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Complete Genome Sequence Analysis of Two Divergent Groups of *Sweet potato chlorotic fleck virus* Isolates Collected from Korea

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The *Sweet potato chlorotic fleck virus* (SPCFV), of the genus *Carlavirus* (family Betaflexiviridae), was first detected as one of several viruses infecting sweet potatoes (*Ipomea batatas* L.) in Korea. Out of 154 sweet potato samples collected in 2012 that were showing virus-like symptoms, 47 (31%) were infected with SPCFV, along with other viruses. The complete genome sequences of four SPCFV isolates were determined and analyzed using previously reported genome sequences. The complete genomes were found to contain 9,104-9,108 nucleotides, excluding the poly-A tail, containing six putative open reading frames (ORFs). Further, the SPCFV Korean isolates were divided into two groups (Group I and Group II) by phylogenetic analysis based on the complete nucleotide sequences; Group I and Group II had low nucleotide sequence identities of about 73%. For the first time, we determined the complete genome sequence for the Group II SPCFV isolates. The amino acid sequence identity in coat proteins (CP) between the two groups was over 90%, whereas the amino acid sequence identity in other proteins was less than 80%. In addition, SPCFV Korean isolates had a low amino acid sequence identity (61% CPs and 47% in the nucleotide-binding protein [NaBp] region) to that of *Melon yellowing-associated virus* (MYaV), a typical *Carlavirus*.

Keywords **:** Complete genome sequences, phylogenetic analyses, sweet potato chlorotic fleck virus

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Sweet potatoes (*Ipomea batatas* L., family Convolvulaceae) are grown extensively throughout tropical and temperate regions and are the seventh most important food crop in the world in terms of production. According to Food and Agriculture Organization Corporate Statistical Database (FAOSTAT) data from 2014, sweet potatoes have been cultivated across 8.4 million ha, with the production of 107 million tons worldwide. In Korea, sweet potatoes are cultivated across 20,515 ha, resulting in the production of 322,071 tons, with output increasing every year (FAOSTAT, 2014).

Viral diseases affecting sweet potatoes have become widespread, causing serious crop losses around the world. To date, more than 30 viruses have been identified that infect sweet potatoes (Brunt et al., 1996; Clark et al., 2012). In Korea, eight viruses have been reported, including the *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato virus* C (SPVC), *Sweet potato virus* G (SPVG), *Sweet potato virus* 2 (SPV2), and *Sweet potato latent virus* (SPLV) belonging to the genus *Potyvirus* in the family Potyviridae; *Sweet potato leaf curl virus* (SPLCV), belonging to the genus *Begomovirus* in the family Geminiviridae; *Sweet potato symptomless virus* 1 (SPSMV-1), belonging to the genus *Mastrevirus* in the family Geminiviridae; and *Sweet potato chlorotic fleck virus* (SPCFV), belonging to the genus *Carlavirus* in the family Betaflexiviridae (Kwak et al., 2006, 2014). Most sweet potato virus symptoms result from a mixed infection of at least two of these eight viruses (Kwak et al., 2014). Among these eight viruses, SPFMV and SPVC are very prevalent in Korea (Kwak et al., 2014).

SPCFV was first detected in sweet potatoes showing fine

chlorotic spots in Peru. It has since been detected in several countries of South America, Asia, and South Africa, and the complete genome sequence of an isolate from Uganda has been characterized (Aritua et al., 2007). SPCFV reportedly causes a synergistic disease when sweet potatoes are co-infected with *Sweet potato chlorotic stunt virus* (SPCSV) (Untiveros et al., 2007). In Korea, SPCFV occurred in 31% of samples collected from most areas of Korea in 2012 (Kwak et al., 2014). However, the incidence rates of SPCFV decreased to 0% and 2.5% in 2013 and 2014, respectively (Kim et al., 2017).

SPCFV is a positive-sense single-stranded RNA virus with a genome of approximately 9.1 kb that potentially includes six open reading frames (ORFs). ORF1 encodes a viral replicase (Rep), ORF2 to ORF4 encode triple gene block (TGB) proteins (TGB1, TGB2, and TGB3), and ORF5 encodes the coat protein (CP). Further, ORF6 encodes a putative nucleotide-binding protein (NaBp) (Aritua et al., 2007).

In this study, to analyze the genetic structure and variability of Korean SPCFV isolates, we determined the complete genome sequences of four SPCFV isolates based on geographic location from sweet potato samples collected in 2012 (Kwak et al., 2014).

Total RNA was extracted from the infected sweet potato leaf, petiole, and stem samples using an Easy-spin™ total RNA extraction kit (Intron, Korea) according to the manufacturer's instructions. Reverse transcription polymerase chain reaction (RT-PCR) was performed as a two-step procedure; RT was conducted using *Avian myeloblastosis virus* (AMV) reverse transcriptase (Promega, USA), and PCR was carried out using high-fidelity LA taq polymerase (Takara, Japan). Specific PCR primer pairs were designed for primer walking and subsequent sequencing to obtain the complete genome sequences based on previously reported SPCFV nucleotide sequences (Table 1, 2). End sequences of each RNA segment were obtained according to the 5′ and 3′ rapid amplification of cDNA ends (RACE) protocol (BM, Germany). cDNA clones containing the 5′ end of the genome were obtained using the Xec primer (5′-AAAGAATTCCCCCCCCCCCCC-3′) and SPCFV_5′-race-R primer complementary to nucleotides 262-239 of the SPCFV genome. In addition, cDNA clones containing the 3′ end of the genome were obtained using a SPCFV

Table 1. Full sequencing primers for Group I of SPCFV

Fragment	Name	Sequence (5' → 3')	Loci*	Size (bp)
P1	spcfv-full-1f	TGCTGAAGAGGCACTATCCTCC	86-107	1060
	spcfv-full-1r	AGCAGACTGAACATCTGGCTTC	1145-1124	
	spcfv-full-2f-1	TGCAATGATGGTACWGTCTATAGTG	897-921	1173
	spcfv-full-2r-1	CCTTTGAGRCTTCTARWGCTTC	2070-2049	
	spcfv-full-3f	ACGGGCTCATTTGGTTCTTG	1810-1829	1027
	spcfv-full-3r	GCTTCAGTGTCCCTGGATTCAC	2836-2815	
	spcfv-full-4f	AGATGCCAAGAGATTTCCAAGAG	2629-2651	1050
	spcfv-full-4r	AGGTGCCACCTCCTCTAGCTC	3678-3658	
P2	spcfv-full-5f-1	TTTGATATWTGCGGGGTGTGC	3462-3482	1016
	spcfv-full-5r	GCGAGGGCACGACATTCCT	4477-4459	
	spcfv-full-6f-1	CAAAGTGATTATGACTCTCTGCG	4254-4276	1108
	spcfv-full-6r-1	TCGAAGTCCTGYACAGCCTTATC	5361-6340	
	spcfv-full-7f	AGAGATTTGCAGCCATCTACCC	5149-5161	1027
	spcfv-full-7r	ATTGCATAATTATCCAGGCATTC	6175-6153	
	spcfv-full-8f	AACACTTAAGACAGAGCATGATGG	5978-6001	1085
	spcfv-full-8r	TTAGAATAGTCCGCGGGTGG	7063-7044	
P3	spcfv-full-9f	GGCCACAAGAAGTGAGGGG	6894-6912	1061
	SPCFV 1R	AAGGTCTGTAGTTTTCCATGTACC	7954-7931	
	SPCFV 1F	ATGTTTGCTGGGGAGAGTCAGG	7555-7576	
	SPCFV 2F-1	AAAGTGGAACAGGGAGCCCG	8067-8086	1038
	SPCFV 2R	GCTCAAAAGTACTTTAAAACATGC	9104-9081	
RACE	SPCFV 3'-F	GTGATTGGGAYYGYTGTCTT	8718-8737	387
	SPCFV 5'-R	GGTGAAACAGGTGAGAGGTATATC	262-239	262

*Reference sequence: SPCFV- Hoima 4 (AY461421)

Table 2. Full sequencing primers for Group II of SPCFV

Fragment	Name	Sequence (5' → 3')	Loci*	Size (bp)
P1	spcfv-full-1f	TGCTGAAGAGGCACTATCCTCC	86-107	1060
	spcfv-full-1r	AGCAGACTGAACATCTGGCTTC	1145-1124	
	SPCFV(II)-2F	ATTCGCCCACCACTTAATTAGTATC	944-968	1089
	SPCFV(II)-2R	TGGTGATTCAACTGCATTCGGGTC	2032-2009	
	SPCFV(II)-3F	AAGGGCAAGTGGTTTTCAAGTAAC	1850-1870	1032
	SPCFV(II)-3R	GGGCCTCATGACTACCAGAGTCAG	2881-2858	
	SPCFV(II)-4F	GCCTGAGGGGTTTCAGGAGAAGT	2638-2660	1103
	SPCFV(II)-4R	GCAGAAGGTCCAAAATGTAGTCCTT	3740-3720	
P2	SPCFV(II)-5F	GCAAGATCGAAGCACTCAAGGATC	3602-3625	744
	SPCFV(II)-5R	CGGAACCAAGGCTCTACACTCATC	4483-4460	
	spcfv-full-6f-1	CAAAGTGATTATGACTCTCTGCG	4254-4276	1108
	spcfv-full-6r-1	TCGAAGTCCTGYACAGCCTTATC	5361-6340	
	SPCFV(II)-7F	ATTGACCAATGCCGCAGAGAGG	5126-5146	1096
	SPCFV(II)-7R	GGTAGGACCTTCTCTCCCAA	6222-6200	
	spcfv-full-8f	AACACTTAAGACAGAGCATGATGG	5978-6001	1085
	spcfv-full-8r	TTAGAATAGTCCGCGGGTGG	7063-7044	
P3	SPCFV(II)-9F	ACTACAGCCTATTTGGCCAAGC	6738-6760	1149
	SPCFV(II)-9R	GGAGATCTCACCCTGCGCA	7887-7879	
	SPCFV 1F	ATGTTTGCTGGGGAGAGTCAGG	7555-7576	
	SPCFV 2F-1	AAAGTGGAACAGGGAGCCCG	8067-8086	1038
	SPCFV 2R	GCTCAAAAGTACTTTAAAACATGC	9104-9081	
RACE	SPCFV 3'-F	GTGATTGGGAYYGYTGTCTT	8718-8737	387
	SPCFV 5'-R	GGTGAAACAGGTGAGAGGTATATC	262-239	262

*Reference sequence: SPCFV- Hoima 4 (AY461421)

3′-race-F primer complementary to nucleotides 8,718-8,737 and an anchor primer (5′-GACCACGCGTATCGATGTC-GACTTTTTTTTTTTTTTTV-3′).

The complete genomes of Korean SPCFV isolates ranged from 9,104 to 9,108 nucleotides, excluding the poly-A tails, and encoded six ORFs. The genomic organization of the Korean SPCFV isolates was typical for members of the *Carlavirus* genus. ORF1 encodes a viral replicase (2,090 aa in size), and ORF2 to ORF4 encode TGB proteins, including TGB1, TGB2, and TGB3 (240 aa, 108 aa, and 67 aa in size, respectively). ORF5 encodes the CP (299 aa), and ORF6 encodes a NaBp (133 aa). The 5′ and 3′ non-coding regions (NCR) are 65 and 52 nucleotides in length, respectively. The full-length genome sequences are available in the GenBank database, with the accession numbers listed in Table 3.

To analyze phylogenetic relationships, the complete nucleotide sequences and deduced amino acid (aa) sequences were aligned using the ClustalX2 program and Geneious methods in Geneious Pro 8 and compared to those of previously reported isolates (Table 3). *Melon yellowing-associated virus* (MYaV, accession no. AY373028) was used as an outgroup. Genius Pro 8 software was used to calculate the percentages of nucleotide and aa identities. Phylogenetic analyses were performed according to the maximum-likelihood method implemented in MEGA 6 (Tamura et al., 2013). All phylogenic tests were conducted using the best substitution model for nucleotides or amino acids, with 1,000 bootstrap replicates.

The complete nucleotide and deduced aa sequences of 4 SPCFV isolates were compared to those previously reported for virus isolates. According to a previous report, SPCFV is divided geographically into African and Peruvian isolates and Asian isolates (Aritua et al., 2009). Phylogenetic analysis indicated that Korean SPCFV isolates are clustered into the Asian isolates, including Chinese, Taiwanese, and Australian isolates. Furthermore, Korean SPCFV isolates could be largely divided into two groups (Group I and Group II) (Fig. 1). The nucleotide and aa sequence identities between SPCFV isolates are summarized in Table 4. Based on the full-length and partial genome nucleotide sequences, SPCFV isolates can be split into two groups with low nucleotide sequence identities of about 73-78%, while the intragroup nucleotide sequence identity

Table 3. Database of the complete and partial nucleotide sequences of SPCFV isolates infecting sweet potato

Virus	Isolate[c]	Origin	Genome size (nt)	NCBI accession No.
SPCFV[a]	**SC20**	Sacheon, Korea	9,104	KP115606
	UN210	Muan, Korea	9,104	KP115607
	HN83	Haenam, Korea	9,104	KP115605
	HG176	Muan, Korea	9,108	KP715159
	Hoima 4	Uganda	9,104	AY461421
	KBL38	Uganda		EU375903
	94-15	Kenay		EU375900
	KY5	Kenay		EU375904
	CIP	Peru		EU375899
	Tar	Tanzania		AJ781296
	Gwangzhu1	China		EU375901
	B-Guangdong-11-5	China		KC130184
	B-Jiangxi-11-4	China		KC130185
	AusCan	Australia		EF990647
	TN340	Taiwan		EU375898
MYaV[b]	MB-10	Brazil		AY373028

[a]*Sweet potato chlorotic fleck virus* (SPCFV), [b]*Melon yellowing-associated virus* (MYaV), [c]Isolates analyzed in this study are shown in boldface.

ranged from 86% to 98%. Especially, the Korean SPCFV-SC20 isolate belonging to Group I showed 88.4% nucleotide sequence identity with the Uganda isolate, SPCFV-Hoima 4; 73% nucleotide sequence identity with SPCFV-HG176, belonging to Group II; and 61% nucleotide sequence identity with the closest *Carlavirus*, MYaV. The

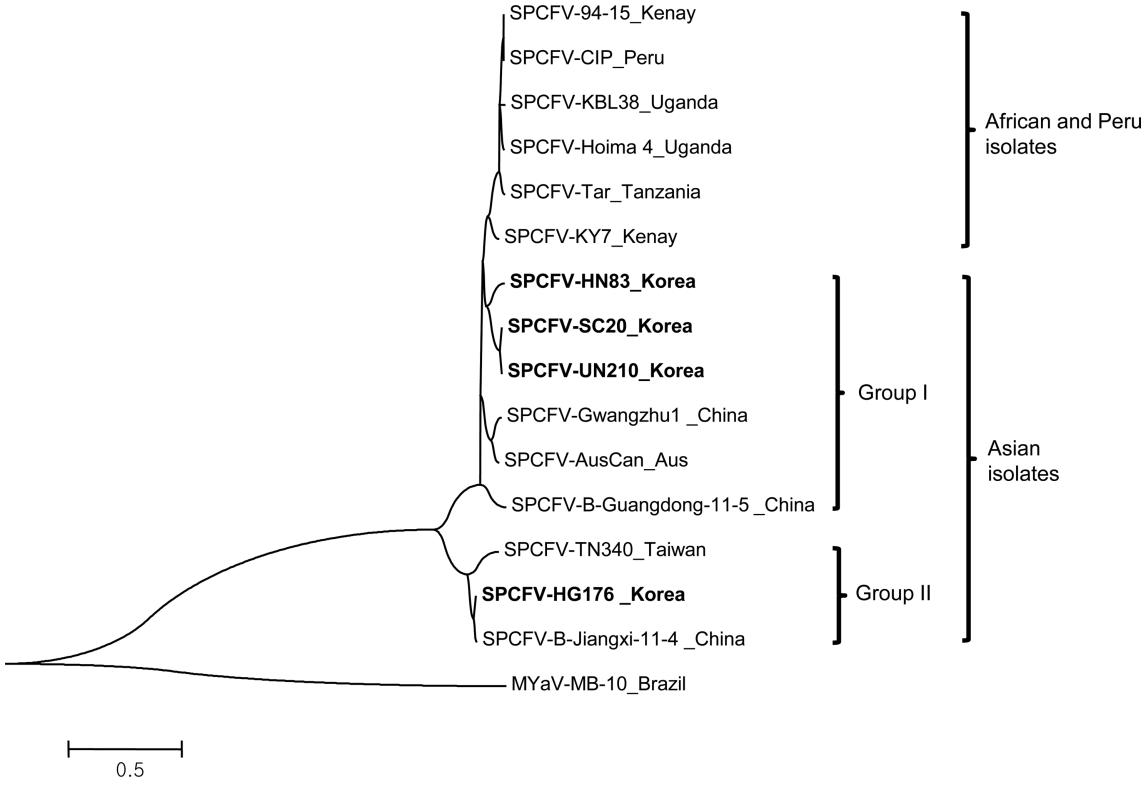

Fig. 1. Phylogenetic trees reconstructed using the complete and partial nucleotide sequences of the SPCFV isolates. Phylogenetic trees were reconstructed using the maximum-likelihood method in MEGA 6.

Table 4. Nucleotide and amino acid sequence identities (%) between Korean isolates SPCFV-SC20 and other SPCFV isolates infecting sweet potato

Virus isolate	Genome (nt)	5'UTR (nt)	Rep (aa)	TGB1 (aa)	TGB2 (aa)	TGB3 (aa)	CP (aa)	NaBp (aa)	3'UTR (nt)
UN210-Korea	98.4	100	98.7	100	100	97	99.7	99.2	100
HN83-Korea	88.5	93.8	93.1	91.3	97.2	92.5	97.7	94	98.1
Hoima 4-Uganda	88.4	96.8	92.9	91.3	96.3	86.6	96	93.2	98.1
KBL38-Uganda	88.4		-	-	-	-	95.7	88.7	96.2
94-15-Kenay	88.7	-	-	-	-	-	95.7	91.7	98.1
Tar-Tanzania	88.7	-	-	-	-	-	95.3	92.5	98.1
CIP -Peru	88.7	-	-	-	-	-	95.7	91.7	98.1
KY5-Kenay	89.5	-	-	-	-	-	96.7	91	94.2
Gwangzhu1-China	88.5	-	-	-	-	-	96.3	90.3	98.1
AusCan -Aus	86.3	-	-	-	-	-	95.7	-	-
B-Guangdong-11-5-China	85.9	-	-	-	-	-	95.7	-	-
HG176-Korea	73.2	90.8	78.4	77.6	89.8	68.7	93.3	77.4	96.2
B-Jiangxi-11-4-China	76.5	-	-	-	-	-	92.6	-	-
TN340-Taiwan	77.6	-	-	-	-	-	91.3	78.9	98.1
MB-10-Brazil*	61.3	-	-	52.6	69.7	24.6	61.9	46.7	69.1

*MYaV (*Melon yellowing-associated virus*) as an outgroup

complete genome sequence of Group II SPCFV isolates (HG176) was determined in this study for the first time.

Regarding the deduced aa sequences of six individual proteins, the aa sequence identity in CPs between Group I and Group II was over 90%, whereas the identities in other proteins were less than 80%. The Korean SPCFV-SC20 isolate showed a relatively high aa sequence identity (91-100%) with Group I, including the Uganda isolate, SPCFV-Hoima 4. However, the Korean SPCFV-SC20 isolate showed 69-93% aa sequence identity with Group II, including the Korean isolate, SPCFV-HG176. Further, the Korean SPCFV-SC20 isolate showed low aa sequence identities (61% and 47% in CPs and NaBp region, respectively) with those of MYaV.

According to a report by the International Committee on Taxonomy of Viruses (ICTV), criteria for species classification of the family *Betaflexiviridae* include less than 72% nucleotide identity (or 80% aa identity of the encoded proteins) in the CP or replicase genes (Adams et al., 2011). Two groups of SPCFV Korean isolates belong to the same

Table 5. Recombination in SPCFV isolates

Recombination event No.	Recombinant isolate	Recombination site in genome		Genes affected	Parental isolates[a]	RDP4[b]	P-value[c]
		start	End				
1	Hoima 4	744	4167	Rep	HN83 × AusCan	MCS**3**	2.41E-04
2	HN83	4521	4718	Rep	SC20 × Hoima 4	B**S**	1.04E-05
3	HN83	8725	8969	NaBp	SC20 × AusCan	R**B**MCS3	9.30E-03
4	HN83	6663	6860	TGB1	SC20 × Unknown	B**S**	7.20E-03
5	AusCan	6786	6817	TGB1	Unknown × HN83	MC**3**	5.94E-03
6	Hoima 4	2938	2972	Rep	SC20 × HN83	G**C**	2.16E-02
7	HN83	1048	1254	Rep	Unknown × Hoima 4	**R**S	3.07E-02
8	HN83	2673	2679	Rep	Hoima 4 × HG176	**N**3	3.28E-02
9	Hoima 4	7532	7833	TGB3, CP	Unknown × HN83	MC**S**	6.69E-03
10	Tm37	6836	6851	TGB1	HG176 × Unknown	**S**3	1.03E-10

[a] 'Parental isolates' indicates the most likely isolates among those analyzed; Major parent × minor parent.
[b]RDP4-implemented methods that supported the corresponding recombination site: R (RDP), G (GENECONV), B (BootScan), M (MaxChi), C (Chimaera), and S (SiScan), 3 (3Seq).
[c]The highest P-value among the RDP4-implemented methods is reported. The corresponding method is shown boldface.

species to meet the criteria above.

Recently, the complete genome sequences of two SPCFV isolates (Tm37 and AusCan isolates) and other partial sequences have been reported, and phylogenetic relationships of SPCFV based on CPs and NaBp nucleotide sequences have been analyzed (Maina et al., 2016; Tugume et al., 2016). According to these reports, SPCFV isolates, including Korean isolates, can be divided into East African and Asian groups (Asian 1 and Asian 2) geographically.

Recombination has been shown to significantly contribute to virus diversity. To examine whether recombination events have occurred within the SPCFV isolates, we aligned full-length nucleotide sequences of seven isolates including four Korean isolates, using the Geneious method in Geneious Pro 8 and analyzed them using the RDP, GENECONV, BootScan, MaxChi, Chimaera, SiScan and 3Seq methods implemented in the RDP4 software with a highest acceptable P-value of 0.01. In total, 10 potential recombinant events were detected by at least one of the methods (Table 5). However, most isolates seemed to be 'tentative' recombinants, as they were supported by less than four methods or one of the parental isolates was labeled as 'unknown'. No significant recombination was detected in seven isolates. Korean SPCFV isolate HN83 showed some recombination spots, however, it is likely that genetic exchange by recombination is infrequent in natural populations of SPCFV. Further analyses of genetic population structure of SPCFV with more diverse full-genome sequences of worldwide isolates will be required for in-depth understanding of the evolutionary history of SPCFV.

In conclusion, it is essential to understand the molecular variation of viruses when designing knowledge-based control strategies. This study reports the complete genome sequences of SPCFV Group II isolates (denoted Asian 2 group by Tugume et al., 2016) for the first time. However, all SPCFV isolates found in Korea were infected with multiple viruses. Further, these SPCFV isolates have not been shown to mechanically infect previously reported host plants (Aritua et al., 2009). To better understand the relationship between genetic variation and pathogenicity in Korean SPCFV isolates, full-length infectious clones of two divergent SPCFV isolates will be required to elucidate biological characteristics of the two groups, including symptoms and host range.

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