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Short communication

Distribution and molecular diversity of three cucurbit-infecting poleroviruses in China

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ABSTRACT

Cucurbit aphid-borne yellows virus (CABYV) and Melon aphid-borne yellows virus (MABYV) have been found to be associated with cucurbit yellowing disease in China. Our report identifies for the first time a third distinct polerovirus, tentatively named Suakwa aphid-borne yellows virus (SABYV), infecting Suakwa vegetable sponge. To better understand the distribution and molecular diversity of these three poleroviruses infecting cucurbits, a total of 214 cucurbitaceous crop samples were collected from 25 provinces in China, and were investigated by RT-PCR and sequencing. Of these, 108 samples tested positive for CABYV, while 40 samples from five provinces were positive for MABYV, and SABYV was detected in only 4 samples which were collected in the southern part of China. Forty-one PCR-amplified fragments containing a portion of the RdRp gene, intergenic NCR and CP gene were cloned and sequenced. Sequence comparisons showed that CABYV isolates shared 78.0-79.2% nucleotide sequence identity with MABYV isolates, and 69.7-70.8% with SABYV. Sequence identity between MABYV and SABYV was 73.3-76.5%, In contrast, the nucleotide identities within each species were 93.2–98.7% (CABYV), 98.1–99.9% (MABYV), and 96.1-98.6% (SABYV). Phylogenetic analyses revealed that the polerovirus isolates fit into three distinct groups, corresponding to the three species. The CABYV group could be further divided into two subgroups: the Asia subgroup and the Mediterranean subgroup, based on CP gene and partial RdRp gene sequences. Recombination analysis suggested that MABYV may be a recombinant virus.

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The family *Luteoviridae* is divided into three genera (*Luteovirus*, *Polerovirus* and *Enamovirus*), which can be distinguished on the basis of the arrangement and sizes of the open reading frames (ORFs). Poleroviruses are plant viruses consisting of small, single molecules of infectious, linear, positive-sense ssRNA, with no envelope, and hexagonal particles of approximately 25–30 nm in diameter (D'Arcy and Domer, 2005). Polerovirus particles are largely confined to phloem cells of their hosts. The genome of poleroviruses contains 6 ORFs, has a VPg linked to the 5′-end of the genomic RNA and lacks a Poly(A) tract or a tRNA-like structure on the 3′-end. Poleroviruses possess an ORF0 in the 5′ terminus and a non-coding region of about 200 nt between ORF2 and ORF3. ORFs 0, 1 and 2 are translated from the genomic RNA. Frameshift from ORF1 into ORF2 occurs upstream of the termination of ORF1. ORFs 3, 4 and 5 are expressed from its sgRNA (D'Arcy and Domer, 2005).

Cucurbit aphid-borne yellows virus (CABYV) was the first polerovirus reported to infect cultivated cucurbits naturally and cause a severe disease (Abou-Jawdah and Fayyad, 1997; Lecoq

et al., 1992). This virus has been found in France, Italy, Lebanon, Spain, Iran, Turkey, Tunisia, Slovak and USA (Abou-Jawdah and Fayyad, 1997; Bananej et al., 2009; Juarez, 2004; Lecoq et al., 1992; Tomassoli and Meneghini, 2007; Yardumcı and Özgönen, 2007). The complete genomic sequence of CABYV originated from France and partial sequences of Spanish, Italian and Tunisian isolates have been reported (Guilley et al., 1994; Juarez, 2004; Mnari, 2005). CABYV is transmitted persistently by various aphids and by grafting, but not mechanically. Yield losses of up to 40% have been reported in melon plants when compared with uninoculated control plants. Affected plants characteristically produce fewer fruits per plant, although fruit shape and quality are not impacted greatly (Lecoq et al., 1992; Lemaire et al., 1993).

In China, various species of cucurbits (family *Cucurbitaceae*), especially watermelon, melon, cucumber, squash and cushaw, are cultivated widely for many different purposes. Among the diseases affecting cucurbits, viral diseases can result in lethal syndromes and yellowing phenotypes, and are very difficult to manage. Among these viruses, *Cucumber mosaic virus* (CMV), *Watermelon mosaic virus* (WMV), *Squash mosaic virus* (SqMV) and *Zucchini yellow mosaic virus* (ZYMV) have been reported to be the most destructive in cucurbits in China (Chen, 2003; Gu, 2002). Our preliminary surveys carried out in 2006 definitively confirmed the

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 Table 1

 Locations, crops, dates and persons involved in sample collection, as well as RT-PCR detection results and GenBank accession numbers of viral isolates in this study. Sites are listed in order of latitude from north to south in China.

Locations	Crops	Dates	Collector	Number of samples tested ^a					GenBank accession number
				A	P	С	M	S	
I. Heilong-jiang	Cushaw	2006.10	B. Geng	3	3	3	0	0	EU244321
	Watermelon	2006.10	B. Geng	1	0	0	0	0	
	Muskmelon	2006.10	B. Geng	2	1	1	0	0	
2. Liaoning	Cushaw	2006.10	C.G. Han	1	1	1	0	0	EU262623
	Cucumber	2006.10	C.G. Han	1	1	1	0	0	EU262624
3. Inner Mongolia	Cushaw	2006.9	C.G. Han	2	1	1	1	0	EU262627
	Calabash	2006.9	C.G. Han	1	0	0	0	0	20202027
	Cucumber	2006.9	C.G. Han	1	0	0	0	0	
4. Xinjiang	Muskmelon	2007.7	D. Yang	11	8	8	0	0	EU244315
i. Airijiurig	Cushaw	2007.7	D. Yang	1	0	0	0	0	2021.310
5. Gansu	Cushaw	2006.9	C.G. Han	2	1	1	0	0	EU262625
6. Beijing	Squash	2006.8	H.Y. Xiang	4	3	3	3	0	
	Cushaw	2006.8	H.Y. Xiang	25	13	13	13	0	EU000535
	Wax gourd	2006.8	H.Y. Xiang	10	9	9	9	0	EU000534
	Suakwa	2006.8	H.Y. Xiang	10	5	5	5	0	EF063708
	Muskmelon	2006.8	C. Zhang	4	4	4	4	0	EU091149
	Watermelon	2006.8	C. Zhang	10	2	2	2	0	EU091150
	Calabash	2006.8	Q.X. Shang	9	5	5	0	0	EU091151
	Bitter gourd	2006.8	Q.X. Shang	3	2	2	0	0	EU091148
	Cucumber	2006.8	Q.X. Shang	11	3	3	0	0	EF063706
7. Tianjin	Cushaw	2007.9	Y. He	5	2	2	0	0	EU244323
	Calabash	2007.9	Y. He	1	0	0	0	0	
	Muskmelon	2007.9	Y. He	1	0	0	0	0	
	Suakwa	2007.9	Y. He	2	0	0	0	0	
8. Hebei	Cushaw	2007.8	Y.M. Wei	2	2	2	0	0	
	Suakwa	2007.8	Y.M. Wei	2	0	0	0	0	
9. Shanxi	Cushaw	2007.8	F.S. Shi	1	1	1	0	0	EU244319
	Watermelon	2007.8	F.S. Shi	1	0	0	0	0	
	Muskmelon	2007.8	F.S. Shi	1	0	0	0	0	
10. Shandong	Cushaw	2007.8	Y.H. Han	2	1	1	0	0	EU244318
	Calabash	2007.8	Y.H. Han	1	1	1	0	0	
	Suakwa	2007.8	Y.H. Han	1	0	0	0	0	
11. Shaanxi	Cushaw	2006.9	C.G. Han	3	3	3	0	0	EU244317
	Suakwa	2007.9	Z. Bi	1	0	0	0	0	2021317
	Bitter gourd	2007.9	Z. Bi	1	0	0	0	0	
12. Henan	Cushaw	2007.9	J. Yang	4	2	2	0	0	EU244325
12. Heliali	Suakwa	2007.9	J. Yang	2	2	2	0	0	EU244324
13. Jiangsu	Cushaw	2006.10	C.G. Han	5	3	3	0	0	EU262630
	Suakwa	2006.10	C.G. Han	1	0	0	0	0	10202030
	Cucumber	2006.10	C.G. Han	2	1	1	0	0	EU262629
	Bitter gourd	2006.10	C.G. Han	1	0	0	0	0	
14. Anhui	Calabash	2008.8	Z.M. Gao	1	1	0	1	0	FJ460213
17, Alliiui	Cucumber	2008.8	Z.M. Gao	1	1	1	0	0	FJ460214
15. Shanghai	Suakwa	2006.9	R.H. Peng	2	1	1	0	0	EF063707
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16. Hubei	Cushaw Calabash	2006.10 2006.10	L.Y. Yan L.Y. Yan	2 1	1 0	1 0	0 0	0 0	EF488996
17. Sichuan	Cushaw	2007.4	Q.N. Cai	3	2	1	0	0	EU244320
18. Zhejiang	Cushaw	2006.10	C.C. Wang	1	1	1	0	0	EU262626
	Suakwa	2006.10	C.C. Wang	1	0	0	0	0	
19. Jiangxi	Squash	2008.11	J.X. Jiang	1	1	0	1	0	FJ460215
	Cushaw	2008.11	J.X. Jiang	1	1	1	0	0	FJ460216
	Suakwa	2008.11	J.X. Jiang	1	0	0	0	0	
	Bitter gourd	2008.11	J.X. Jiang	1	0	0	0	0	
	Cucumber	2008.11	J.X. Jiang	1	0	0	0	0	
20. Hunan	Cushaw	2007.7	Q.X. Shang	2	1	1	0	0	EU244322
	Suakwa	2007.7	Q.X. Shang	1	0	0	0	0	
21. Fujian	Cushaw	2008.5	F.P. Yu	3	2	2	0	1	FJ425879
z i, i ujian	Squash	2008.5	F.P. Yu	4	1	1	0	0	FJ425880
22 Vunnan	Cushaw			7			0		EF488997
22. Yunnan	Cushaw	2006.10	Y.J. Li	/	5	5	U	0	EF40099/

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