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Incidence and Distribution of Four Viruses Causing Diverse Mosaic Diseases of Sugarcane in China

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Abstract: Mosaic diseases of sugarcane caused by various viruses have been reported in most sugarcane planting countries and threaten global sugar production. There is a lack of extensive, systematic investigation of mosaic diseases and their causal viruses in China. In this study, a total of 901 leaf samples showing mosaic symptoms were collected from commercial fields in eight provincial regions in China and tested for sorghum mosaic virus (SrMV), sugarcane mosaic virus (SCMV), sugarcane streak mosaic virus (SCSMV), and maize yellow mosaic virus (MaYMV) using RT-PCR with four specific primer pairs. Of 901 tested samples, 38.5% (347/901) of samples were infected with one of the four viruses alone. Infection by two or more viruses was seen for 42.6% (384/901) of samples. The highest incidence of virus-causing sugarcane mosaic disease was SrMV (70.1%), followed by SCMV (33.4%) and SCSMV (30.3%), and the lowest incidence was seen for MaYMV (5.1%). Three viruses (SrMV, SCMV, and SCSMV) were found in eight sugarcane-planting provinces, whereas MaYMV was only found in Fujian, Guangxi, and Sichuan provinces. Mixed infections of the three main viruses, particularly for SrMV + SCMV and SrMV + SCSMV, were commonly found in the sugarcane samples. Our systematic determination of the occurrence and distribution of four RNA viruses associated with sugarcane mosaic diseases can provide evidence to guide the development of strategies for the prevention and control of sugarcane mosaic diseases in China.

Keywords: molecular detection; mosaic disease; prevalence; RNA virus; sugarcane

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1. Introduction

Sugarcane (*Saccharum* spp. hybrid) is an important sugar crop that accounts for 80% of sugar production and is also a renewable energy crop that provides 40% of fuel ethanol worldwide [1]. Sugarcane production in China mainly occurs in the southern and southwestern regions, including three main sugar-producing provinces Guangxi, Yunnan, and Guangdong, which combined produce 88% of sugar in China. Yield losses in sugarcane due to biotic stresses can be up to 20% [2]. Mosaic is a major sugarcane disease that was first detected in Java, Indonesia, in 1892 and then was distributed throughout the world in various major sugarcane-planting countries and regions [3,4]. Sugarcane mosaic disease can reduce the yields of some susceptible sugarcane cultivars by 17% to 50% [3,5].

Mosaic symptoms in sugarcane are currently associated with numerous diseases caused by several different viruses. These diseases include common mosaic caused by sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV), streak mosaic caused by sugarcane streak mosaic virus (SCSMV), mild mosaic caused by sugarcane mild mosaic

virus (SCMMV), and striate mosaic caused by sugarcane striate mosaic-associated virus (SCSMaV) [4,6]. Recently, the maize yellow mosaic virus (MaYMV) was found to be responsible for another mosaic disease in sugarcane [7–9]. MaYMV was first reported in maize crops showing mosaic and foliar yellowing symptoms in Yunnan, China, in 2016 [10], while similar symptoms were shown in sugarcane leaves, whereas they were the mixed infection of MaYMV and other viruses [7,8]. The abovementioned viruses associated with mosaic diseases in sugarcane are assigned to different viral genera and different families: SrMV and SCMV in the genus *Potyvirus* [11] and SCSMV in the genus *Poacevirus* of family *Potyviridae* [12]; SCMMV in the genus *Ampelovirus* (family *Closteroviridae*) [13]; SCSMaV and MaYMV in the subfamily *Quinvirinae* (family *Betaflexiviridae*) and the genus *Polerovirus* (family *Solemoviridae*), respectively (https://talk.ictvonline.org/ accessed on 19 June 2021). These viruses have a wide range of hosts that include maize, sorghum, sugarcane, and other grasses [8,14,15]. Most of these viruses are mainly transmitted through infected cuttings, contaminated mechanical tools, and various aphids [12,16]. SCMMV and MaYMV cannot be mechanically transmitted [14,16], whereas SCMMV is transmitted by the pink mealybug (*Saccharicoccus sacchari*) [14]. The vector for SCSMaV and SCSMV transmission remains unknown [17,18].

The specific causal virus (SrMV, SCMV, or SCSMV) of sugarcane mosaic diseases varies among countries. In the USA, SCMV and SrMV have been found to infect sugarcane in Louisiana, but only SCMV has been reported in Florida [19]. In India, SCSMV was found to be the predominant causal virus of mosaic disease, followed by SCMV alone or a combination of both viruses [5,18]. In Argentina, SCMV is the dominant virus infecting sugarcane, followed by SrMV [20]. In Brazil, SCMV is the only virus of the family *Potyviridae* that infects sugarcane [21]. In China, numerous studies have examined mosaic diseases of sugarcane and their causal viruses. Prior to the 1990s, various SCMV strains infecting sugarcane were identified in Fujian and Guangxi provinces using a host discrimination system [22]. Subsequently, two other viruses, SrMV and SCSMV, were found to infect sugarcane [23,24]. Overall, the infection of the *Saccharum* spp. hybrid is dominated by SrMV or SCSMV, followed by SCMV [11,25], whereas chewing cane (*S. officinarum*) is mainly infected by SCMV [26,27]. However, Xu et al. (2008) showed that SCMV was also the main causal virus in both *Saccharum* spp. hybrid or *S. officinarum* [28]. Recently, infection of *Saccharum* spp. hybrid by MaYMV was found in sugarcane mosaic samples in China [7,8] and in India [9]. SCSMV is distributed in Asian countries [12] and was recently reported in Côte d'Ivoire [29].

There is a lack of systematic studies for the identification and distribution of viruses in sugarcane mosaic diseases in China due to a limited number of samples and coverage of sugarcane-planting regions. Here, we collected 901 sugarcane leaf samples exhibiting diverse mosaic symptoms from 24 counties/regions in eight provinces of China for molecular detection and identification of four major RNA viruses (SrMV, SCMV, SCSMV, and MaYMV). These samples were examined by RT-PCR using virus-specific primer pairs, and the incidence and distribution of these viruses in China were also determined. Our findings provide valuable information for the prevention and control of sugarcane mosaic diseases in China.

2. Materials and Methods

2.1. Survey Area and Leaf Sample Collection

During 2017–2020, 901 sugarcane leaf samples with mosaic symptoms were collected from commercial fields in eight provinces (Fujian, Guangdong, Guangxi, Guizhou, Hainan, Sichuan, Yunnan, and Zhejiang provinces) in China: 99 from Fujian, 45 from Guangdong, 173 from Guangxi, 170 from Guizhou, 51 from Hainan, 181 from Sichuan, 81 from Yunnan, and 101 from Zhejiang. All leaf samples were disinfected with 75% alcohol and then stored at −80 °C for further experiments. Information and locations of the collected samples are shown in Table S1 and Figure S1.

2.2. Total RNA Extraction and RT-PCR Detection

Total RNA extraction from sugarcane leaves was performed using TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA). The quality and quantity of total RNA were measured by 1.0% agarose gel electrophoresis and a Synergy™ H1 Hybrid Multimode Reader (BioTek, Winooski, VT, USA). Four primer pairs were used for virus-specific detection (Table S2): SrMV-F/SrMV-R for SrMV [30], SCMV-F4/SCMV-R3 for SCMV [31], SCSMV-CPF2/SCSMV-CPR2 for SCSMV [32], and MaYMV-F/MaYMV-R for MaYMV [10]. First-strand cDNA was synthesized using a HiScript II 1st Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) with 1.0 μg total RNA as a template and Oligo(dT) $_{23}$VN as a reverse transcription primer. Subsequently, the PCR amplification on mixtures contains 1 μL cDNA, 12.5 μL Premix Taq (*Ex Taq* Version 2.0 plus dye) (TaKaRa, Dalian, China), and 1 μL of each primer (10 μmol/L) in a 25 μL volume. The PCR program was 94 °C for 5 min, followed by 35 cycles at 94 °C for 30 s, 52 °C (SrMV), 65 °C (SCMV), 55 °C (SCSMV), or 54 °C (MaYMV) for 30 s, and 72 °C for 1 min (SrMV, SCMV, and SCSMV), or 70 s (MaYMV). A final extension was performed at 72 °C for 10 min. The PCR product was analyzed by gel electrophoresis on 1.0% agarose gels.

2.3. Cloning and Sequencing of PCR Products

The RT-PCR amplicons were eluted from low melting agarose gel using an E.Z.N.A.® Gel Extraction Kit (OMEGA Bio-Tek, Inc.; Norcross, GA, USA). The purified PCR fragments were ligated into the pMD19-T vector (TaKaRa) and then transformed into *Escherichia coli* DH5*α* competent cells. Three positive colonies from each leaf sample were picked, and the inserted fragments were sequenced by bidirectional sequencing with M13 primers.

2.4. Sequence Alignment and Identity Analysis

Nucleotide sequences obtained in this study were confirmed to be a specific virus (SrMV, SCMV, SCSMV, or MaYMV) using BLAST/N online (http://blast.ncbi.nlm.nih.gov/ (accessed on 19 June 2021)). Multiple sequence alignment of representative sequences of each virus (SrMV = 265, SCMV = 132, SCSMV = 86, and MaYMV = 38) together with individual reference viral sequences were conducted with the ClustalW algorithm supplemented in MEGA7 software [33]. Pairwise sequence identity analysis was performed using BioEdit version 7.1.9 [34]. Reference sequences for SrMV (NC_004035), SCMV (EU196448), SCSMV (NC_014037), and MaYMV (KU248489) were downloaded from the NCBI library (Table S3).

3. Results

3.1. Mosaic Symptoms on Leaves

A total of 901 leaf samples were collected from 24 locations in eight different provinces of China (Figure S1 and Table S1). These samples exhibited various mosaic symptoms. The most distinctive symptom was on the leaves and manifested as obvious yellow, green interphase mosaicism, stripe, and streaks of varying length, size, and irregularity that were surrounded by contrasting light green to yellow and dark green patches. Chlorotic areas were also sometimes present on the lower surface of the leaf midrib. The symptoms differed in intensity with sugarcane variety and species of the virus and whether there was infection with one or multiple types of viruses (Figure 1).

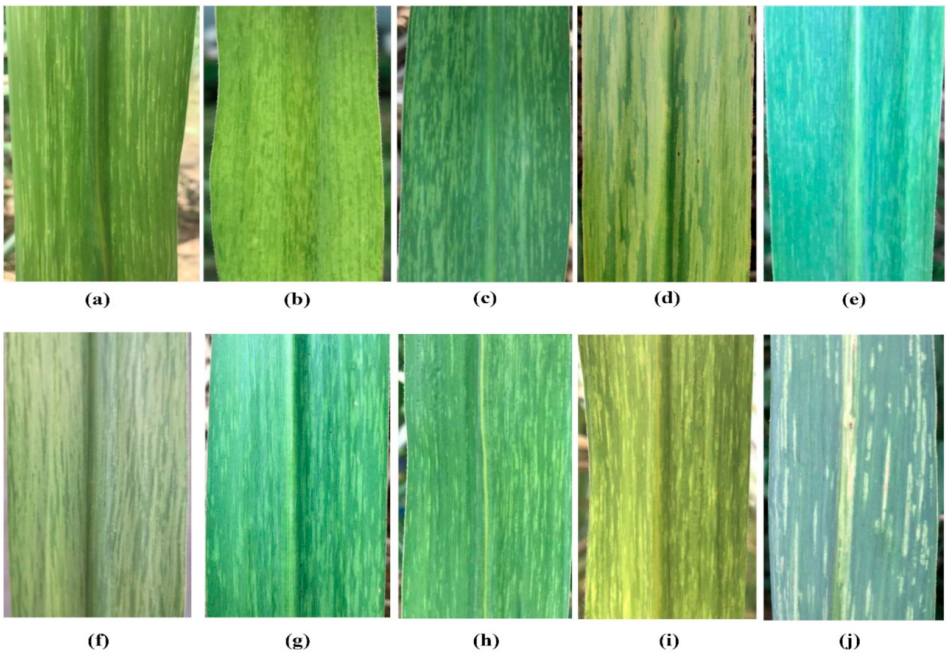

Figure 1. Mosaic symptoms varied in intensity with sugarcane variety and infection with SrMV, SCMV, SCSMV, and MaYMV. (**a**) Single infection by SrMV in *cv.* ROC10 (sample ID HNWZS038); (**b**) Single infection by SCMV in *cv.* Guangdong Huangpi (sample ID ZJWL004); (**c**) Single infection by SCSMV in *cv.* Yunzhe 11-3898 (sample ID GXBS059); (**d**) Single infection by MaYMV in *cv.* Yunrui 10-187 (sample ID GXLB040); (**e**) Mixed infection by SrMV + SCMV + SCSMV in *cv.* Yunzhe 06-189 (sample ID FJFZ067); (**f**) Mixed infection by SrMV + SCMV in *cv.* Guangdong Huangpi (sample ID ZJWL049); (**g**) Mixed infection by SrMV + SCSMV in *cv.* ROC22 (sample ID GXBS060); (**h**) Mixed infection by SCMV + SCSMV in *cv.* Yuetang 03-373 (sample ID HNSY008); (**i**) Mixed infection by SrMV + SCSMV + MaYMV in *cv.* Zhongtang 1201 (smple ID FJFZ040); (**j**) Mixed infection by SrMV + SCMV + SCSMV + MaYMV in *cv.* Yuegan 59 (sample ID FJFZ064). The leaf samples IDs are shown in the Supplementary Table S1.

3.2. RT-PCR Detection and Confirmation of Virus Identities

Four virus-specific primers pairs were used for viral detection of SrMV, SCMV, SCSMV, and MaYMV. RT-PCR results showed that one or more of the four viruses were detected in 81.1% (731/901) of the samples. The size of the amplified fragments for SrMV, SCMV, SCSMV, and MaYMV was ~850, 900, 572, and 753 bp, respectively. The RT-PCR products of the four viruses from partial representative samples were cloned and sequenced. These sequences were verified correctly and originated from each virus using NCBI BLAST/N online. Pairwise sequence identity analysis revealed that nucleotide sequence identities of these targeted sequences shared 72.9–100.0% for SrMV, 90.3–100.0% for SCMV, 88.7–100.0% for SCSMV, and 99.4–100.0% for MaYMV (Figure 2a), whereas nucleotide sequence identities were 75.7–97.4%, 92.9–97.4%, 89.2–99.1%, and 99.2–99.6% among the detected fragments for SrMV, SCMV, SCSMV, and MaYMV, respectively, with individual reference sequences (Figure 2b).

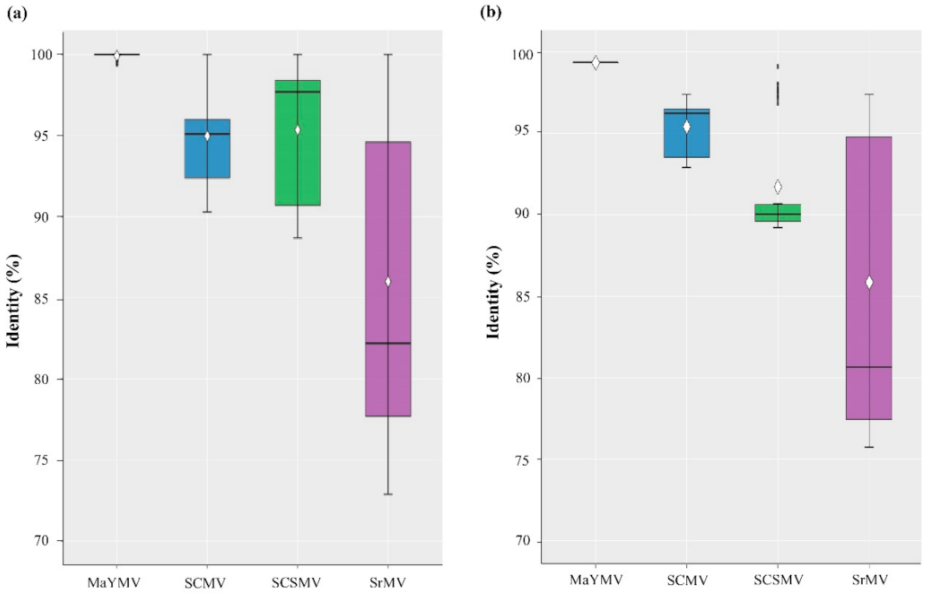

Figure 2. Nucleotide sequence identity of targeted fragments amplified from SrMV, SCMV, SCSMV, and MaYMV with virus reference sequences. (**a**) Comparison of targeted fragment sequences within each virus. (**b**) Comparison of targeted fragment sequences from each virus with corresponding reference sequences (NCBI accession numbers N_C004035, EU196448, NC_014037, and KU248489 were used for SrMV, SCMV, SCSMV, and MaYMV, respectively).

3.3. Distribution Frequency of Four Viruses in Various Provinces

The incidence and distribution of the four viruses across the 901 tested samples from eight provinces in China were determined (Table 1). SrMV had the highest incidence (70.1%, 632/901), followed by SCMV (33.4%, 301/901), SCSMV (30.3%, 273/901), and MaYMV (5.1%, 46/901). Notably, SrMV, SCMV, and SCSMV were found in all eight provinces, while MaYMV was found only in Fujian, Guangxi, and Sichuan provinces. Of the three main viruses (SrMV, SCMV, and SCSMV) associated with mosaic disease, the virus composition, and percentage differed among the eight provinces. Three distribution patterns were present: Guangdong, Guizhou, and Sichuan provinces shared similar distribution frequency with the order SrMV > SCMV > SCSMV; Fujian, Guangxi, and Hainan, and Yunnan provinces had similar distribution frequency with the order SrMV > SCSMV > SCMV; Zhejiang province had a unique distribution frequency with the order SCMV > SrMV > SCSMV.

Table 1. Percentage (%) of SrMV, SCMV, SCSMV, and MaYMV infecting sugarcane in eight provinces in China.

Province	No. of Samples Tested	SrMV	SCMV	SCSMV	MaYMV
Fujian	99	84 (84.8%)	56 (56.6%)	60 (60.6%)	27 (27.3%)
Guangdong	45	25 (55.6%)	23 (51.1%)	16 (35.6%)	0
Guangxi	173	113 (65.3%)	8 (4.6%)	58 (33.5%)	1 (0.6%)
Guizhou	170	111 (65.3%)	43 (25.3%)	11 (6.5%)	0
Hainan	51	30 (58.8%)	7 (13.7%)	19 (37.3%)	0
Sichuan	181	135 (74.6%)	68 (37.6%)	35 (19.3%)	18 (9.9%)
Yunnan	81	77 (95.0%)	30 (37.0%)	46 (56.8%)	0
Zhejiang	101	57 (56.4%)	66 (65.3%)	28 (27.7%)	0
Total	901	632 (70.1%)	301 (33.4%)	273 (30.3%)	46 (5.1%)

3.4. Mixed Infection by Four Viruses in Leaf Samples

Of the 901 samples tested, 170 were not infected with any of four viruses while 731 were infected with at least one virus or had a mixed infection with two or more viruses. Of these 731 samples, incidences of infection with a single virus type were 36.7% (268), 5.1% (37), 4.7% (34), and 1.1% (8) for SrMV, SCMV, SCSMV, and MaYMV, respectively (Figure 3 and Table 2). Meanwhile, 52.2% (384/731) of samples had a mixed infection with two or more viruses. Some samples were infected by two viruses: 14.7% (132) SrMV + SCMV, 11.4% (103) SrMV + SCSMV, 1.0% (9) SrMV + MaYMV, 1.9% (17) for SCMV + SCSMV, and 0.1% (1) for SCMV + MaYMV. Some samples were infected by three viruses: 10.7% (96) for SrMV + SCMV + SCSMV, 0.3% (3) for SrMV + SCMV + MaYMV, 0.9% (8) for SrMV + SCSMV + MaYMV, and 0.2% (2) for SCMV + SCSMV + MaYMV. Only 1.4% (13) samples were infected by four viruses (SrMV + SCMV + SCSMV + MaYMV) together. Overall, SrMV + SCMV was the most common mixed infection (14.7%), followed by SrMV + SCSMV (11.4%), and SrMV + SCMV + SCSMV (10.7%) (Table 2). Notably, no samples showed mixed infection with SCSMV and MaYMV (Figure 3).

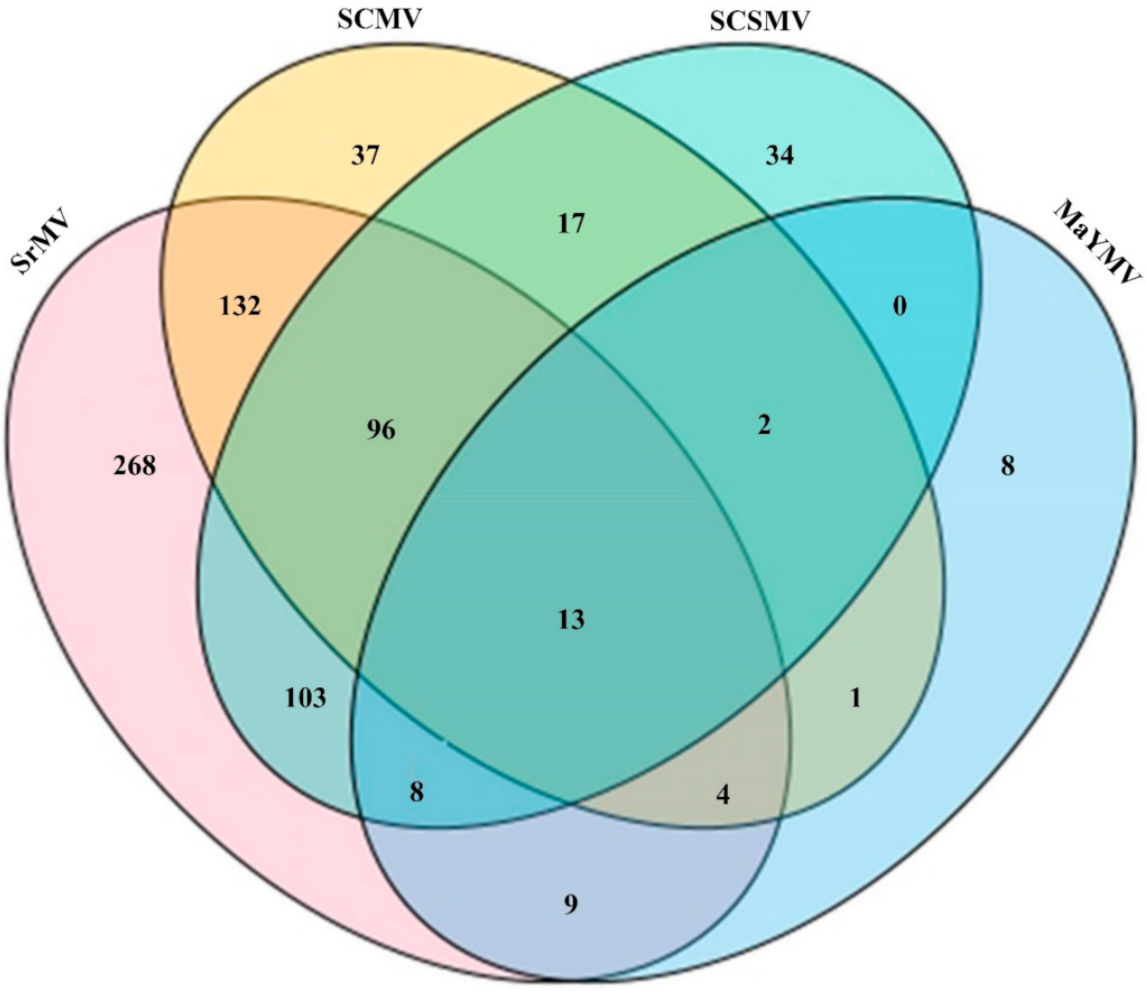

Figure 3. Venn diagram for single or mixed infection by four viruses (SrMV, SCMV, SCSMV, and MaYMV) in 731 virus-positive sugarcane leaf samples determined by RT-PCR.

Table 2. Percentage (%) of mixed infections by SrMV, SCMV, SCSMV, and MaYMV in sugarcane in eight provinces in China.

Province	No. of Samples Tested	No Infection by Any Four Viruses [a]	Single Infection by Any Four Viruses [a]	Mixed Infection by Two or More Viruses [b]										
				(1) + (2)	(1) + (3)	(1) + (4)	(2) + (3)	(2) + (4)	(3) + (4)	(1) + (2)+ (3)	(1) + (2) + (4)	(1) + (3) + (4)	(2) + (3) + (4)	(1) + (2)+ (3) + (4)
Fujian	99	3 (3.0%)	11 (11.1%)	21 (21.2%)	16 (16.2%)	6 (6.1%)	5 (5.1%)	0	0	17 (17.2%)	1 (1.0%)	8 (8.1%)	2 (2.0%)	9 (9.1%)
Guangdong	45	19 (42.2%)	2 (4.4%%)	8 (17.8%)	1 (2.2%)	0	1 (2.2%)	0	0	14 (31.1%)	0	0	0	0
Guangxi	173	39 (22.5)	91 (52.6%)	5 (2.9%)	35 (20.2%)	0	0	0	0	3 (1.7%)	0	0	0	0
Guizhou	170	43 (25.3%)	94 (55.3%)	26 (15.3%)	1 (0.6%)	0	1 (0.6%)	0	0	5 (2.9%)	0	0	0	0
Hainan	51	14 (27.5%)	22 (43.1%)	1 (2.0%)	8 (15.7%)	0	2 (3.9%)	0	0	4 (7.8%)	0	0	0	0
Sichuan	181	29 (16.0%)	81 (44.8%)	30 (16.6%)	10 (5.5%)	3 (1.7%)	0	1 (0.6%)	0	21 (11.6%)	2 (1.1%)	0	0	4 (2.2%)
Yunnan	81	1 (1.2%)	19 (23.5%)	17 (21.0%)	31 (38.3%)	0	1 (1.2%)	0	0	12 (14.8%)	0	0	0	0
Zhejiang	101	22 (21.8%)	27 (26.7%)	24 (23.8%)	1 (1.0%)	0	7 (6.9%)	0	0	20 (19.8%)	0	0	0	0
Total	901	170 (18.9%)	347 (38.5%)	132 (14.7%)	103 (11.4%)	9 (1.0%)	17 (1.9%)	1 (0.1%)	0	96 (10.7%)	3 (0.3%)	8 (0.9%)	2 (0.2%)	13 (1.4%)

[a] Four viruses include SrMV, SCMV, SCSMV, and MaYMV. [b] The numbers present different viruses: (1) SrMV, (2) SCMV), (3) SCSMV, and (4) MaYMV.

Different compositions of mixed infections were seen for different provinces. Mixed infection with both SrMV and SCSMV was commonly found in Guizhou, Sichuan, Fujian, and Zhejiang provinces, whereas mixed infection with both SCMV and SCSMV were commonly present in Yunnan, Guangxi, and Hainan provinces. Infection with three viruses (SrMV + SCMV + SCSMV) was seen in Guangdong province (Table 2).

4. Discussion

Sugarcane mosaics are among the most harmful and prevalent diseases in sugarcane-planting regions in China. The prevalence of mosaics can be caused by long-term cultivation of limiting sugarcane cultivars and continuous cropping, as well as by insufficient quarantine efforts for the exchange of sugarcane planting materials across commercial sugarcane-planting regions [12]. Mosaic disease symptoms can differ in intensity with sugarcane variety, growing conditions, and species in addition to the strains of the causative virus [3]. Disease symptoms can also be aggravated by the synergism of plant viruses [5,35]. Single or mixed viral infections of sugarcane produce similar disease symptoms and are difficult to identify visually, which presents challenges for field diagnosis and prevention and disease control [8].

Since the 2010s, four plant viruses have been found to infect sugarcane and be causal agents of mosaic diseases in China. In this present study, SrMV was the predominant pathogen related to mosaic diseases, followed by SCMV and SCSMV. A lower frequency of MaYMV in commercial sugarcane-planting regions was seen. These results are consistent with our previous studies by Luo et al. (2016) [11] and Sun et al. (2021) [8]. In addition, Xu et al. (2014) [36] and Zhou et al. (2014) [32] examined commercial fields from two main sugarcane-planting provinces (Guangxi and Yunnan), respectively, which occupy about 88% of total sugarcane-planting areas in China, and obtained similar results to the present study. However, SCSMV was the primary pathogen causing sugarcane mosaic diseases in the National Germplasm Resources Nursery (Kaiyuan, Yunnan province) [37] and in leaf samples of new sugarcane varieties from national regional trials (Kaiyuan, Mile, and Yuanjiang, Yunnan province) [25]. Variations in viruses associated with mosaic in different regions may be due to interactions of the viruses with the cultivars and with insect vectors under particular plant growing conditions [11]. Here we found that SCMV was prevalent in Zhejiang province, and this result may be because a majority of leaf samples collected in this province were from chewing cane (*S. officinarum*) that is vulnerable to attack by SCMV but not by SrMV [26,27].

Notably, none of the four viruses were detected in some leaf samples (19%) in this study. In an earlier study by Grisham et al. (2007) [38], 8% of sugarcane samples with mosaic symptoms were found not to be infected by SCMV and SrMV. These results suggest that other viruses that can cause sugarcane mosaic may be circulating in China. Other viruses such as SCSMaV and SCMMV are also associated with sugarcane mosaic disease [39], but both viruses have not been reported in sugarcane in China to date. Therefore, viral metagenomics approaches could be employed to investigate these putative novel viruses. For example, Filloux et al. (2018) [19] and Fernandez et al. (2020) [40] used a viral metagenomics approach to reveal that SCMMV infected sugarcane in Florida (USA) and in the CIRAD's sugarcane quarantine program at Montpellier (France), respectively. In addition, the PCR-based technique could be an alternative approach for the detection of both SCMMV [40] and SCSMaV [41]. However, it is challenging to design ideal primers of two viruses for molecular detection because the information of nucleotide sequences of both viruses is very limited, and low viral titers occur in plants. To date, SCMMV and SCSMaV have only occurred in a minority of sugarcane-planting countries.

Another notable finding of the current study is the frequent occurrence of mixed infection by two or more viruses (SCMV, SCSMV, SrMV, and MaYMV) in samples exhibiting sugarcane mosaic symptoms. This result agrees with several previous investigations in China [8,11] and India [5]. Different compositions of mixed infections caused by diverse viruses are prevalent in sugarcane. These mixed infections could be transmitted between

sugarcane plants by insect vectors and harvesters, which both allow diverse viruses to readily accumulate in sugarcane, a vegetatively propagated crop [35]. However, little is known about how to interact with each other of these viruses in mixed infections and how to synergistically cause mosaic symptoms in sugarcane.

There are still no truly effective methods for controlling viral disease in sugarcane, although the use of resistant cultivars and healthy seedlings are two partially effective means of disease control [12]. Eliminating insect transmission in the field using pesticides is another measure used to control mosaic diseases caused by the viruses (SrMV, SCMV, and MaYMV), which are transmitted by a variety of aphids [3,4,16] and SCMMV, which is transmitted by the mealybug (*Saccharicoccus sacchari*) [14]. Lastly, quarantine programs and molecular detection and identification of viruses are of foremost importance for managing viral diseases associated with mixed infections in plants, particularly in sugarcane, which is a vegetatively propagated crop [8,35]. Collectively, this study provides insight into the control of mosaic diseases in sugarcane and could serve as a reference point for strategies to promote healthy seedling regulation and disease quarantine in China.

5. Conclusions

This study determined the four types of viruses (SrMV, SCMV, SCSMV, and MaYMV) associated with mosaic diseases in sugarcane in China. The RT-PCR amplification of the four viruses indicated that both single and mixed infections occurred in eight provinces. Meanwhile, the mixed infection of SrMV with SCMV and SCSMV in sugarcane crops is ubiquitous. These findings lay a good foundation for mosaic disease diagnosis and integrated disease management. However, the mechanistic dissections of synergistic interactions among four different sugarcane-infecting viruses need to be further explored.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12020302/s1, Table S1 Origin of leaf samples collected from 24 locations in eight provinces in China between 2017 and 2020. Table S2 Specific primer pairs used for RT-PCR detection of SrMV, SCMV, SCSMV, and MaYMV. Table S3 Sources of SrMV, SCMV, SCSMV, and MaYMV sequences obtained in this study and four reference sequences (highlighted in yellow) of each virus derived from the NCBI library. Figure S1 Distribution plot of sugarcane leaves showing mosaic symptoms sampled from 24 locations in China between 2017 and 2020. The numbers of collected samples are shown in brackets.

Author Contributions: E.-Q.H. and W.-Q.B. performed the experiments, analyzed the data, and wrote a first draft of the manuscript. S.-R.S., C.-Y.H., J.-S.C. and Z.-W.B. participated in partial experiments. Y.X. participated in leaf sample collection. J.-J.L. and S.-J.G. conceived and supervised the project and critically reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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