Identification and Distribution of *Soybean mosaic virus* Strains in Southern China

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ABSTRACT

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From 1,417 leaf samples with *Soybean mosaic virus* (SMV)-like symptoms collected from about 1,500 fields of 117 counties of 13 provinces in Southern China during 2004 to 2006, 201 SMV isolates were obtained after biological purification with single-lesion isolation and serological determination. Based on disease reactions on 10 differentials used in China as a representative from various reported differential cultivar systems, the isolates were grouped into 12 strains. Among them, eight were the same as those previously reported from Northeast China and the Huang-Huai Valleys while the other four were new and were designated as SC18, SC19, SC20, and SC21. Strains SC15 and SC18, which were predominant and widespread in Southern China, accounted for 32.3 and 26.4% of the total isolates and were distributed in 9 and 10 provinces, respectively. Thus far, 21 strains have been identified in the three soybean-producing regions in China under the uniform differentials system. According to their responses on the 10 differentials, the strains were clustered into five groups at similarity coefficient 0.6. Among them, SC15 in cluster V is the most virulent strain because it infects all differentials. Therefore, specific attention should be placed on control of its prevalence in soybean production.

Soybean (*Glycine max* (L.) Merr.) is a native nutritional crop in China due to its high protein and oil content, as well as its functional elements, such as isoflavones, and has been used as both human food and animal feed. *Soybean mosaic virus* (SMV; genus *Potyvirus*, family *Potyviridae*) causes one of the two most important diseases nationwide and also is the most prevalent virus disease of soybean in the three major soybean-producing regions in China (i.e., Northeast China, Huang-Huai Valleys, and Southern China). SMV infection causes seed quality deterioration and yield loss of about 35 to 50% under natural field conditions (19). Investigations of incidence and distribution of SMV are necessary for developing diagnostic systems and appropriate control procedures. Also, knowledge about which strains are likely to be most prevalent in a particular region allows farm advisors to guide growers in disease management. Soybean production in Southern China accounts for one-fourth of the total production in China. Soybean in this region is grown in various seasons from spring to winter under differ-

ent cropping systems scattered in a wide range of areas. This results in a continuous supply of young plants that serve as a source of virus for spread by aphids, resulting in early infection and more severe disease symptoms in this region than in other regions. Accordingly, SMV is one of the most common and serious diseases in Southern China. However, very little knowledge about SMV strains and their distribution in Southern China is available.

Conover (7) first recognized that soybean mosaic disease was caused by more than one strain of SMV in 1948. Since then, various SMV strain differential systems have been reported (Table 1). In the United States and Korea, the same set of soybean differentials was used whereas, in Japan, another set of differentials was used. In China, however, different researchers have established their own differential systems for SMV strain classification, and have not established a nationwide uniform set of SMV differentials (Table 1). Unfortunately, the use of many differential systems has not facilitated the establishment of breeding programs for resistance to SMV but, instead, has led to confusion among soybean breeders in identification of resistance sources, exchange of resistance information, and utilization of the nationwide germplasm resources. A uniform differential system is needed for identifying SMV strains so that scientists can exchange information and resistance materials based on a common understanding. Wang et al. (22) at the National Center for Soybean Im-

provement started to establish a new, nationwide uniform differential system based on both international and domestic differential systems. They evaluated various differential systems in Table 1 and attempted to collect all differentials. Unfortunately, some could not be obtained. Finally, in total, 25 differentials previously reported were collected. A wide range of SMV isolates from the Huang-Huai Valleys and Middle and Lower Changjiang Valleys (northern part of Southern China) were tested on the 25 differentials, and nine were chosen as a new set of SMV strain differentials, including Nannong 1138-2 (NN1138-2), Youbian 30, 8101, Tiefeng 25, Davis, Buffalo, Zaoshu 18, Kwanggyo, and Qihuang No. 1. Eight SMV strains (SC1-SC8) were identified from 81 isolates collected from the Huang-Huai Valleys and Middle and Lower Changjiang Valleys in China using this new set, which consisted of the nine differentials (22). Yang (25) identified SMV isolates from the Middle and Lower Changjiang Valleys based on this set of differentials during 2000 to 2001, and found that Wang et al.'s (22) differentials showed stable responses to the tested SMV isolates among replicated tests and could satisfactorily differentiate the isolates. But Yang (25) also found one severely virulent strain, SC9, which could infect all nine differentials proposed by Wang et al. (22). Accordingly, Dabaima, with resistance to SC9, was added into the differential system set by Wang et al. (22). Yang (25) also showed that Kefeng No. 1 had the same response patterns to SMV isolates as Dabaima, and that these two differentials could be used interchangeably. Based on this, Zhan et al. (27) used 10 differentials (9 from Wang et al. [22] plus Kefeng No. 1) as a set of differentials to further identify the SMV isolates collected from the Huang-Huai Valleys, and identified 10 strains (SC1 to SC10). Later, Guo et al. (8) and Wang et al. (23) also used Zhan et al.'s (27) set of differentials to classify the SMV strains from Northeast China and the central and northern Huang-Huai Valleys. As a result, seven new strains (SC11 to SC17) were identified. This set of differentials contains two to three from each of the reported differential systems in Table 1 except Takahashi's in Japan (21) (Table 1, boldface differentials) and, therefore, should cover a wide range of situations both in China and abroad, to satisfactorily differentiate various SMV strains.

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The SMV differential systems discussed above were established in Northeast China and the Huang-Huai Valleys, and there are no reports of SMV strain distribution from Southern China, though it is one of the major soybean-producing areas in China. Because SMV causes an especially severe disease in this region, the knowledge of SMV strains, including constitution, distribution, and corresponding management strategies, is important in controlling its occurrence and prevalence. Therefore, the objective of the present study was to make a thorough study of SMV strains in Southern China based on previous work by Wang et al. (22), Yang (25), Zhan et al. (27), Guo et al. (8), and Wang et al. (23). SMV samples were collected extensively from 13 provinces in Southern China, and isolates were purified from the samples by single-lesion isolation and serological verification. The isolates were tested on the set of 10 differentials established by Wang et al. (22), Yang (25), and Zhan et al. (27) (abbreviated as WYZ) to identify the SMV strains.

MATERIALS AND METHODS

Sources and maintenance of virus samples. In total, 1,417 leaf samples with mosaic-like symptoms were collected at R1/R2 from 13 provinces in Southern China during April to August of 2004 to 2006 (Fig. 1). Diseased leaf samples were collected from an average of 9 counties in each province, with at least 12 soybean

production fields in each county, in a total of about 117 counties and 1,500 fields. No samples were taken in fields without SMV-like symptoms. Each leaf sample was taken from a single soybean plant with SMV-like symptoms. Leaf samples were dried in anhydrous calcium chloride desiccators, then transferred to vinyl bags, cooled and transported on ice, and stored

at -80°C before being used for inoculation. All samples to be tested were inoculated onto and individually maintained on the susceptible cv. NN1138-2 in an aphid-free greenhouse or an insect-free nylon net room.

Biological purification and serological determination. The bean *Phaseolus vulgaris* cv. Topcrop was used to purify virus

Fig. 1. Location of the 13 provinces in China from which samples of soybean were collected for sources of *Soybean mosaic virus*. Numbers to the left of the province name correspond to the map location. Numbers to the right of the province name are the total number of samples collected in each province.

Table 1. Cultivars and lines of *Glycine max* used as differential hosts to describe and identify strains of *Soybean mosaic virus* in China and other countries

Country (region)	Reference	Soybean differential cultivars or lines ^a	Strains reported
United States	5	Clark, Rampage, Davis , York, Marshall, Ogden, Kwanggyo , Buffalo	G1, G2, G3, G4, G5, G6, G7
	4	Same	G3A, G5H
	2	Same	G7A
	13	Same	C14
Korea	10	Clark, Rampage, Davis , York, Marshall, Ogden, Kwanggyo , Buffalo	G5HD
	11	Same	G7H
Japan	21	Harosoy, Shiromame, Ou13, Tokachinagaha	A, B, C, D, E
China (Jiangsu)	17	Nannong 493-1, Hefeng 23, Nannong 133-3, Qihuang No. 1 , Xudou No. 1, Kexi No.8, Kwanggyo , Dabaima	Sa, Sb, Sc, Sd, Se, Sf
	3	Same	Sg, Sh
China (Jiangsu)	24	Williams, Rampage, York, Marshall, Ogden, Kwanggyo , Buffalo , PI96983, Suweon 97, and SS741185	G1, G2, G3, G4, G5, G6, G7, G8, G9, G10
China (Northeast)	14	Hefeng 23, Jilin 27, Jiunong No.9, Qihuang No. 1 , Tiefeng 18, Youbian 30 , Kexi No.8	N1, N2, N3
	28	Hefeng 25, Jilin 21, Jilin 26, Tiefeng 25 , Kefeng 34, Zaoshu 18 , Ludou 10, 8101	S1, S2-1M, S2-1N, S2-2M, S2-2N, S2-3M, S2-3N, S3-1M, S3-1N, S3-2M, S3-2N, S3-3M, S3-3N
China (Shandong)	15	Qihuang 22, Ludou No.4, Youbian 30 , Qihuang 10, Wenfeng No.5, Nannong 1138-2	Sd1, Sd2, Sd3, Sd4, Sd5, Sd6
China (Huang-Huai Valleys)	20	Nannong 1138-2 , Wenfeng No.5, Qihuang 10, Xudou No. 1, Youbian 30 , Ludou No.4, Qihuang 22, Mijia No. 1	Y1, Y2, Y3, Y4, Y5, Y6, Y7
China (Huang-Huai Valleys, Middle and Lower Changjiang Valleys)	22	Nannong 1138-2 , Youbian 30 , 8101 , Tiefeng 25 , Davis , Buffalo , Zaoshu 18 , Kwanggyo , Qihuang No. 1	SC1, SC2, SC3, SC4, SC5, SC6, SC7, SC8
	25	Nannong 1138-2 , Youbian 30 , 8101 , Tiefeng 25 , Davis , Buffalo , Zaoshu 18 , Kwanggyo , Qihuang No. 1 , Dabaima (or Kefeng No. 1)	SC9
	27	Nannong 1138-2 , Youbian 30 , 8101 , Tiefeng 25 , Davis , Buffalo , Zaoshu 18 , Kwanggyo , Qihuang No. 1 , Kefeng No. 1	SC10
China (Northeast China)	8	Same	SC11, SC12, SC13, SC14, SC15
	23	Same	SC16, SC17

^a Boldface entries were used as differentials in the present study.

isolates. Seed were sown in plastic pots. When the primary leaves were fully expanded, seedlings were transferred to dark conditions for 24 h to increase sensitivity to the virus. Diseased young leaves of soybean NN1138-2 with SMV-like symptom were homogenized with a sterilized ice-chilled mortar and pestle in 3-5 ml of 0.01 M phosphate buffer (mixture of sodium phosphate and potassium phosphate, pH 7.4). Each extract was mixed with a small amount of Carborundum (600-mesh) and applied to one-half of a detached *P. vulgaris* cv. Topcrop leaf with a paintbrush. Immediately afterward, inoculated leaves were rapidly rinsed with running tap water. Finally, they were put into petri dishes containing moist filter paper and incubated in a growth chamber (HPG-320H; Yitong Science & Technology Development [Tianjin] Co., Ltd.) at 25 to 30°C, 90% humidity, and 48 to 72 h of continuous incandescent lighting. After 2 to 3 days, obvious vein necrosis or local necrotic spots developed (Fig. 2), and single diseased spots were cut from leaves with sterilize scissors, then extracted into 10 mM K-phosphate and inoculated back onto NN1138-2 plants as outlined above. Each necrotic spot was designated as a purified isolate and each virus sample was purified at least three times by passage through *P. vulgaris* cv. Topcrop.

The double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was used for serological determination according to Clark and Adams (6) using antisera provided by the General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. Extracts from non-inoculated healthy leaves and those from NN1138-2 leaves inoculated with strain SC3, a widespread strain in the Huang-Huai Valleys (22), were used as negative and positive controls, respectively. Samples were considered positive if the optical density was twofold or more higher than that of negative controls. Finally, 201 isolates were obtained, while those that proved to be not SMV were discarded.

Differentials and strain identification.

The 10 differentials used in the present study were NN1138-2, Youbian 30, 8101, Tiefeng 25, Davis, Buffalo, Zaoshu 18, Kwanggyo, Qihuang No. 1, and Kefeng No. 1, provided by the National Center for Soybean Improvement, Nanjing Agricultural University.

Twenty NN138-2 plants were inoculated for each isolate and the symptoms appeared approximately 7 days post inoculation. When obvious mosaic symptoms were observed, 15 trifoliolate leaves of each isolate were collected from the diseased plants and homogenized for inocula as described above. Seed ($n = 12$ to 15) of each differential soybean genotype were sown into plastic pots (11 cm in diameter). When the primary leaves fully emerged,

they were inoculated as described above. Throughout the experiments, NN1138-2 was used as a susceptible check and as cultivar for increase of the virus isolates.

All inoculation experiments were conducted in an aphid-free greenhouse or an insect-free nylon net room at 20 and 30°C. Each of the 201 SMV isolates was tested on the 10 differentials at least twice. Inoculations were repeated a third or more times in the case of negative or uncertain results. Symptom expression in inoculated and noninoculated leaves of soybean differentials was monitored, scored, and recorded 7 to 30 days post inoculation. Both mosaic and necrosis symptoms were observed on inoculated and systemic leaves of the differential hosts. Based on the results obtained, a provisional grouping of the isolates was made, and two to three representative isolates of each strain (or group of isolates with the same symptoms on differentials) were selected for further verification of the strain's infection pattern to ensure the accuracy of classification.

Cluster analysis of strains. The phenotypic data for the 4 strains newly found in the present study plus those for 17 SMV strains previously reported were used for cluster analysis to explore their interrela-

tionships. Similarity coefficients among strains were calculated according to Nei and Li (16) and a dendrogram based on similarity matrix and unweighted pair group method with arithmetic means clustering procedure was obtained by using the software NTSYS-pc (18).

RESULTS

SMV isolates classification. From 1,417 leaf samples with mosaic-like symptoms on SMV-susceptible soybean NN1138-2, 476 were recovered (transmitted two or more times to NN1138-2) and 201 isolates were obtained after single-lesion isolation and serological determination (Table 2).

The 201 SMV isolates showed 12 different reaction patterns and, therefore, were classified into 12 strains based on the inoculation tests on the 10 differentials. Each strain was represented by a different number of isolates from different provinces (Tables 3 and 4). Strain no. 1 was least virulent because it systemically infected only NN1138-2 and 8101, whereas strain no. 12 systemically infected all 10 differentials, including the putatively most resistant cv. Kefeng No. 1 which, therefore, was the most virulent strain (Table 3). According to their pathogenicity, the 12

Fig. 2. Necrotic venal lesions on detached leaves of *Phaseolus vulgaris* cv. Topcrop 2 to 3 days post inoculation. Lesions were cut from the leaf and inoculated onto *Glycine max* cv. Nannong 1138-2 to recover purified isolates of *Soybean mosaic virus*.

Table 2. Isolation of *Soybean mosaic virus* (SMV) from Southern China provinces

Code, province	No. of collected symptomatic samples	No. of isolates recovered ^a	No. of biologically purified isolates ^b	No. of isolates serologically positive for SMV (%)
1 Shaanxi	97	31	4	4 (2.0)
2 Jiangsu	31	18	14	10 (5.0)
3 Shanghai	73	40	33	18 (8.9)
4 Sichuan	73	28	12	11 (5.5)
5 Chongqing	80	17	9	7 (3.5)
6 Zhejiang	155	80	39	31 (15.4)
7 Jiangxi	162	18	19	8 (4.0)
8 Hunan	179	60	120	56 (27.7)
9 Fujian	136	48	27	16 (8.0)
10 Guizhou	57	15	13	2 (1.0)
11 Yunnan	57	16	9	6 (2.9)
12 Guangxi	77	25	21	13 (6.5)
13 Guangdong	240	80	31	19 (9.5)
Total	1,417	476	351	201 (100.0)

^a Number of isolates recovered from NN1138-2.

^b Number of biologically purified isolates from *Phaseolus vulgaris* cv. Topcrop.

strains are grouped into three categories. Strains no. 1 to 3 were less virulent, infecting one to three differentials, and accounted for 29.4% of the isolates. Strains no. 4 to 10 were moderately virulent, infecting 5 to 8 differentials, and accounted for 33.3% of the isolates. Because strain no. 11 can infect 9 of the 10 differentials and no. 12 can infect all 10 differentials, these two strains were the most virulent

and accounted for 37.3% of the total isolates. Thus, the most virulent and moderately virulent strains were predominant in Southern China.

For a further verification of the above results, two to three isolates from each of the 12 SMV strains were tested again on the differentials. The results were the same as in the previous tests, with only a few cases showing symptoms changed between

systemic mosaic and systemic necrosis. However, it does not change the determination of its reaction pattern because both systemic mosaic and systemic necrosis are classified as infected here.

To identify germplasm resistant to strain no. 12, 10 soybean accessions known to be resistant to most SMV strains (1,9,12,26) were inoculated and tested. None of the germplasm so far was resistant (Table 5).

Table 3. Identification of 12 strains of *Soybean mosaic virus* (SMV) from 201 isolates from Southern China, based on responses of 10 soybean differential cultivars^a

Strain no.	NN 1138-2	Youbian 30	8101	Tiefeng 25	Davis	Buffalo	Zaoshu 18	Kwang-gyo	Qihuang No. 1	Kefeng No. 1	No. of isolates	Designation
1 ^b	-/M	-/-	-/M	-/-	-/-	-/-	-/-	-/-	-/-	-/-	53	SC18
2	-/M	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/-	-/-	2	SC3
3	-/M	-/-	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/-	4	SC11
4 ^b	-/M	N/N	-/M	-/-	-/M	-/M	-/-	-/-	-/-	-/-	6	SC21
5 ^b	-/M	N/N	-/M	-/-	-/N	N/N	N/N	-/-	-/-	-/-	12	SC20
6 ^b	-/M	N/N	-/M	-/-	-/N	N/N	-/N	-/-	-/M	-/-	5	SC19
7	-/M	-/M	-/M	-/M	-/M	-/M	-/-	-/-	-/-	-/-	2	SC6
8	-/M	N/M	-/M	-/M,N	-/M	-/M	-/-	N/N	-/-	-/-	4	SC13
9	-/M	-/M	-/M	-/M	-/M	-/M	-/M	-/-	-/-	-/-	14	SC7
10	-/M	-/M,N	-/M	-/M	-/M	-/M,N	-/M	-/-	-/M	-/-	24	SC10
11	-/M	-/M	-/M	-/M	-/M	-/M	-/M	-/N/M,N	-/M	-/-	10	SC9
12	-/M	-/M	-/M	-/M	-/M	-/M	-/M	-/N/M,N	-/M	-/M	65	SC15

^a Format: reactions on inoculated primary leaves/reactions on noninoculated upper leaves; - = symptomless: no mosaic or necrosis symptom on leaves above the inoculated primary leaves on all the tested plants; M = mosaic symptoms on the leaves above the primary leaves on most of the plants tested; N = necrosis symptoms on the leaves above the primary leaves on more than two-thirds of the plants tested; M,N = both mosaic and necrosis symptoms appear on the leaves above the primary leaves on most of the plants tested; -, N = symptomless and local necrosis.

^b Newly found SMV strains in Southern China.

Table 4. Frequency distribution of *Soybean mosaic virus* (SMV) strains isolated from 13 provinces in Southern China

Code, province	SC3	SC6	SC7	SC9	SC10	SC11	SC13	SC15	SC18	SC19	SC20	SC21
1 Shaanxi	...	...	...	...	1	...	...	...	3	...	...	...
2 Jiangsu	...	...	1	...	2	2	...	1	4	...	...	...
3 Shanghai	...	...	3	3	8	...	...	...	1	2	1	...
4 Sichuan	...	...	...	1	1	...	...	1	8	...	...	...
5 Chongqing	...	...	2	1	2	...	...	...	2	...	...	...
6 Zhejiang	1	...	...	1	7	2	...	...	19	1	...	...
7 Jiangxi	...	...	1	...	...	...	...	4	3	...	...	...
8 Hunan	...	1	4	3	1	...	...	33	3	...	7	4
9 Fujian	1	...	...	...	1	...	2	3	7	1	1	...
10 Guizhou	...	...	...	...	...	...	...	2	...	...	...	...
11 Yunnan	...	1	...	...	...	...	1	4	...	...	...	...
12 Guangxi	...	...	1	1	1	...	...	6	3	...	1	...
13 Guangdong	...	...	2	...	...	...	1	11	...	1	2	2
Total ^a	2	2	14	10	24	4	4	65	53	5	12	6
Percent ^b	1.0	1.0	6.9	5.0	11.9	2.0	2.0	32.3	26.4	2.5	6.0	3.0

^a Number of SMV isolates.

^b Percentage of strains in each category, relative to total number of isolates tested.

Table 5. Pathogenicity of *Soybean mosaic virus* (SMV) strain SC15 on soybean cultivars and germplasm reported to be resistant to SMV

Soybean cultivar	Strains for which resistance reported	Reference	Response to SC15	
			Symptom ^a	Infection ratio ^b
PI96983	G1, G2, G3, G4, G5 ^c , G6 ^c , SC14	9,12,26	-/M	30/30
Dongda No.2	SC3, SC8 ^c , SC11, SC12, SC13 ^c , SC17 ^c	1	-/M	28/30
Bindou 95-20	SC3, SC8, SC11, SC12, SC13, SC17	1	-/M	28/29
Zhongpin 02-046	SC3, SC8, SC11, SC12, SC13, SC17	1	-/M	25/26
Dongda No.4	SC3, SC8, SC11, SC12, SC13, SC17	1	-/MN	26/26
Hengsheng No. 1	SC3, SC8, SC11, SC12, SC13, SC17	1	-/MN	29/29
BN101	SC3, SC8, SC11, SC12, SC13, SC17	1	N/N	30/32
Zhongpin 661	SC3, SC11, SC12, SC13, SC17	1	-/MN	28/30
Zhongzuo00-683	SC3, SC11, SC12, SC13, SC17	1	-/M	25/26
Zhongpin 662	SC3, SC17	1	-/M	27/30

^a Format: reactions on inoculated primary leaves/reactions on noninoculated upper leaves; - = symptomless; N = necrosis; M = mosaic; R = resistant; S = susceptible.

^b Number of plants showing symptoms/total inoculated plants.

^c Virulent strains that reported in the previous literature.

Strain distribution. The distribution of SMV strains in each province in Southern China is shown in Table 4. Eight strains were identified in Hunan Province, the most of any province. Among them, only SC18 was in the less-virulent class; the other seven strains were highly or moderately virulent. The most virulent strain, SC15, was predominant and accounted for 58.8% of the total isolates in the province. The complexity of SMV strain constitution in terms of number of strains decreased in order for Fujian, Guangxi, Guangdong, Zhejiang, Shanghai, Jiangsu, Sichuan, and Chongqing provinces (7, 6, 6, 6, 6, 5, 4, and 4 strains, respectively), with SC15 or SC18 predominant in all provinces except for Shanghai, where SC7, SC9, and SC10 were predominant. The constitution of SMV strains in Jiangxi, Yunnan, Shaanxi, and Guizhou was relatively simple, consisting of only 3, 3, 2, and 1 SMV strains, respectively. Among the 12 strains, SC7, SC9, SC10, SC15, and SC18 were detected in 7, 6, 9, 9, and 10 provinces. Strains SC15 and SC18 comprised 65 and 53 isolates and accounted for 32.3 and 26.4% of the total isolates, respectively. These two strains were especially predominant in Southern China.

Virulence differentiation and similarity among SMV strains from all regions in China. Twenty-one SMV strains have been identified in China using the WYZ set of differentials (Table 6). Here, the phenotypes were grouped in two categories, with and without systematic symptoms. A dendrogram showing relationships among the 21 strains is shown in Figure 3. There were five clusters with similarity coefficients of 0.60 or more. Cluster I contains nine less virulent strains—SC1 to SC4, SC11, SC12, SC14, SC16, and SC18—which can infect only one to four

differentials. Among them, SC1 and SC2 plus SC3 and SC18 had high similarity in differential responses, whereas SC11 and SC16 differed only in their response on the nondifferential cv. Wenfeng No. 5. Cluster II contains nine moderately virulent strains—SC5 to SC8, SC10, SC13, and SC19 to SC21—which infected five to eight differentials. Of these, SC5 and SC6, SC8 and SC13, and SC20 and SC21 had high similarity in their differential responses. Clusters III, IV, and V contain SC9, SC17, and SC15, respectively. These were the most virulent strains, infecting 9 to 10 differentials, including the most resistant differential, Kefeng No. 1. SC15 of

cluster V can infect all 10 differentials and is the most virulent strain at present.

DISCUSSION

Evaluation of the differentials system of SMV strains. Previous reports demonstrated that the WYZ set of differentials for SMV strain determination was effective in classifying SMV strains in the Huang-Huai Valleys and Northeast China (8,22,23,25). In the present study, the same set of differentials was also used in identifying and classifying SMV isolates in Southern China.

The reaction patterns of the 12 SMV strains on the 10 differentials in this study

Fig. 3. Relationships among 21 *Soybean mosaic virus* strains in China. The dendrogram was generated using unweighted pair group method with arithmetic means cluster analysis based on Nei and Li's similarity estimates (16,18). Here, "Coefficient" means coefficient of similarity.

Table 6. Summary of responses of 10 differential soybean cultivars to 21 *Soybean mosaic virus* strains in China^a

Strain	NN 1138-2	Youbian 30	8101	Tiefeng 25	Davis	Buffalo	Zaoshu 18	Kwanggyo	Qihuang No. 1	Kefeng No. 1
SC1	-/M	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
SC2	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
SC3 ^b	-/M	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/-	-/-
SC4	-/M	-/M	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/-
SC5	-/M	-/M	-/M	-/M	-/M	-/-	-/-	-/-	-/-	-/-
SC6 ^b	-/M	-/M	-/M	-/M	-/M	-/M	-/-	-/-	-/-	-/-
SC7 ^b	-/M	-/M	-/M	-/M	-/M	-/M	-/M	-/-	-/-	-/-
SC8	-/M	-/M	-/M	-/M	-/M	-/M	-/M	N/M	-/-	-/-
SC9 ^b	-/M	-/M	-/M	-/M	-/M	-/M	-/M	N/M	-/M	-/-
SC10 ^b	-/M	-/M,N	-/M	-/M	-/M	-/M,N	-/M	-/-	-/M	-/-
SC11 ^b	-/M	-/-	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/-
SC12	-/M	-/-	-/M	-/M	-/-	-/M	-/-	-/-	-/-	-/-
SC13 ^b	-/M	N/M	-/M	-/M,N	-/M	-/M	-/-	N/N	-/-	-/-
SC14	-/M	-/-	-/M	-/M	-/-	-/-	-/-	-/-	-/-	-/M
SC15 ^b	-/M	-/M	-/M	N/M	-/M	-/M	N/M	N/M	-/M	-/M
SC16	-/M	-/-	-/M	-/M	N/-	N/-	-/-	N/-	-/-	-/-
SC17	-/M	N/M	-/M	-/M	-/M	N/M	-/M	N/N	-/-	-/M
SC18 ^b	-/M	-/-	-/M	-/-	-/-	-/-	-/-	-/-	-/-	-/-
SC19 ^b	-/M	N/N	-/M	-/-	-/N	N/N	-/N	-/-	-/M	-/-
SC20 ^b	-/M	N/N	-/M	-/-	-/N	N/N	N/N	-/-	-/-	-/-
SC21 ^b	-/M	N/N	-/M	-/-	-/M	-/M	-/-	-/-	-/-	-/-

^a Data are from previous studies (8,22,23,25,27) and from strains identified from 13 provinces in this study. Format: reactions on inoculated primary leaves/reactions on noninoculated upper leaves; - = symptomless, M = mosaic, N = necrosis.

^b Strains detected in 13 provinces in Southern China.

(Table 3) were compared with previously reported SMV strains (8,22,23,25,27), and the results indicated that eight strains identified in this study were previously identified. Strains no. 2, 3, 7, 8, 9, 10, 11, and 12 corresponded to strains SC3, SC11, SC6, SC13, SC7, SC10, SC9, and SC15, respectively, while the remaining four had unique reaction patterns and are proposed to be new strains. Following Wang et al.'s (22) nomenclature system, we propose that these four be designated SC18, SC19, SC20, and SC21, corresponding to strains no. 1, 4, 5, and 6, respectively. Our results indicate that the WYZ set of differentials not only can identify satisfactorily the various SMV strains but also can detect satisfactorily the new ones in Southern China. Because this set of differentials was selected from most of the previously reported different systems, it has a wide genetic background and, therefore, can identify a wide range of SMV variation. Accordingly, we suggest using this set of differentials as a uniform system, at least in China, for better communication and exchange of research information and breeding materials regarding SMV resistance. However, among the 21 strains in China, SC11 and SC16 show similar responses on the 10 differentials, with difference only on nondifferential cv. Wenfeng No. 5 (Table 6) (i.e., SC11 does not infect Wenfeng No. 5, whereas SC16 does; 8,23). Therefore, we suggest they be treated as one strain, designated as SC11.

Comparisons of constitution and distribution of SMV strains among Northeast China, the Huang-Huai Valleys, and Southern China. In Southern China, SC15 and SC18 were recognized as predominant and widespread strains, with the former being the most virulent, accounting for 32.3% of the total isolates in 9 provinces, and the latter being less virulent, accounting for 26.4% of the total isolates in 10 provinces. SC7, SC9, and SC10 were detected in 7, 6, and 9 provinces, respectively, and were widespread virulent strains in Southern China. From the above information, it can be concluded that the widespread SMV strains, except SC18 in Southern China, are all the most and moderately virulent compared with the situation of less and moderately virulent strains predominant in Northeast China and the Huang-Huai Valleys (8,23). This explains why the mosaic disease caused by SMV is especially serious in Southern China. Therefore, the five major strains in Southern China should be treated as major targets in breeding for SMV resistance in this region. In Northeast China, Wang et al. (23) classified 112 SMV isolates into eight strains and indicated that SC11 was the major and most widespread strain. In the Huang-Huai Valleys, Guo et al. (8) grouped 88 SMV isolates into 10 strains and showed that SC11 and SC13 were the most widespread strains, whereas SC8 and

SC11 were the predominant strains. Thus, the major SMV strains and their distribution in various regions are different, especially between Northeast and Southern China, with the most widespread and predominant strain in Northeast China being strain SC11 whereas, in Southern China, strain SC15 is predominant. The constitution of SMV strains becomes more complex and the SMV disease more severe from north to the south in China. This might be due to the fact that, in Southern China, soybean cultivars are grown in multiple cropping systems, including spring, summer, fall, and even winter planting types. Because there is a warmer and longer growing season, some aphid species of genus *Aphis*, such as *Aphis glycines*, the major vector of SMV, could transmit the virus from one season's crop to another.

The most virulent strain, SC15, is predominant and widespread in Southern China and no resistance source has been identified; therefore, soybean breeders should focus attention on strategies to cope with this strain.

By comparing the results of virulence clustering analysis of 21 SMV strains in China to various soybean ecological regions, SC11 in cluster I was the major strain in Northeast China (23); while SC3 in cluster I and SC7 and SC11 in cluster II were the major strains in Huang-Huai Valley (8,22,27). SC18, SC7 and SC10, SC9, and SC15 in clusters I, II, III, and V, respectively, were the major strains in Southern China. Although the presence of less virulent and virulent strains overlapped among the three major soybean-producing regions, less virulent strains in cluster I predominate in Northeast China; less and moderately virulent strains in cluster I and II predominate in the Huang-Huai Valleys; whereas moderately and most virulent strains in cluster II, III, and V predominate in Southern China. Therefore, the trend of virulence of SMV strains in China appeared to gradually increase from the north to the south.

SMV purification and serological determination. In this study, all diseased samples were collected from soybean in fields under natural conditions. Each sample may include two or more SMV genotypes, or even may be a mixture of several kinds of viruses. Therefore, in our study, isolates were biologically purified and serologically verified to ensure that each isolate was a purified and separate SMV genotype. In many cases, multiple isolates from the same tissue sample were not the same SMV strain or even were not all SMV, although they infected *P. vulgaris* cv. Topcrop and induced necrotic spots. This result indicated that strains of virus in soybean fields were complex. For example, sample k6024 was purified by using *P. vulgaris* cv. Topcrop, from which four isolates were obtained and designated as k6024-1, k6024-2, k6024-3, and k6024-4

(data not shown). Isolates k6024-1, k6024-3, and k6024-4 were serologically verified as SMV whereas isolate k6024-2 was negative for SMV. Isolates k6024-1, k6024-3, and k6024-4 were assigned to SC7, SC10, and SC15, respectively. Unfortunately, biological purification and serological determination were not performed in some reports on classification of SMV strains (5,14,15,20), and this may account for some inconsistencies in the results of these studies. Interestingly, we also found a few symptomatic samples that could not infect *P. vulgaris* cv. Topcrop and induce necrotic spots although they were proved to be SMV positive by ELISA. This result did not agree with that of Cho and Goodman (5), who reported that all SMV can infect *P. vulgaris* cv. Topcrop and induce necrotic spots. This phenomenon should be further studied.

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