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Genomic evidence of intraspecific recombination in sugarcane mosaic virus

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Abstract The sugarcane mosaic virus (SCMV) of the genus *potyvirus*, which primarily affects maize, sugarcane, sorghum, abaca, and grasses, occurs worldwide and causes significant economic loss. Using the full genome sequences of SCMV and several recombination detection methods, in this study we report that recombination is the major driving force in the evolution and emergence of several new variants of SCMV. We reported eight highly significant ($P < 0.001$) recombination break points, majority of which are located within 6K1-VPg-NIaPro-NIb region, thus indicating a region for recombination hotspot. The observation of commonalities of same recombination events among the SCMV isolates between the countries (Spain and Mexico), and within the country (within China, and within Mexico), suggests common origin of the isolates in respective regions.

Keywords Potyvirus · SCMV · Genome · Evolution · Recombination · Phylogeny

The family *Potyviridae*, which includes approximately 200 species of economically important group of the plant viruses, causes significant losses in agricultural, pasture, horticultural, and ornamental crops [1]. The *Sugarcane mosaic virus* (SCMV) sub-group, which consists of five species such as SCMV, *Maize dwarf mosaic virus* (MDMV), *Sorghum mosaic virus* (SrMV), *Johnson grass mosaic virus* (JGMV), and *Zea mosaic virus* (ZeMV) of the genus *Potyvirus*, is the causative agent of mosaic disease in Poaceae [2–5]. These viruses have worldwide distribution and share similar morphology, host range, and modes of transmission [6]. They are transmitted by several aphids in a non-persistent manner and by mechanical inoculation [4]. The SCMV and SrMV have been reported to infect sugarcane, maize, sorghum, and other important crops [2, 7–11]. Recent study reported the evidence of co-infection of sugarcane by SCMV and SrMV [10]. Interestingly, while sugarcane plants that were infected with both SCMV and SrMV showed mosaic symptom, plants infected with a single virus (SCMV or SrMV) were either symptomatic or asymptomatic [10]. Thus, indicating that viral mixture was more virulent than single infections [10]. When animals/plants were co-infected with multiple viral strains, the possibility of emergence of new viral strains due to genetic recombination may not be ruled out [12, 13]. Recombination has been proven to be one of the important evolutionary forces in the evolution and divergence of several positive sense RNA viruses, including several members of *Potyviridae* [12, 14]. The evolution of virulent viral strains by recombination mechanism can have devastating consequences on the host population structure and ultimately can jump to new host. Identifying genomic regions that are hotspot for frequent recombination is, therefore, crucial in understanding the putative role of recombination in the evolution and emergence of new viral strains.

The members of the *Potyviridae* are the positive sense single stranded RNA viruses with the genome size of approximately 10 kb in length and are characterized by an untranslated region (5′ UTR), a large open reading fame (ORF), and a 3′ UTR region. The ORF comprises ten

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functional proteins such as P1 (first protein), HC-Pro (helper complement protein), P3 (third protein), 6K1, CI (cylindrical inclusion protein), 6K2, VPg (viral protein genome-linked), NIa-Pro (major protease of small nuclear inclusion protein -NIa), NIb (large nuclear inclusion protein), and CP (coat protein) [15]. The genome-wide detection of recombination, however, is limited by the availability of whole genome sequences. Here we report a genome-wide assessment of recombination among a collection of 20 complete genome sequences of SCMV subgroups. We demonstrate that intraspecific recombination in SCMV is much more common and may show a bias for certain types of genes.

Twenty full-length potyviral genomes were retrieved from Genbank and were aligned using MEGA ver 4 [16]. To determine the genomic regions that are affected by the possible recombination events, all the 20 full-length SCMV sub-group sequences were screened with five different algorithms implemented in the RDP3 program [17]. The individual programs RDP [17], GENECONV [18], MaxChi [19], chimaera [20], and 3seq [21] were implemented for the analysis. Since no single program provided optimal performance under all conditions, any event that supported all the five methods with P-values $\leq 10^{-3}$ was the criteria used for positive recombination breakpoints identification. The breakpoint position and the putative parental sequences were also determined. Neighbor joining tree based on the full-length genome was reconstructed using the maximum composite likelihood model implemented in MEGA 4 [16].

Of the 20 full-length sequences analyzed, seven SCMV sequences showed strong evidence of recombination ($P < 0.001$; Table 1, Fig. 1). Interestingly, all these recombinant sequences were clustered together (Fig. 2). Although none of the genes were free of recombination event, VPg, NIa-pro, and NIb appeared to be the hotspot for recombination (Fig. 1). Eight strong recombination events were detected in seven sequences representing China, Mexico, and Spain (Table 1; Fig. 1). Recombination event 1, which spans the VPg-NIa-pro- NIb regions, appeared to be unique to three sequences (AF494510, AY149118, and AY042184). The major parent (AJ297628) of these three sequences also appeared to be from China. Recombination event 2, which covers 6K1-CI-6K2-VPg-NIa-NIb, is also unique to a sequence from China. Interestingly, recombination event 7, which covers VPg-NIa-pro- NIb, is unique to two Mexican and to the lone Spain isolate; however, their major parent is from China. While recombination events 3 and 4 were restricted to Mexican isolates, recombination events 5, 6, and 8 were restricted to Chinese isolates (Fig. 1; Table 1).

Using several recombination detection methods and utilizing the 20 full-length genome sequences of SCMV sub-groups, this study reports the evidence of several recombination events which were frequently detected in 6K1-VPg-NIaPro-NIb region, demonstrating a recombination 'hotspot'. This genomic region has also been recently detected as hotspot for recombination in several other members of potyvirus such as *Sweet potato feathery mottle virus* [22], *Soybean mosaic virus* (SMV) [23], and *Watermelon mosaic virus* [24]. Taken together with the previous studies [2, 11, 12, 14, 25], this study has also revealed that recombination is the major driving force in the emergence of new variants of SCMV. Our results provide further

Table 1 Detection of recombination by five different methods implemented in RDP3

Recombination event number	Recombinant sequence(s)	Detection methods				
		RDP	GENECONV	Maxchi	Chimaera	3Seq
1	AY149118_SCMV AF494510_SCMV AY042184_SCMV	2.12E−103	7.58E−96	1.13E−49	6.55E−40	1.27E−13
2	AY569692_SCMV	5.76E−71	4.36E−12	7.23E−07	4.62E−10	5.06E−13
3	GU474635_SCMV	1.25E−06	1.77E−19	1.72E−13	2.62E−08	4.45E−50
4	EU091075_SCMV GU474635_SCMV	5.28E−09	1.99E−39	9.21E−25	3.67E−19	2.23E−46
5	AY042184_SCMV	6.12E−23	4.03E−09	1.33E−17	7.53E−06	1.29E−40
6	AY042184_SCMV	7.61E−27	1.21E−29	1.90E−13	6.99E−14	1.27E−13
7	AM110759_SCMV GU474635_SCMV EU091075_SCMV	6.87E−15	2.94E−17	1.09E−07	9.70E−17	1.25E−15
8	AF494510_SCMV	1.77E−08	1.24E−05	9.07E−08	1.61E−05	6.10E−14

P-values under each detection method for the respective recombination event are mentioned. P-value < 0.001 is considered significant

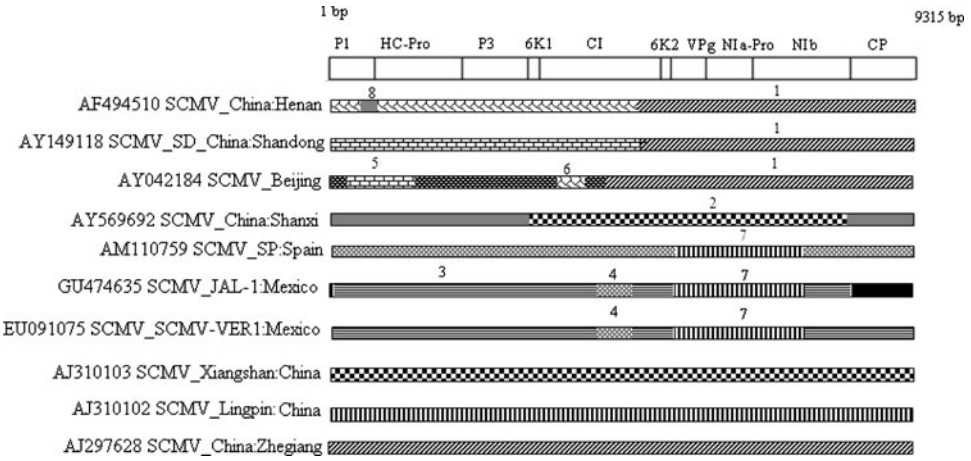

Fig. 1 Recombination breakpoint map of the isolates detected by different methods implemented in RDP3 program. The nucleotide position (in alignment) for the entire coding region is shown. Regions that correspond to different isolates are color-coded. Of the 20 full genome sequences, seven sequences showed strong evidence of recombination. The numbers above the breakpoints are corresponding to the recombination event number as shown in Table 1

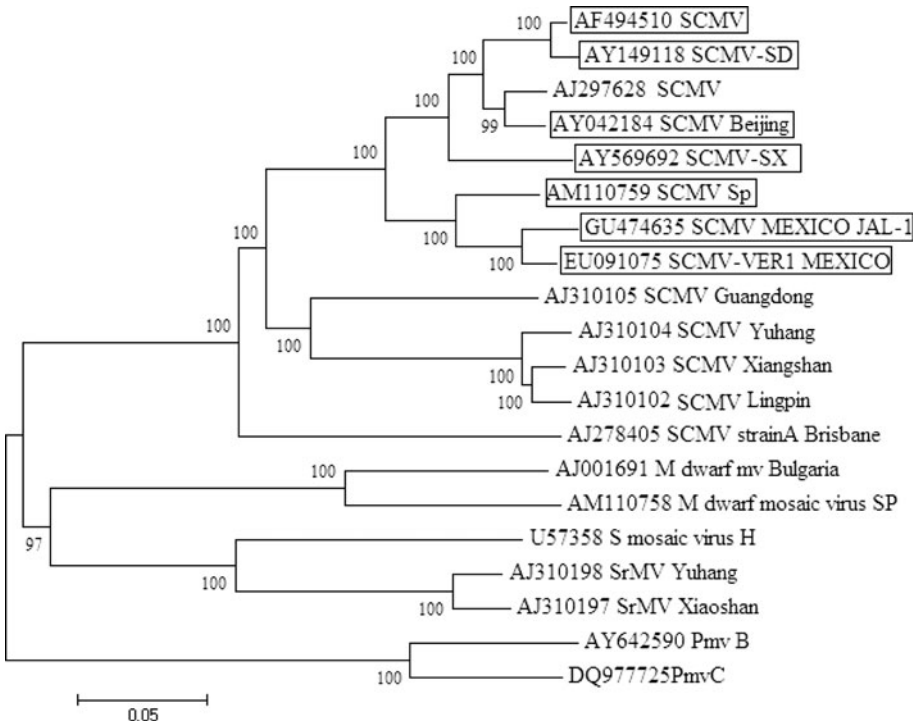

Fig. 2 Neighboring tree based on the full-length genome sequence data depicting phylogenetic relationships among different species of the genus *potyvirus*. All the recombinant isolates are boxed

evidence for the occurrence of natural recombinants for SCMV [2, 11]. The results revealed SCMV isolates from China are not clustered together; rather the recombinant China SCMV isolates were more closely related to the corresponding recombinant isolates from Mexico and Spain. Our study clearly delineates recombination is the significant force for such pattern. It is unclear, however, as to what factors are responsible for such intraspecific recombination among the intercontinental geographically separated SCMV populations. Since SCMV can be transmitted through seed [2, 26], the movement of germplasm can be one of the possible explanations for such high sequence similarities observed between the intercontinental isolates of SCMV.

In summary, our results demonstrate that recombination is the major driving force in the evolution, and emergence of new variants of SCMV and 6K1-VPg-NIaPro-NIb region is detected to be the hotspot for recombination.

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