common mosaic virus* (BCMV), is prevalent in China but not detected in other regions (Yang et al., 2011, 2014). Therefore, to gain a more complete understanding on the evolutionary history and patterns of SMV, it is necessary to study Chinese SMV strains/isolates in terms of their genomic sequences. The present study reports a total of 18 newly sequenced genomes of Chinese SMV strains/isolates, most of which were collected in the summer of 2013. Together with 65 other SMV genomes available in GenBank (18 were deposited after 2009 and have not been analyzed systematically), the combined data represents SMVs sampled from all over the world and enabled us to see a better picture on the evolutionary history of SMV.

2. Materials and methods

2.1. Virus sample collection and preservation

A total of 370 soybean leaf samples with typical mosaic symptoms were collected from four provinces, representing three major soybean growing areas of China in the summer of 2013, with 92 samples collected from Heilongjiang province that represented Northeast area, 156 samples from Jiangsu province that represented Huang-Huai Valley area, and 71 and 51 samples from Hubei and Jiangxi provinces that represented the Southern Yangzi River area. Each leaf sample with mosaic symptoms was taken from a single soybean plant and usually several samples from distant locations in an individual field were collected. All the leaf samples were dried in vinyl bags of anhydrous calcium chloride, transported in a thermoelectric cooler box (−10–0 °C) and preserved at a −80 °C freezer until further analysis.

The SMV strains SC6-N and SC7-N were provided by the National Center for Soybean Improvement of China, Nanjing Agricultural University in 2010; the SMV isolate SX-Z was field collected in 2012 at Yangling, Shanxi Province; and the strain NE-N1 was obtained in 2013 from Dr Q.S. Chen of the Northeast Agricultural University. These four SMV strains/isolates were recovered and maintained in a sensitive cultivar NanNong 1138-2 (NN1138-2) for further usage.

2.2. RNA extraction, reverse transcription–polymerase chain reaction (RT–PCR), SMV detection, and genome sequencing

Total RNA of soybean mosaic leaves was extracted using the RNeasy Plant Mini kit (Qiagen, Hilden, Germany), following the manufacturer's instruction. The first strand cDNA was synthesized by using the SuperScript™ III First-Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA). SMV-infected leaf samples were then detected using detection primers (Zhou et al., 2014): DF, 5'-AAGTATGATGGYAGCATGC-3' and DR, 5'-CCAACCATRCAAACNCGTTC-3' with high-fidelity *Fast-Pfu* DNA polymerase (Transgen, Beijing, China) via RT–PCR. Positive PCR products (~1.7 kb) were sequenced next (Genescript, Nanjing, China) to confirm SMV identity. To avoid samples that are co-infected by two or more kinds of virus isolates, only those samples showing clean sequencing chromatograms were selected for genome sequencing. Three primer pairs were designed to amplify three overlapping fragments of SMV genome based on available sequences in GenBank (Table 1). The 5' fragment (~3.3 kb) was amplified by primers SMV-NF (5'-AAATTAATACTMSTYATAAGA-3', nt position: 1–22) and SMV-NR (5'-CCYTGCARYACACTAGTCATTG-3', nt position: 3231–3253), the middle fragment (~3.6 kb) by primers SMV-MF (5'-CTCCACATACGGARAAATG-3', nt position: 2974–2992) and SMV-MR (5'-CCAACCATRCAAACMCGTTC-3', nt position: 6576–6595), and the 3' fragment (~3.2 kb) by primers SMV-CF (5'-ATGTTTGGGGTYGGCTATGG-3', nt position: 6351–6370) and SMV-CR (5'-AGGACAACAAACATTGCCGYACCT-3', nt position: 9565–9588). The positions of primers were given according to the genome sequence of SMV G1 strain (FJ640977). Adjacent regions of these PCR fragments were overlapped by at least 250 bp to ensure that they were from the same SMV genome. Each fragment was purified, sequenced bi-directionally, and contigs were assembled, as described in previous study (Zhou et al., 2014).

2.3. Sequence alignment and genetic variation of SMV-encoded genes

The SMV genome contains a long open reading frame (ORF) and a small overlapping ORF, known as "pipo" (Chung et al., 2008). Upon translation, the single, large polypeptide is processed by three self-encoded proteases (P1, HC-Pro, and NIa-Pro), yielding 10 functional proteins: P1, HC-Pro, P3, 6K1, CI, 6K2, NIa-VPG, NIa-Pro, NIb, and CP (Urcuqui-Inchima et al., 2001). To explore the genetic variation of these genes, the 18 newly obtained SMV genomic sequences (with primer sequences removed on both ends) were aligned together with the 65 SMV genomic sequences available in GenBank (Table 1) using ClustalW implemented in Mega 5.0 (Tamura et al., 2011). For a better effect, the long ORF sequences were aligned according to the amino acid sequences. For each encoded gene, the overall mean similarity of analyzed SMV strains/isolates were calculated in Mega 5.0 at both nucleotide and amino acid levels (Tamura et al., 2011).

2.4. Recombination analyses and phylogenetic reconstruction

Based on previous studies (Gibbs and Ohshima, 2010; Wylie and Jones, 2011), 14 sequences of six potyviruses that were closely-related to SMV, including Calla lily latent virus (CLLV_BM19,

Download English Version:

<https://daneshyari.com/en/article/3428129>

Download Persian Version:

<https://daneshyari.com/article/3428129>

[Daneshyari.com](https://daneshyari.com)