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The Current Incidence of Viral Disease in Korean Sweet Potatoes and Development of Multiplex RT-PCR Assays for Simultaneous Detection of Eight Sweet Potato Viruses

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Sweet potato is grown extensively from tropical to temperate regions and is an important food crop worldwide. In this study, we established detection methods for 17 major sweet potato viruses using single and multiplex RT-PCR assays. To investigate the current incidence of viral diseases, we collected 154 samples of various sweet potato cultivars showing virus-like symptoms from 40 fields in 10 Korean regions, and analyzed them by RT-PCR using specific primers for each of the 17 viruses. Of the 17 possible viruses, we detected eight in our samples. *Sweet potato feathery mottle virus* (SPFMV) and *sweet potato virus* C (SPVC) were most commonly detected, infecting approximately 87% and 85% of samples, respectively. Furthermore, *Sweet potato symptomless virus* 1 (SPSMV-1), *Sweet potato virus* G (SPVG), *Sweet potato leaf curl virus* (SPLCV), *Sweet potato virus* 2 (SPV2), *Sweet potato chlorotic fleck virus* (SPCFV), and *Sweet potato latent virus* (SPLV) were detected in 67%, 58%, 47%, 41%, 31%, and 20% of samples, respectively. This study presents the first documented occurrence of four viruses (SPVC, SPV2, SPCFV, and SPSMV-1) in Korea. Based on the results of our survey, we developed multiplex RT-PCR assays for simple and simultaneous detection of the eight sweet potato viruses we recorded.

***Keywords* :** disease incidence, sweet potato viruses, multiplex RT-PCR

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Sweet potato (*Ipomea batatas* L.), of the family *Convolvulaceae*, is grown extensively from tropical to temperate regions and is an important food crop worldwide. According to FAOSTAT data, there are 8 million ha of sweet potato in cultivation worldwide, with approximately 103 million metric tons produced in 2012. Major sweet potato-producing nations include China, Tanzania, Nigeria, Uganda, and Indonesia; production in China alone is around 73 million metric tons, or more than 70% of the worldwide total. Korea currently produces approximately 260,000 metric tons from 19,000 ha, with production increasing every year.

Viral diseases of sweet potato have become widespread, causing serious crop losses around the world. In total, more than 30 viruses have now been reported to infect sweet potato (Brunt et al., 1996; Clark et al., 2012). Among these, 23 have been assigned a formal taxonomic position by the International Committee on Taxonomy of Viruses (ICTV) (Table 1). The number continues to increase as virus detection methods are improved. Only a few of the viruses are considered to be of major economic importance. The most severe disease in sweet potato is caused by coinfection with the whitefly-transmitted *Sweet potato chlorotic stunt virus* (SPCSV) and the aphid-transmitted *Sweet potato feathery mottle virus* (SPFMV), which results in the synergistic sweet potato virus disease (SPVD) (Gibson et al., 1998; Karyeija et al., 2000; Mukasa et al., 2006). Synergism has also been observed between SPCSV and the possibly whitefly-transmitted *Sweet potato mild mottle virus* (SPMMV) (Gutiérrez et al., 2003; Hahn, 1979). SPCSV caused synergistic diseases in sweet potato with many other sweet potato viruses (Untiveros et al., 2007).

In Korea, SPFMV, *Sweet potato virus* G (SPVG), and *Sweet potato latent virus* (SPLV), all belonging to the family *Potyviridae*, and *Sweet potato leaf curl virus* (SPLCV), a member of the *Geminiviridae*, have been detected (Kwak

Table 1. Major viruses infecting sweet potato

Virus name	Abb.	Family (genus)	Transmission
Sweet potato feathery mottle virus	SPFMV	Potyviridae (*Potyvirus*)	Aphid (non-persistent)
Sweet potato virus G	SPVG	Potyviridae (*Potyvirus*)	Aphid (non-persistent)
Sweet potato latent virus	SPLV	Potyviridae (*Potyvirus*)	Aphid (non-persistent)
Sweet potato leaf curl virus[a]	SPLCV	Geminiviridae (*Begomovirus*)	Whitefly (persistent)
Sweet potato mild speckling virus	SPMSV	Potyviridae (*Potyvirus*)	Aphid (non-persistent)
Sweet potato mild mottle virus	SPMMV	Potyviridae (*Ipovirus*)	Whitefly (persistent)
Sweet potato chlorotic stunt virus	SPCSV	Closteroviridae (*Crinivirus*)	Whitefly(non-persistent)
Sweet potato collusive virus	SPCV	Caulimoviridae(*Cavemovirus*)	*
Sweet potato virus 2	SPV2	Potyviridae (*Potyvirus*)	Aphid (non-persistent)
Sweet potato virus C	SPVC	Potyviridae (*Potyvirus*)	Aphid (non-persistent)
Sweet potato symptomless virus 1	SPSMV-1	Geminiviridae (*Mastrevirus*)	*
Sweet potato chlorotic fleck virus	SPCFV	Betaflexiviridae (*Carlavirus*)	*
Sweet potato vein clearing virus	SPVCV	Caulimoviridae (*Solendovirus*)	*
Sweet potato pakakuy virus	SPPV	Caulimoviridae (*Badnavirus*)	*
Sweet potato C6 virus	SPC6V	Betaflexiviridae (*Carlavirus*)	*
Sweet potato leaf speckling virus	SPLSV	Luteoviridae (*Polerovirus*)	*
Cucumber mosaic virus	CMV	Bromoviridae (*Cucumovirus*)	Aphid (non-persistent)

[a]*Sweet potato leaf curl virus* has been classified into seven species: *Sweet potato leaf curl virus* (SPLCV), *Ipomoea yellow vein virus* (IYVV), *Sweet potato leaf curl Georgia virus* (SPLCGoV), *Sweet potato leaf curl China virus* (SPLCV-CN), *Sweet potato leaf curl Lanzarote virus* (SPLCLaV), *Sweet potato leaf curl Canary virus* (SPLCCaV), and *Sweet potato leaf curl Spain virus* (SPLCESV) by ICTV.
*Not reported.

et al., 2006). Our previous nationwide survey revealed that, in 2003, about 73% of samples were infected with at least one of these four viruses (Kwak et al., 2006). SPFMV and SPVG were especially prevalent (40% and 16%, respectively), and coinfection with SPFMV and SPVG was detected in 11% of diseased sweet potatoes. Although SPCSV was reported by Yun et al. (2002), it has not subsequently been detected in Korea.

In the present study, we established detection methods for 17 major sweet potato viruses using reverse transcription-polymerase chain reaction (RT-PCR), and investigated the current incidence of viral disease in Korean sweet potatoes by analyzing 154 samples of various cultivars showing virus-like symptoms collected from 40 fields in 10 regions. Finally, we developed multiplex RT-PCR methods for simple and simultaneous detection of the eight sweet potato viruses known to occur in Korea.

Materials and Methods

Survey and sample collection. In 2012, we carried out a survey of sweet potato viruses in 40 seedling-cultured fields of sweet potatoes in 10 regions of 5 different Korean provinces (Fig. 1). We collected samples of 154 sweet potato leaves (including petiole and stem) showing virus-like symptoms. The collected samples were treated with insecticides to remove potential insect vectors and maintained in pots in a greenhouse at 20–25°C (Table 2). All samples were inspected for disease symptoms for at least 30 days and analyzed for virus infection by RT-PCR.

Total nucleic acids extraction and *virus detection* by RT-PCR. We extracted total nucleic acids from the infected leaf, petiole and stem samples using the Viral gene-spin^{TM} viral DNA/RNA extraction kit (iNtRON, Korea), according to the manufacturer's instructions. Typical RT-PCR assays were carried out using the primers shown in Table 3 in a two-step procedure using AMV reverse transcriptase (Promega, USA) for RT and Go-taq polymerase (Promega, USA) for PCR, as described by Kwak et al. (2013). Multiplex RT-PCR assays were performed using two-step RT-PCR or one-step RT-PCR. In the case of two-step RT-PCR, RT reactions were carried out at 42°C for 30 min in a final 5 μl reaction obtained by combining 0.5 μl total RNA (approx. 0.5 μg), 0.5 μl of a mixture of equal amounts of 32 μM reverse primers for four viruses, 1 μl 5 × RT buffer, 0.5 μl 2.5 μM dNTP, 0.1 μl BSA (10 mg/ml), 8 U RNase inhibitor, 0.5 U AMV reverse transcriptase (Promega, USA), and sufficient dH_2O to bring the total to 5 μl. RT reactions were terminated by heating at 95°C for 5 min. When RT was completed, we added 20 μl of a solution comprising 0.5 μl of a mixture of equal amounts of 32 μM

Fig. 1. Geographic location of the sweet potato fields surveyed in 2012. A total of 154 sweet potato specimens were collected from 40 cultivated fields in 10 regions of 5 Korean provinces, as indicated.

forward primers for four viruses, 5 µl 5 × PCR buffer, 2.5 µL 25 mM $MgCl_2$, 0.4 µl BSA (10 mg/ml), 1 U Go-Taq DNA polymerase (Promega, USA), and dH_2O to the tube containing RT products. PCR was performed in a thermal cycler (Bio-Rad, USA) with the following conditions: predenaturing step at 94°C for 5 min; 35 cycles of a denaturing step at 94°C for 30 s; an annealing step at 55°C for 30 s; an extension step at 72°C for 60 s; and a final extension at 72°C for 10 min. One-step RT-PCR was performed in a 20 µl reaction containing total RNA (approx. 0.5 µg), a mixture of equal amounts of 32 µM forward and reverse primers for four viruses, and RT-PCR master mix (GenetBio, Korea) under the following conditions: 30 min at 50°C; 10 min at 94°C; and 35 cycles consisting of 20 s at 94°C, 30 s at 55°C, 60 s at 72°C, and 5 min at 72°C. PCR products were analyzed by electrophoresis on a 1.5% agarose gel at 100 V for 90 min, stained with ethidium bromide, and DNA bands were visualized using a UV transilluminator.

Primer design. Specific primers for the detection of sweet potato viruses were designed based on the reported nucleotide sequences of various sweet potato virus isolates retrieved from the GenBank of the National Center for Biotechnology Information (Table 3). In particular, the primers for multiplex RT-PCR were designed to have similar melting temperatures and selected so as not to produce nonspecific bands. Two sets of the multiplex detection primers and the expected sizes of the amplicons are listed in Table 3.

Table 2. Legend of sweet potato specimens analyzed in this study

Province	Regions[a]	No. of fields investigated	No. of samples[b] collected
Jeollanam-do	Haenam	8	40
	Yeongam	4	10
	Muan	7	37
Jeollabuk-do	Iksan	3	16
	Gimje	6	14
Gyeongsangnam-do	Sancheong	2	13
	Sacheon	1	4
Chungcheongbuk-do	Cheongwon	2	7
Chungcheongnam-do	Nonsan	2	3
	Boryeong	5	10
Total	10	40	154

[a]Regions and fields selected for sample collection were within major Korean sweet potato production areas in 2012.
[b]Samples of sweet potato leaves (including petiole and stem) showing virus-like symptoms were collected.

Results

Incidence of viral disease in Korean sweet potatoes in 2012. To investigate the current incidence of viral disease in Korean sweet potatoes, we collected 154 samples from various seedling-cultured sweet potato cultivars in 10 regions of 5 Korean provinces in 2012. We observed various representative symptoms on the infected sweet potato samples, including vein clearing, vein banding, chlorotic local lesions, purpling, malformation, and leaf curling (Fig. 2). We analyzed samples by RT-PCR using specific primers designed to detect 17 major viruses infecting sweet potato (Table 3). In case of SPLCV, universal primers were used to detect seven SPLCV species (Table 1). Among the 17 viruses tested, we detected 8, including 4 viruses not previously detected in Korea (*Sweet potato virus* C [SPVC], *Sweet potato virus* 2 [SPV2], *Sweet potato chlorotic fleck virus* [SPCFV], and *Sweet potato symptomless virus* 1 [SPSMV-1]; Table 4). SPFMV and SPVC were detected most often, in about 87% and 85% of samples, respectively, including in mixed infections with other viruses (Table 4). Furthermore, SPSMV-1, SPVG, SPLCV, SPV2, SPCFV, and SPLV were detected in 67%, 58%, 47%, 41%,

Table 3. Primers used in multiplex and single RT-PCR assays for the detection of major sweet potato-infecting viruses

Virus	Primer[a]	Sequence (5'→3')	Loci	Size (nt)
Multiplex RT-PCR primer set 1				
SPLCV	SPLC-u1	TCTGCCGTCGATCTGGAACTC	2315–2335	507
	SPLC-d1	GTGCCCGCCTTTGGTGGAC	2821–2803	
SPFMV	SPFMV 1-F	TACACACTGCTAAAACTAGG	9073–9092	356
	SPFMV 1-R	AGTTCATCATAACCCCATGA	9428–9409	
SPVG	SPG 3-F	CAATGCCAAATGGAAGAATAG	9945–9965	286
	SPG 3-R	GCATGATCCAATAGAGGTTTTA	10230–10209	
SPLV	SPLV 1-F	GGAGTCAGTTCAATCAATGGTA	9340–9361	184
	SPLV 1-R	AGTGGCTTTATTGGGTATGAT'	9523–9503	
Multiplex RT-PCR primer set 2				
SPCFV	SPCFV 2F	AGCTGCTCAAACAAGCAAGAGG	8526–8547	579
	SPCFV 2R	GCTCAAAAGTACTTTAAAACATGC	9104–9081	
SPVC	SPVC-F	ATTCTTGAATGGGATAGATCACATG	9353–9377	447
	SPVC-R	AGCTTCACGAAGCGCAGC	9799–9782	
SPV2	SPV2-F1	ATGTGTTGAACCATCAGCTGAA	9414–9435	369
	SPV2-R1	GTAACTTGCCTTGGGCTACG	9782–9763	
SPSMV-1	SPSMV-1 F1	ACCGTGTATTTGATGACGATGTAC	352–375	230
	SPSMV-1 R	GGGAAGTTCTGGTAGAACGTATC	581–559	
Single RT-PCR primers[b]				
SPMMV	SPMMV 3-F	CCGCGCCAACAA AGGAACTA	9842–9861	298
	SPMMV 3-R	TTGATGGGGTAATAAAGCACT	10140–10120	
SPCSV	SPCSV-uni-f1	GGGAAGAMGAGAYATGGAGTTAA	4484–4506	583
	SPCSV-uni-r1	CCTTGTTACAAAGAGCGTTCCT	5066–5045	
SPMSV	SPMSV 1-F	GCCAAAACCAACAAGCATCA	105–124	275
	SPMSV 1-R	ATTCGCATTTCCTCATCATCT	380–360	
SPCV	SPCV F	AGGAAATCCCAGTATTATTCAAC	4267–4289	922
	SPCV R	ATTTCTAATTTGGTTTACTAATCC	5188–5165	
SPVCV	SPVCV-F	ATCCATTGCCAAATAAGATATTAAGA	5844–5870	308
	SPVCV-R	CTTCTTAAGCAATGTTTCATGCTC	6151–6128	
SPPV	SPPV-F	ATGAGGAGAA(C)CAGGGGCC	1486–1503	722
	SPPV-R	CCAACG(A)TTTGGAGTGTTGGAT	2207–2187	
SPC6V	SPC6V-F1	AAAAGCTTGTTGGCAATTTGTG	6804–6825	590
	SPC6V-R1	TTGGCATTTCGATTGTCCC	7393–7376	
SPLSV	SPLSV-F	ATGAGTACGGTCGTGGTTAGAAAC	1-24	612
	SPLSV-R	CTACCTATTTGGGTTCTGGAAGG	612–590	
CMV	CMV-DP u	CGTCGTGGTTCCCGCTCCG	1309–1327	474
	CMV-DP d2	AGCGCGCATCGCCGAAAGAT	1782–1763	

[a]Primers were designed based on the nucleotide sequences of sweet potato viruses registered in Genbank. [b]RT-PCR conditions are as follows ; 30 min at 42ºC, 5 min at 94ºC, and 35 cycles consisting of 20 s at 94ºC, 30 s at 55ºC, 60 s at 72ºC, and 5 min at 72ºC.

31%, and 20% of the collected samples, respectively (Table 4). While SPFMV, SPVC, SPVG, SPV2, and SPLCV were detected in all surveyed areas, SPLV and SPSMV-1 were not detected in Boryeong and Sancheong, respectively, and SPCFV was not detected in Nonsan or Boryeong (Table 4). All samples but one were infected with at least one of the eight viruses (Table 5). Only 3.9% of the samples were singly infected with one of the eight viruses, while 95.5% of the samples were found in mixed infections (Table 5). The total rates of double, triple, quadruple, quintuple, sextuple, septuple, and octuple infections were 8.4%, 12.3%, 20.8%, 32.5%, 19.5%, 1.3%, and 0.6%, respectively. The most prevalent infection type was quintuple infection with SPFMV, SPVC, and other viruses (Table 5).

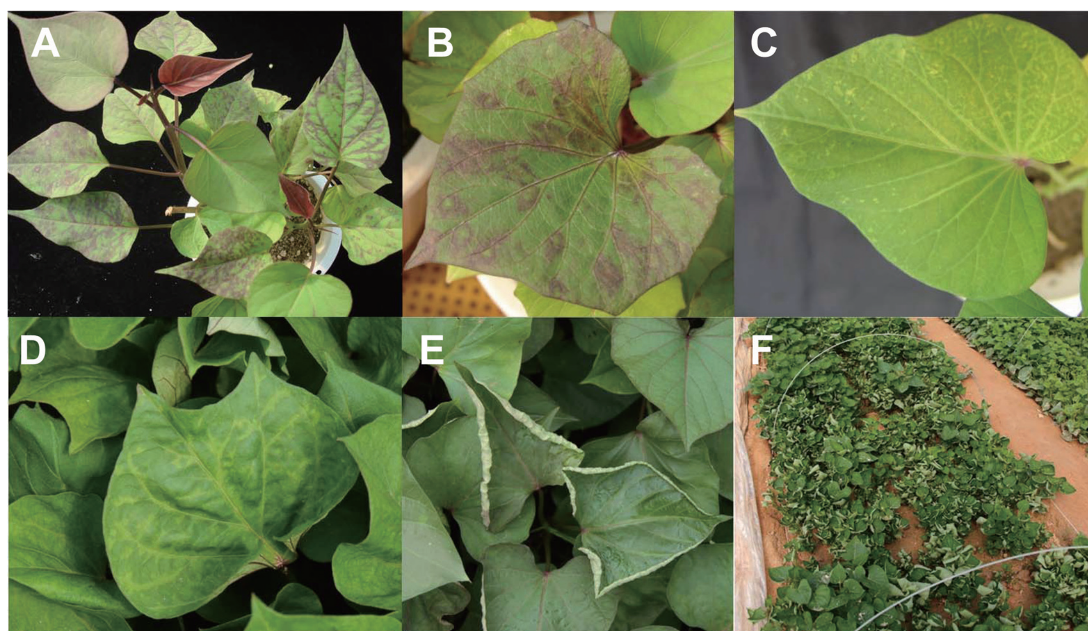

Fig. 2. Representative virus symptoms observed in sweet potato: (A) vein banding and purpling; (B) chlorotic local lesions and purpling; (C) vein clearing and mottle; (D) vein clearing and leaf malformation; (E) leaf curling; (F) leaf curling in a cultivated field.

Table 4. Incidence of sweet potato viruses in samples of seedling-cultured sweet potatoes collected in 2012

Province/Region		No. of samples	No. of virus detections by RT-PCR [a]							
			SPFMV	SPVC	SPVG	SPV2	SPLV	SPCFV	SPLCV	SPSMV-1
Jeollanam-do	Haenam	40	40	40	17	17	8	17	20	29
	Yeongam	10	10	10	7	8	2	1	3	10
	Muan	37	25	27	13	3	2	12	22	34
Jeollabuk-do	Iksan	16	13	7	10	7	1	1	6	6
	Gimje	14	10	11	8	4	6	3	7	11
Gyeongsangnam-do	Sancheong	13	13	13	12	8	6	9	4	0
	Sacheon	4	4	4	4	2	3	2	2	1
Chungcheongbuk-do	Cheongwon	7	7	6	6	4	1	2	5	5
Chungcheongnam-do	Nonsan	3	3	3	3	2	1	0	1	2
	Boryeong	10	9	10	9	8	0	0	3	5
Total	10	154	134	131	89	63	30	47	73	103
Ratio (%)			87	85	58	41	20	31	47	67

[a]Including single and mixed infections.

Development of multiplex RT-PCR assays for simultaneous detection of sweet potato viruses. For simultaneous detection of sweet potato-infecting viruses occurring in Korea, we developed two multiplex RT-PCR assays for the eight major viruses including SPLCV, SPFMV, SPVG, and SPLV in one set, and SPCFV, SPVC, SPV2, and SPSMV-1 in another (Table 3). The primer sets of the assays were designed to generate different sizes of amplicons for the target viruses (Table 3). Each primer pair was highly specific and did not cross-amplify to other viral or sweet potato nucleic acids (Figs. 3 and 4, lanes 1–4). The specificity of each primer pair was confirmed by sequencing the RT-PCR products. Simultaneous detection of all possible combinations among SPLCV, SPFMV, SPVG, and SPLV was performed by multiplex RT-PCR using the singly or mixed-infection field samples (Fig. 3). Likewise, we also performed simultaneous detection of all possible combinations among SPCFV, SPVC, SPV2, and

Table 5. Mixed infection types of eight sweet potato viruses

Mixed infection type	Detected viruses									
	SPFMV	SPVC	SPVG	SPV2	SPLV	SPCFV	SPLCV	SPSMV-1	Subtotal	Total (%)
Octuple	+	+	+	+	+	+	+	+	1	1 (0.6)
Septuple	+	+	+	+	+	+	+	–	1	2 (1.3)
	+	+	+	–	+	+	+	+	1	
Sextuple	+	+	+	+	+	+	–	–	4	30 (19.5)
	+	+	+	+	+	–	+	–	6	
	+	+	+	+	+	–	–	+	3	
	+	+	+	+	–	+	+	–	1	
	+	+	+	+	–	–	+	+	6	
	+	+	+	–	+	+	+	–	2	
	+	+	+	–	+	–	+	+	3	
	+	+	+	–	–	+	+	+	1	
	+	+	–	+	–	+	+	+	2	
	+	+	–	–	+	+	+	+	2	
Quintuple	+	+	+	+	+	–	+	–	4	50 (32.5)
	+	+	+	+	–	+	–	–	3	
	+	+	+	+	–	–	+	–	6	
	+	+	+	+	–	–	–	+	12	
	+	+	+	–	+	+	–	–	1	
	+	+	+	–	–	+	+	–	1	
	+	+	+	–	–	–	+	+	8	
	+	+	–	+	–	+	–	+	2	
	+	+	–	–	–	+	+	+	13	
Quadruple	+	+	+	+	–	–		–	6	32 (20.8)
	+	+	+	–	–	+	–	–	1	
	+	+	+	–	–	–	+	–	2	
	+	+	+	–	–	–	–	+	7	
	+	+	–	+	–	–	+	–	1	
	+	+	–	+	–	–	–	+	2	
	+	+	–	–	–	+	–	+	7	
	+	+	–	–	–	–	+	+	2	
	+	–	+	+	–	–	–	+	1	
	+	–	+	–	+	+	–	–	1	
	+	–	+	–	+	–	+	–	1	
	–	+	–	–	–	+	+	+	1	
Triple	+	+	+	–	–	–	–	–	2	19 (12.3)
	+	+	–	+	–	–	–	–	2	
	+	+	–	–	–	–	+	–	1	
	+	+	–	–	–	–	–	+	7	
	+	–	–	–	–	–	+	+	1	
	–	+	–	–	–	–	+	+	4	
	–	+	+	–	–	–	–	+	1	
	–	–	–	–	–	+	+	+	1	
Double	+	–	+	–	–	–	–	–	1	13 (8.4)
	+	–	–	–	–	–	+	–	1	
	+	–	–	–	–	–	–	+	4	
	–	+	–	–	–	–	–	+	2	
	–	–	+	–	–	–	–	+	2	
	–	–	–	–	–	+	–	+	1	
	–	–	–	–	–	–	+	+	2	
Single	+	–	–	–	–	–	–	–	1	6 (3.9)
	–	–	–	–	–	–	+	–	1	
	–	–	–	–	–	–	–	+	4	
No detection	–	–	–	–	–	–	–	–	1	1 (0.6)
Total									154	

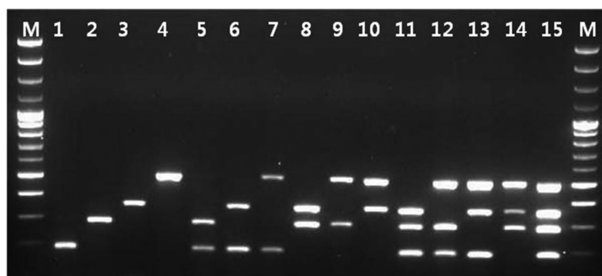

Fig. 3. Simultaneous detection of all possible combinations among SPLCV, SPFMV, SPVG, and SPLV by multiplex RT-PCR. Lane M: 100 bp ladder; Lane 1: SPLV; Lane 2: SPVG; Lane 3: SPFMV; Lane 4: SPLCV; Lane 5: SPLV and SPVG; Lane 6: SPLV and SPFMV; Lane 7: SPLV and SPLCV; Lane 8: SPVG and SPFMV; Lane 9: SPVG and SPLCV; Lane 10: SPFMV and SPLCV; Lane 11: SPLV, SPVG, and SPFMV; Lane 12: SPLV, SPVG, and SPLCV; Lane 13: SPLV, SPFMV, and SPLCV; Lane 14: SPVG, SPFMV, and SPLCV; Lane 15: SPLV, SPVG, SPFMV, and SPLCV.

Fig. 4. Simultaneous detection of all possible combinations among SPCFV, SPVC, SPV2, and SPSMV-1 by multiplex RT-PCR. Lane M: 100 bp ladder; Lane 1: SPCFV; Lane 2: SPVC; Lane 3: SPV2; Lane 4: SPSMV-1; Lane 5: SPCFV and SPVC; Lane 6: SPCFV and SPV2; Lane 7: SPCFV and SPSMV-1; Lane 8: SPVC and SPV2; Lane 9: SPVC and SPSMV-1; Lane 10: SPV2 and SPSMV-1; Lane 11: SPCFV, SPVC, and SPV2; Lane 12: SPCFV, SPVC, and SPSMV-1; Lane 13: SPCFV, SPV2, and SPSMV-1; Lane 14: SPVC, SPV2, and SPSMV-1; Lane 15: SPCFV, SPVC, SPV2, and SPSMV-1.

SPSMV-1 by multiplex RT-PCR (Fig. 4). Various field samples of virus-infected sweet potatoes were subjected to the multiplex RT-PCR assays developed in this study, resulting in reliable and sensitive detection and identification of major sweet potato viruses (data not shown).

Discussion

In Korea, SPFMV, SPLV, SPVG, and SPLCV have previously been reported to infect sweet potato (Kwak et al., 2006). In this study, a nationwide survey was performed to investigate the current incidence of viral diseases in Korean sweet potatoes. To this end, we analyzed 154 samples of sweet potatoes showing virus-like symptoms by single or multiplex RT-PCR assays to detect 17 major viruses. Our survey revealed that eight viruses (including four that were previously undocumented: SPVC, SPV2, SPSMV-1, and SPCFV) infect sweet potatoes in Korea. However, other nine viruses (SPCSV, SPMSV, SPMMV, SPCV, SPVCV, SPPV, SPC6V, SPLSV, and CMV) were not detected in our survey. Although the primers used in this study were designed based on the highly conserved regions of each target virus, we could not excluded the possibility that the primers failed to amplify the targets efficiently because positive controls for RT-PCR detection of these viruses were not available in this study. Therefore, we note that our survey results did not conclude that the examined samples were not infected with the undetected viruses.

Six viruses (SPFMV, SPVC, SPVG, SPV2, SPLV, and SPMSV) are members of the genus *Potyvirus*, in the family *Potyviridae* (Adams et al., 2011; Clark et al., 2012). Among these potyviruses, five viruses but SPMSV had a high incidence (96%, 148/154 samples collected) in Korea, and SPFMV was especially prevalent. SPFMV has been divided into four representative strains: Russet Crack (RC), Ordinary (O), East Africa (EA), and Common (C) (Abad et al., 1992; Kreuze et al., 2000; Kwak et al., 2007). However, because SPFMV strain C has a relatively low homology with the other SPFMV strains, it was reclassified as a new species, SPVC, by ICTV in 2010. A previous study showed that Korean SPFMV isolates are similar to the RC and O strains, but did not detect any EA-like isolates (Kwak et al., 2007). The EA strain has been detected exclusively in East Africa as a distinct group (Kreuze et al., 2000; Mukasa et al., 2003). However, it was also found in Peru (Untiveros et al., 2008), Vietnam (Ha et al., 2008), Easter Island (Rännäli et al., 2009) and recently in China (Qin et al., 2013). The other potyviruses infecting sweet potato (SPVG, SPLV, and SPV2) have been poorly studied; however, their complete genome sequences were recently reported and compared to SPFMV (Li et al., 2012a; Rodriguez Pardina et al., 2012; Wang et al., 2013). Similar to SPFMV, SPLV was reported to cause synergistic disease when coinfected with SPCSV (Untiveros et al., 2007). SPV2 was first isolated from Taiwan and Nigeria (Rossel et al., 1988) and has distinct biological, serological, and genetic characteristics from SPFMV (Ateka et al., 2007; Li et al., 2012).

Begomoviruses infecting sweet potato are widely distributed worldwide. Twelve sweet potato viruses belonging to the genus *Begomovirus* in the family *Geminiviridae* have been reported (Clark et al., 2012), of which seven have

been accepted as virus species by the ICTV (Table 1). Incidence of SPLCV in Korea increased markedly from 5% in 2003 to 47% in 2012 (Kwak et al., 2006). This study provides the first record of SPSMV-1 (of the genus *Mastrevirus* in the family *Geminiviridae*) in Korea; we detected it frequently (~67%) in most areas, with the exception of Sancheong, in Gyeongsangnam-do. Several SPSMV-1 isolates have been identified from Peru, Tanzania, and China, showing 100% homology in the CP-MP region (Clark et al., 2012; Kreuze et al., 2009). SPCFV (a member of the genus *Carlavirus* in the family *Betaflexiviridae*) was first detected in sweet potato 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). Untiveros et al. showed that SPCFV could cause a synergistic disease when sweet potatoes were coinfected with SPCSV. The present study provides the first report of SPCFV in Korea, and we found that it occurred in ~31% of samples collected in most areas except Chungcheongnam-do (Table 4).

Most of the samples were in mixed infections with at least two of the eight viruses (Table 5). The single and mixed infection rates were 3.9% and 95.5%, respectively. The most prevalent infection type (32.5%) was quintuple infection with SPFMV, SPVC, and other viruses. We did not detect single infection of SPVC, SPVG, SPV2, SPLV, or SPCFV (Table 5).

In comparison, among 179 sweet potato samples surveyed in 2003, 73% were infected with SPFMV, SPGV, SPLV, and SPLCV, whereas 27% remained unidentified despite showing virus-like symptoms. However, of the samples collected in 2012, all but one was infected with one or several of the eight aforementioned viruses. In particular, we confirmed that six out of seven samples, which were thought to be virus-free, were actually infected with new viruses (data not shown). Although we detected some differences in virus type and in the degree of multiple infection among sample locations, overall infection rates were consistently high. Consequently, it is essential to test for at least these eight viruses in order to produce virus-free sweet potato seedlings in Korea.

Recently, viral diseases have caused severe damage to sweet potato crops. Thus, detection and identification of viral pathogens is vital for producing virus-free sweet potato seedlings and preventing the spread of viruses. Besides, because most of sweet potatoes were infected with a different combination of viruses, multiplex RT-PCR methods for simultaneous detection of several viruses in one reaction have been developed (Li et al., 2012b; Opiyo et al., 2010; Rukarwa et al., 2010). For rapid, simple, and simultaneous detection of sweet potato-infecting viruses in Korea, we developed two multiplex RT-PCR assays for eight major viruses, including SPLCV, SPFMV, SPVG, and SPLV in one set, and SPCFV, SPVC, SPV2, and SPSMV-1 in another set. We performed these multiplex RT-PCR methods via two-step or one-step reactions and applied them to a variety of sweet potato samples. In case of primer set 1 designed for detecting SPLCV, SPFMV, SPVG and SPLV, two-step multiplex RT-PCR performed well but unfortunately one-step multiplex RT-PCR did not work. Also, in case of primer set 2 designed for detecting SPCFV, SPVC, SPV2 and SPSMV-1, both one-step and two-step multiplex RT-PCRs were successfully. To evaluate the specificity of the assays, we confirmed the amplified products by sequencing. The multiplex RT-PCR assays developed in this study will provide a rapid, convenient, and cost-efficient method for the detection of multiple viruses in sweet potato.

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