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Genetic Diversity of *Sweet potato feathery mottle virus* from Sweet Potatoes in Korea

Hae-Ryun Kwak[1], Mi-Kyeong Kim[1], Mi-Nam Jung[2], Su-Heon Lee[1], Jin-Woo Park[1], Kook-Hyung Kim[3], Sug-Ju Ko[4] and Hong-Soo Choi[1]*

[1]*Department of Plant Pathology, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea*
[2]*Mokpo Experiment Station, National Institute of Crop Science, Mokpo 293-5, Korea*
[3]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea*
[4]*Jeonnam Agricultural Research and Extension Services, Naju 520-715, Korea*

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***Sweet potato feathery mottle virus* (SPFMV) is one of the most prevalent viruses infecting sweet potatoes and occurs widely in sweet potato cultivating areas in Korea. To assess their genetic variation, a total of 28 samples infected with SPFMV were subjected to restriction fragment length polymorphism (RFLP) analysis using DNAs amplified by RT-PCR with specific primer sets corresponding to the coat protein (CP) region of the virus. The similarity matrix by UPGMA procedure indicated that 28 samples infected with SPFMV were classified into three groups based on the number and size of DNA fragments by digestion of CP-encoding regions with 7 enzymes including *Sal*I, *Alu*I, *Eco*RI, *Hin*dIII, *Fok*I, *Sau*3AI, and *Dra*I bands. Four primer combinations out of 5 designed sets were able to differentiate SPFMV and *sweet potato virus* G infection, suggesting that these specific primers could be used to differentiate inter-groups of SPFMV. Sequence analysis of the CP genes of 17 SPFMV samples were 97-99% and 91-93% identical at the intra-group and inter-groups of SPFMV, respectively. The N-terminal region of the CP is highly variable and examination of the multiple alignments of amino acid sequences revealed two residues (residues 31 and 32) that were consistently different between SPFMV-O and SPFMV-RC.**

Keywords : genetic diversity, RFLP, SPFMV, sweet potato, 3'-UTR nucleotide sequences

Sweet potato feathery mottle virus (SPFMV), a member of the genus *Potyvirus*, has flexuous particles of 805-880 nm in length containing a positive-sense ssRNA of about 11.6 kb (Brunt et al., 1996). It is one of the most damaging viruses in sweet potato growing areas around the world and is spread naturally by aphids in a non-persistent manner (Aritua et al., 1998; Gibson et al., 1998; Karyeija et al., 1998; Moyer and Salazer, 1989). Many isolates of SPFMV from different parts of the world have been characterized (Aritua et al., 1998; Cali et al., 1981; Colinet et al., 1993; Gibson et al., 1998; Gibbs et al., 1993; Karyeija et al., 1998; Kim et al., 1998; Kreuze et al., 2000; Moyer and Salazer, 1989; Nishiguchi et al., 1995; Park et al., 1995; Usugi et al., 1991). Phylogenetic analysis of the coat protein (CP) sequences divided SPFMV isolates into four strains including russet crack strain (RC), group O, group C, and East Africa (EA) group (Abad et al., 1992; Colinet et al., 1994; Kreuze et al., 2000; Mori et al., 1994, 1995). Strains of RC group may cause internal corkiness in the tuberous roots of certain sweet potato cultivars and have been found in China, Japan, Korea, USA, and Egypt. Isolates of group O strain have been described from Niger, Nigeria, Japan, Korea, China, and Argentina. Group C strains have been shown 75.8-78.3% nucleotide sequence identity to other strains and have been founded in Argentina, China and USA. SPFMV isolates from East Africa were exclusively placed to the fourth strain group (EA). No SPFMV isolate belonging to the EA strain group has been found outside East Africa and no strain of the other strain groups has been reported in East Africa, which makes East Africa unique as far as the occurrence of SPFMV strains is concerned (Kreuze et al., 2000). However, these results are exclusively based on analysis of ten SPFMV isolates from Uganda and one isolate from Madagascar.

SPFMV is wide spread and causes a damaging disease and thus significant yield losses. Their disease incidences are closely correlated with the widespread cultivation of sweet potato crops (Kwak et al., 2006). Previously, two SPFMV isolates causing systemic foliar symptoms of typical chlorotic spots with strong dense purple pigmented boundaries were isolated. They also caused internal cork symptoms in fleshy roots and thus were not distinguishable each other visually on infected sweet potato plants in Korea (Park et al., 1994, 1995; Ryu et al., 1994). The sequence analysis of the SPFMV CP genes of two Korean isolates,

*Corresponding author.
Phone) +82-31-290-0435, FAX) +82-31-290-0434
E-mail) hschoi@rda.go.kr

SPFMV-K1 and SPFMV-K2, showed 94.1% and 97.8% sequence identity at the nucleotide and amino acid levels, respectively (Kim et al., 1998).

The quasispecies nature of RNA viruses implies a high adaptive potential, allowing for the rapid selection of biologically distinct sequence variants with the highest fitness in new environments. In spite of the high potential for variability of RNA viruses, most RNA virus populations analyzed so far are genetically stable with relatively low diversity. Various methods have been used to analyze the variability among different RNA viruses. These include the host range assay, PCR-based methods, restriction mapping, CP peptide mapping, dot blot hybridization, serological analyses, RNase protection assays, and nucleotide sequence analysis. RFLP is a method that can be used to differentiate isolates of viruses without the expenses of cloning and sequencing and its effectiveness relies on polymorphisms within restriction enzyme-recognition sites (Craig et al., 2004). Interestingly, Kim et al. (2004) reported that *Soybean mosaic virus* (SMV) strains (G2, G5, G5H, G7, and G7H), a type member of the genus *Potyvirus*, family *Potyviridae*, could be differentiated by RT-PCR/RFLP analysis. In this paper, the genetic diversity of SPFMV isolates from major sweet potato cultivating areas in Korea was evaluated by RT-PCR RFLP and sequencing analyses.

Materials and Methods

Virus sources. A total of 28 sweet potato plant samples were collected from 9 different areas where severe virus infection occurred in 2003 (Table 1, Kwak et al., 2006), based on the symptoms on leaves and roots of various sweet potato cultivars including Sinhwangmi, Sinyulmi, Yulmi, Geonmi, Hayanmi, Jinhongmi, Jami, and Borami

Table 1. SPFMV isolates and the mixed infection isolates of SPFMV used in this study

Virus isolate	Virus strain[a]	Area	Variety[b]	Sample	Symptom[c]
96-4	SPFMV-O	Muan	Sinhwangmi	Leaves	cl, nl
108-1	SPFMV-RC	Muan	–	Leaves	cl, nl
166	SPFMV- RC	Yeoju	–	Leaves	cl, nl
209	SPFMV-RC+ SPFMV-O	Iksan	–	Leaves	cl, nl
295	SPFMV-RC	Jecheon	–	Leaves	cl, nl
303	SPFMV-RC	Yeongju	–	Leaves	cl, nl
342	SPFMV-RC+ SPFMV-O	Yokjido	–	Leaves	cl, nl
473	SPFMV-RC	Yeoju	Geonmi	Roots	No symptoms
495	SPFMV-RC	Yeoju	Yeonmi	Roots	No symptoms
520-3	SPFMV-RC	Yeoju	Geonmi	Roots	Russet crack
552	SPFMV-RC	MES	Borami	Roots	No symptoms
583-1	SPFMV-O	MES	Hayanmi	Roots	Russet crack
585-3	SPFMV-O	MES	Hayanmi	Roots	Russet crack
588	SPFMV-RC	MES	Hayanmi	Roots	No symptoms
607-2	SPFMV-O	MES	Borami	Roots	String
625	SPFMV-RC	MES	Yeonmi	Roots	Russet crack
654-1	SPFMV-RC	MES	Jami	Roots	No symptoms
659-1	SPFMV-RC	MES	Jami	Roots	No symptoms
667	SPFMV-RC	MES	Borami	Roots	No symptoms
Sinyulmi	SPFMV-RC	Yeoju	Sinyulmi	Roots	String
44-b	SPGV+ SPFMV-RC	Iksan	–	Leaves	cl, nl
118-b	SPGV+ SPFMV-RC+ SPFMV-O	Haenam	–	Leaves	cl, nl
152	SPGV+ SPFMV-O	Yeoju	–	Leaves	cl, nl
256	SPGV+ SPFMV-RC+ SPFMV-O	Chungju	–	Leaves	cl, nl
487	SPGV+ SPFMV-RC	Yeoju	Geonmi	Roots	Russet crack
512	SPGV+ SPFMV-RC	Yeoju	Jinhongmi	Roots	No symptoms
644	SPGV+ SPFMV-RC	MES	Yeonmi	Roots	Russet crack
665	SPGV+ SPFMV-RC	MES	Jami	Roots	No symptoms

[a]SPFMV, *Sweet potato feathery mottle virus*; SPFMV-O, SPFMV O strain; SPFMV-RC, SPFMV russet crack strain; SPGV, *Sweet potato G virus*.
[b]–, unknown.
[c]cl, chlorotic local; nl, necrotic local.

Table 2. The sense and antisense primer pairs used for specific SPFMV in RT-PCR

Primer name	Locus	Sequence	Expected size
SPFMV 1-F	9073-9092	5' TACACACTGCTAAAACTAGG 3'	355bp
SPFMV 1-R	9428-9409	5' AGTTCATCATAACCCCATGA 3'	
SPFMV 2-F	9465-9483	5' GGACCAAGCCCCATACAAT 3'	347bp
SPFMV 2-R	9812-9795	5' GGAATGGTTGCGGGTTGC 3'	
SPFMV 3-F	9788-9807	5' GAGCTAAGCAACCCGCAACC 3'	225bp
SPFMV 3-R	10013-9994	5' TCATATGTTGACAAGAGTTG 3'	
SPFMV 4-F	10127-10148	5' TGAATGGATTAATGGTTTGGTG 3'	261bp
SPFMV 4-R	10388-10370	5' GCATATCGCGCAAGACTCA 3'	
SPFMV 5-F	10381-10402	5' CGATATGCATTTGATTTCTACG 3'	270bp
SPFMV 5-R	10651-10631	5' TTAAAGGCATACTAAAGATAA 3'	

Table 3. SPFMV-sequences used in this study

Virus isolates	Strain	Origin	Accession No.
SPFMV-54/9S	EA	Kenya (Kakamega)	AY459592
SPFMV-54/7S	EA	Kenya (Kisii)	AY459593
SPFMV-Putisrabe	EA	Madagascar	AY459597
SPFMV-Portugal	EA	Portugal	AY459599
SPFMV-Canar3	EA	Spain (Canary Islands)	AY459600
SPFMV-XN3	RC	China	AY459602
SPFMV- S	RC	Japan	D86371
SPFMV-TZ4	RC	Tanzania	AY459598
SPFMV-K1	RC	Korea	AF015540
SPFMV-K2	O	Korea	AF015541
SPFMV-CH	O	China	S69825
SPFMV-O	O	Japan	D16664
SPFMV-Arua10a	O	Uganda	AY459595
SPFMV-Sor	C	Uganda	AJ539129
SPFMV-C	C	U.S.A	S43451
SPFMV-CH2	C	China	AJ001440
SPFMV-strain 6	C	Argentina	U96625

that are widely grown in Korea (Kwak et al., 2006). Collected samples were simultaneously tested by ELISA and RT-PCR analyses. All twenty-eight samples were infected with SPFMV including 11 leaves and 17 roots samples. Eight samples of 4 leaves and 4 roots had a mixed infection with SPFMV and *Sweet potato virus* G (SPGV) (Table 1).

RT-PCR and RFLP analysis. For RT-PCR, total RNA was extracted from each sample as described previously (Choi et al., 2005). Viral and antiviral SPFMV specific primer sets were designed using previously reported SPFMV sequences assembled from data in GenBank (Tables 2 and 3). The 3'-terminal region of SPFMV comprising part of the CP gene and the 3'-untranslated region (UTR) was amplified as described previously (Kwak et al., 2006) using SPFMV primer set. The RT-PCR amplified DNA products of SPFMV were digested with seven enzymes including *Sal*I, *Alu*I, *Eco*RI, *Hin*d, *Fok*I, *Sau*3AI, and *Dra*I. The RFLP fragments were separated on 1.5% agarose gel in 0.5x TBE buffer. The similarity matrix was analyzed by the upweighted pair-group method with arithmetic average (UPGMA) procedure of the NTSYS-pc, Vision 2.0 to create a dendrogram (Rohlf, 1992).

Cloning and sequencing. Each PCR amplified fragment was cloned and sequenced as described previously (Choi et al., 2005) using the ABI PRISM 3700 Genetic analyzer (Perkin Elmer, USA) according to the manufacturer's instructions. Multiple sequence alignments of the SPFMV nucleotide sequences were obtained with alignment application of DNAMAN version 4.0 (Lynnon Biosoft, Canada) and Geneious version 2.5.3 (Biomatters Ltd, New Zealand) full optimal sequence alignments and neighbor-joining method options of Saitou and Nei (1987) with 1000 bootstrap (Felstein, 1985) replications and were phylogenetically compared to those of other viruses (GenBank and EMBL). Percent nucleotide (nt) and ORF amino acid (aa) sequence identities between virus isolates were calculated using the distance between all pairs of sequences in the multiple alignments. CP-UTR sequences used for comparison and their database accession numbers were as follows: AF015540 (SPFMV-K1 isolate), AF015541 (SPFMV-K2 isolate), AY459592 (SPFMV-54/9S isolate), AY459593 (SPFMV-54/7S isolate), AY459595 (SPFMV-Arua10a isolate), AY459597 (SPFMV-Putisrabe isolate), AY459598 (SPFMV-TZ4 isolate), AY459599 (SPFMV-Portugal isolate), AY459600 (SPFMV-Canar isolate), AY459602 (SPFMV-XN3 isolate), S69825 (SPFMV-CH isolate), D16664 (SPFMV-O strain), and D38543 (SPFMV-severe strain).

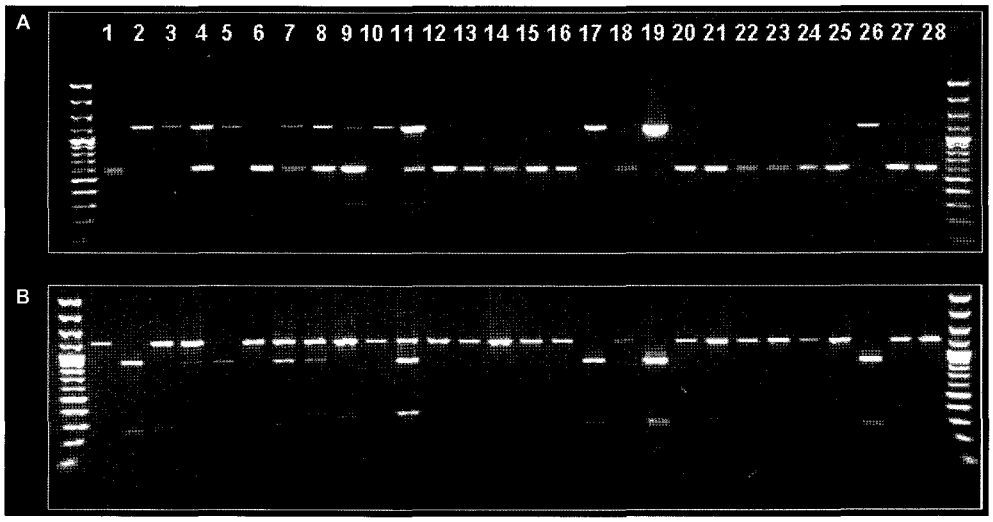

Fig. 1. RFLP analysis for SPFMV samples by *SalI* and *DraI* enzymes: lane 1, 44-b; lane 2, 96-4; lane 3,108-1; lane 4, 118-b; lane 5, 152; lane 6, 166; lane 7, 209; lane 8, 256; lane 9, 295; lane 10, 303; lane 11, 342; lane 12, 473; lane 13, 487; lane 14, 495; lane 15, 512; lane 16, 520-3-b; lane 17, 585-3; lane 18, 588; lane 19, 607-2; lane 20, 644; lane 21, 654-1; lane 22, 659-1; lane 23, 667-2; lane 24, Sinyulmi; lane 25, 552-2; lane 26, 583-1; lane 27, 625; lane 28, 665. Molecular mass marker, 100 bp DNA ladder.

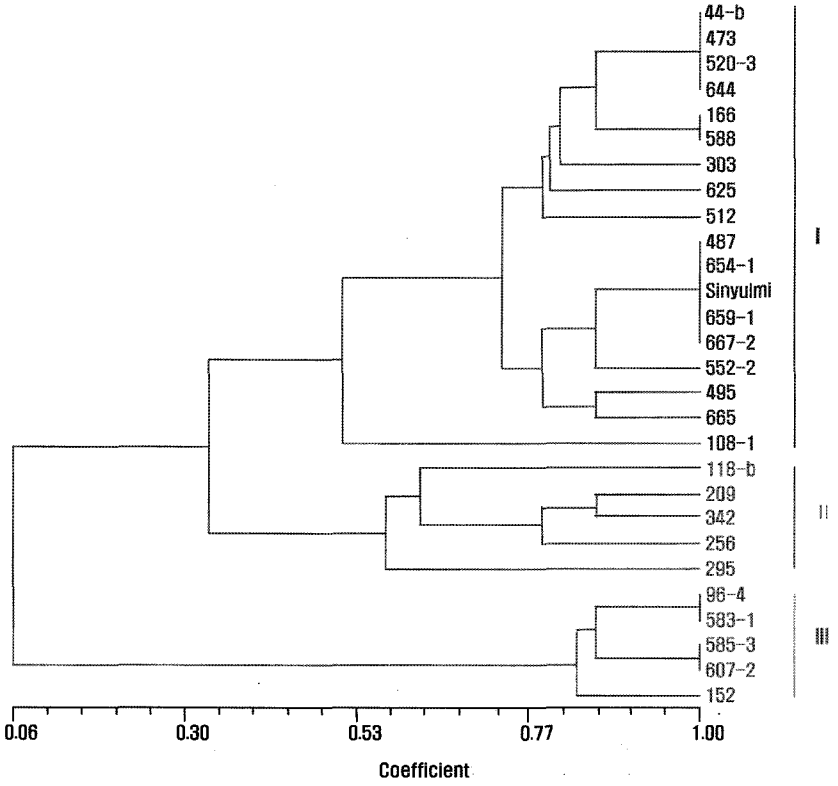

Fig. 2. Comparison of UPGMA phenograms of 28 samples for SPFMV.

Results

Detection of SPFMV by RT-PCR. A total of 28 samples for SPFMV and the mixed infection of SPFMV and SPGV collected and analyzed by RT-PCR. The strain and symptom of the viruses were shown not to have any relationship in cultivars and area grown (Table 1). Most symptoms observed included chlorotic/necrotic local lesion in leaves, no symptom, russet crack, and string in roots (Table 1). For detection of SPFMV, 5 primer sets were designed and used (Table 2). Four out of five primer combinations were able to differentiate single or mixed

infection of SPFMV and SPGV. However, SPFMV 4-F/ SPFMV 4-R primer combination could not distinguish SPFMV from SPGV even in samples where both viruses were present (data not shown).

RFLP analysis. The population structure of 28 samples for SPFMV and the mixed infection of SPFMV and SPGV were investigated by RFLP analysis of the 1.147bp for SPFMV (Fig. 1). The similarity matrix by UPGMA procedure was used to differentiate the 28 SPFMV-infected samples. These samples were classified into three groups based upon analysis of digested bands of CP-encoding region with *Sal*I, *Alu*I, *Eco*RI, *Hin*d, *Fok*I, *Sau*3AI, and *Dra*I when we truncated a dendrogram at similarity value of 0.50. The genetic similarity coefficient for all accessions ranged from 0.06 to 1.00. Eighten out of the 28 samples were grouped into I, whereas each 5 samples into Group II and III (Fig. 2). Because Sal I and Dra I gave a combination of groups I and III, group II probably represented a mixture of both group I and III (Fig. 2).

Sequence analysis of SPFMV. In SPFMV sequence analysis, all the cloned cDNAs were 1,147 bp comprising 167 nt of the nuclear inclusion protein (NIP), 945 nt CP and 34 nt UTR. Phylogenetic analysis of the nt and the predicted aa sequences subgrouped the SPFMV isolates into two groups (Fig. 3). In aspect of SPFMV sequence identity, seventeen samples appeared to be divided into two groups, their identity indexes of intra-group and inter-group of SPFMV were 97-99% and 91-93%, respectively (Fig. 4). Five samples (96-4, 152, 342, 583-1, and 607-2) were designated as group O and also contained isolates K2 (Korea), O (Japan), TZ4 (Tanzania) and Arua10a (Uganda). The CP sequences of these isolates were 97.4-99.6% and

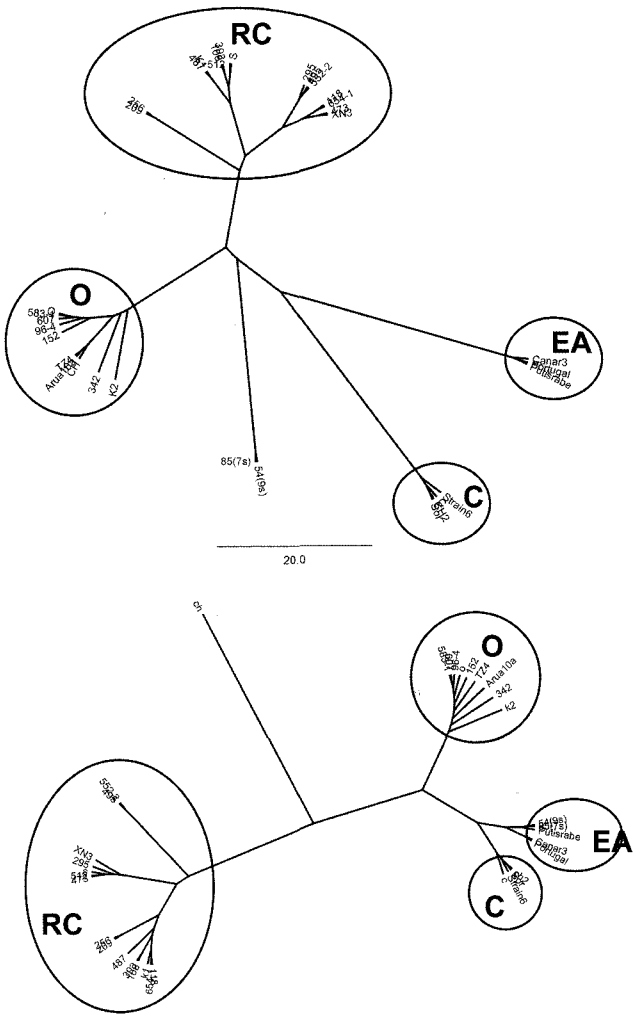

Fig. 3. Phylogenetic analysis of the nucleotides (A) and amino acid (B) sequences of the coat protein of SPFMV samples.

aa / nt	495	108	118	209	256	295	303	473	487	654-1	512	552-2	96-4	152	342	583-1	607-2
495		96.8	95.9	97.1	96.5	97.1	97.8	97.8	97.8	97.8	96.5	98.4	94.0	94.6	95.9	94.6	94.6
108	97.6		96.5	98.4	97.1	97.1	99.0	98.4	98.4	98.4	98.4	97.8	94.9	95.6	97.8	95.6	95.5
118	98.0	98.7		96.8	95.6	96.2	96.8	97.5	96.8	97.5	96.2	96.8	93.7	94.3	95.6	94.3	94.3
209	97.7	98.4	98.6		98.7	97.5	98.7	98.7	98.7	98.7	98.1	98.1	95.2	95.9	97.5	95.9	95.9
256	97.5	98.0	98.2	98.5		96.8	97.5	97.5	98.1	97.5	96.8	97.5	94.6	95.2	96.8	95.2	95.2
295	97.1	96.9	97.2	96.9	96.9		97.5	98.1	98.1	98.1	96.8	98.1	94.3	94.9	96.2	94.9	94.9
303	98.0	98.9	98.7	98.6	98.4	97.2		98.7	98.7	98.7	98.7	98.7	95.2	95.9	98.1	95.9	95.9
473	97.5	97.6	98.0	97.7	97.2	97.8	97.8		98.7	99.4	98.7	98.7	95.5	96.2	97.5	96.2	96.2
487	98.1	98.6	98.8	98.7	98.5	97.4	99.0	97.9		98.7	98.1	98.7	95.2	95.9	97.5	95.9	95.9
654-1	98.1	98.6	99.0	98.7	98.3	97.4	98.8	98.1	98.9		98.1	98.7	95.6	96.2	97.5	96.2	96.2
512	97.2	97.8	97.8	97.7	97.2	97.6	98.0	99.2	97.9	97.9		97.5	94.6	95.2	97.5	95.2	95.2
552-2	98.8	98.1	98.5	98.2	98.0	97.7	98.7	97.8	98.8	98.6	97.6		94.9	95.6	96.8	95.6	95.5
96-4	91.2	91.1	91.1	91.4	91.0	90.7	91.1	91.3	91.2	91.2	91.0	91.1		98.4	97.1	99.4	99.4
152	91.2	91.3	91.3	91.4	90.9	90.7	91.1	91.3	91.2	91.2	91.0	91.1	98.0		97.8	99.0	99.0
342	92.5	92.8	92.6	92.9	92.4	92.0	92.8	92.7	92.7	92.7	92.7	92.4	97.4	97.7		97.8	97.8
583-1	91.3	91.2	91.2	91.5	91.1	90.8	91.2	91.4	91.3	91.3	91.1	91.2	99.3	98.1	97.5		100.0
607-2	91.1	91.0	91.1	91.3	90.9	90.6	91.0	91.2	91.1	91.1	90.9	91.0	99.3	98.1	97.5	99.6	

Fig. 4. Amino acid sequence (upper) and nucleotide sequence (lower) identity of the coat protein of SPFMV samples.

		1	11	21	31	41	51	61	71
RC	118	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	473	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	654-1	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	495	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	AIIPESYGRD	TSKEKESIVG	ASSKGMRDKD
	295	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSEGMRDKN
	552-2	SSERTEFKDA	GANPPAPKPK	DIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKSMRDKD
	487	SSERTEFKDA	GANPPAPNPK	NIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGMRDKD
	108	SSERTEFKDA	GANPPAPKPT	NIPPPPTITE	VTDSEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	303	SSERTEFKDA	GANPPAPKPT	NIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	512	SSERTEFKDA	GANPPAPKPT	NIPPPPTITE	VTDPEDPKQA	ALRAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	209	SSERTEFKDA	GADPSAPKPK	NIPPPPTITE	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	ASSKGVRDKD
	256	SSERTEFKDA	GADPSAPKPK	NIPPPPTITG	VTDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSSKGMRDKD
O	342	SSERTEFKDA	GANPPAPKPT	NIPPPPTITE	IVDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSSKGVRDKD
	152	SSENTEFKDA	GANPPDPKSK	INPPPPTITE	IVDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSSKGVRDKD
	96-4	SGEKTEFKDA	GANPPDPKSK	INPPPPTITE	IVDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSLKGVRDKD
	583-1	SGEKTEFKDA	GANPPDPKSK	INPPPPTITE	IVDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSSKGVRDKD
	607	SGEKTEFKDA	GANPPDPKSK	INPPPPTITE	IVDPEDPKQA	ALKAARAKQP	ATIPESYGRD	TSKEKESIVG	TSSKGVRDKD

Fig. 5. Alignments of the N-terminal amino acids of the coat protein of SPFMV samples. Amino acid variations within group RC isolates are indicated by bold letters and underlines. The DAG motif involved in aphid transmissibility is enclosed in dot boxes.

Table 4. The sense and antisense primer pairs used for differentiation of SPFMV-RC and SPFMV-O

Primer name	Strain	Sequence (5'->3')	Expected size
SPFMV-RC-F	SPFMV- RC	TGATCCAGAAGACCAAAGC	738bp
SPFMV-RC-R		CGATTTTTCGCATTCTTAA	
SPFMV-O-F	SPFMV-O	GCGTGATACGAGCAAAGAA	212bp
SPFMV-O-R		CGCAACTTCATACTGCTCTGGT	

97.1-100% identical at the nt and aa levels, respectively. Many samples (70.6%) including isolates K1 (Korea), XN3 (China), and S (Japan) designated as group RC. The CP sequences of group RC were 96.9-99.2% identical at the nt and 95.9-99.4% identical at aa levels. However, no SPFMV strains belonging to group EA and C have been found in Korea. All SPFMV CPs consisting of 945 nucleotides (315 amino acids) start from TCT and terminate with an amber stop codon UAA. The DAG motif was present in all samples (Fig. 5). There were three regions that show aa variations within group RC isolates, i.e. N13 → D and P15 → S substitution in the 2 isolates (209 and 256), a V76 → M substitution in 5 samples (256, 495, 295, 487 and 552-2), and a K20 → T substitution in the other 3 isolates (108, 303 and 512). In contrast, all 5 samples (342, 152, 96-4, 583-1, 607) in group O contained the common substitutions V31 → I, T32 → V and A71 → T. Interestingly, isolate 342 was very similar to that of samples 108, 303 and 512 of group RC in the other aa regions, whereas the other samples of group O showed the R4 → N/K and A16 → D substitutions (Fig. 5).

Specific primer for SPFMV-RC and –O differentiation. The specific primer sets to differentiate SPFMV group RC from group O were designed using SPFMV sequences assembled from seventeen samples obtained in this work. The variable 5' terminal half of the CP gene was amplified. Depending on the group to be detected, primer combinations shown in Table 4 were used. The sizes of the amplified RT-PCR fragments are shown in Fig. 6. With the SPFMV-RC primers, the 738 bp fragment was expected to be amplified for SPFMV-RC isolates, while no fragment was expected to be amplified for the SPFMV-O isolates. In

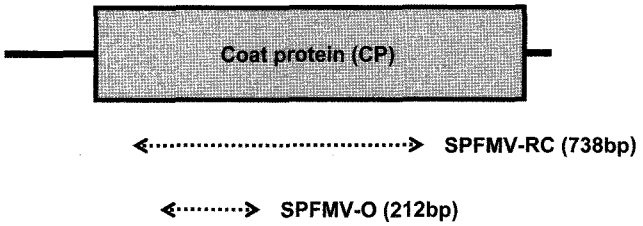

Fig. 6. Schematic map and genomic location of the specific primer for differentiation of SPFMV-RC and SPFMV-O.

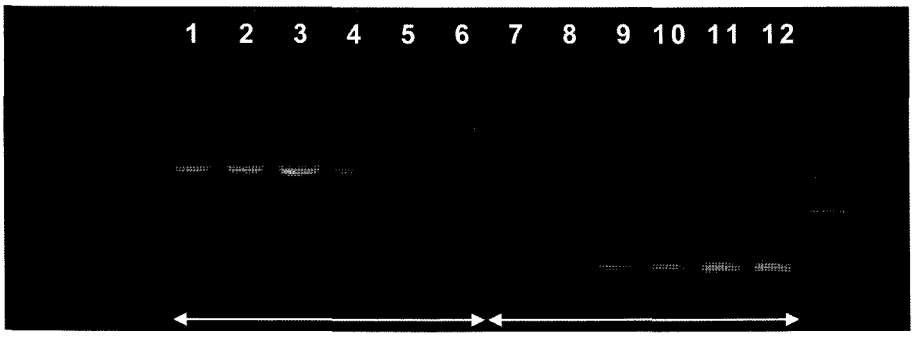

Fig. 7. RT-PCR amplifications by using the specific primer of SPFMV-RC (lane 1-6) and SPFMV-O (lane 7-12): lane 1 and 7, 654-1; lane 2 and 8, 552-2; lane 3 and 9, 256; lane 4 and 10, 342; lane 5 and 11, 583-1; lane 6 and 12, 607-2. Molecular mass marker, 100 bp DNA ladder.

contrast, with the SPFMV-O primers, the 212 bp fragment was expected to be amplified for all SPFMV-O isolates with no expected amplification for SPFMV-RC isolates (Fig. 7). In single infection of SPFMV, all twenty samples comprising 7 leaf samples from fields and 13 root seedlings were infected 14 with SPFMV-RC, 4 with SPFMV-O and 2 with SPFMV-O+SPFMV-RC. In 8 samples that were mixed infected with SPFMV and SPGV, 62.5% were infected with SPGV+SPFMV-RC, 12.5% with SPGV+SPFMV-O and 25% with SPGV+SPFMV-O+SPFMV-RC. However, even though the variation in SPFMV groups was evident, there were any significant relationships between SPFMV groups and symptoms (Table 1).

Discussion

In a previous paper (Kwak et al., 2006) as the results of a survey of sweet potato viruses, the major viruses infecting sweet potato in Korea were SPFMV. By results of electron microscopy and RT-PCR, sweet potatoes in the fields were infected with more than one virus species. Although it was reported that the SPFMV caused no symptom in sweet potatoes in East Africa (Gibson et al., 1998). Park et al. (1994) reported that SPFMV caused systemic foliar symptoms such as typical chlorotic spots associated with strong dense purple pigmented boundaries and internal cork symptoms in fleshy roots in Korea. On the other hand, Kwak et al. (2006) reported that the incidence and severity of the sweet potato viral diseases were shown not to have any relationship in cultivars and areas grown and despite the high incidence, severity was very low as there was no marked reduction in yield (Kwak et al., 2006). In this study, we did not observe any relationship between SPFMV infection or mixed infection of SPFMV/ SPGV and symptom appearances. In addition, no relationship was observed for russet crack symptoms in roots and SPFMV-RC infections (data not shown). It remained to be further elucidated what exactly causes russet crack symptoms in sweet potato roots in many sweet potato cultivating areas.

Genetic variability among the Korean SPFMV samples by UPGMA procedure revealed that SPFMV isolates were classified into three subgroups. In SPFMV, 18 of 28 samples were grouped into group I, whereas each 5 samples into group II and III. It is tempting to speculate that group II probably represents a mixture of both group II and III as evidenced by RFLP analysis. We showed that SPFMV groups could be differentiated by RT-PCR/RFLP analysis, as observed by Craig et al. (2004) and Kim et al. (2004). SPFMV isolates might be divided into two groups. In aspect of sequence identity, homology analysis of CP sequences, SPFMV was similar to the results of genetic variability by UPGMA. Therefore, we can conclude that group I by UPGMA belongs to SPFMV-RC, Group II to SPFMV-O+SPFMV-RC and Group III to SPFMV-O.

Alignment of CP nucleotide (nt) and amino acid (aa) sequences revealed a varying degree of sequence identity grouping. While the same tendency to the sequence data for CP gene of the two Korean isolates of SPFMV by Ryu et al. (2003), most of SPFMV were clustered with russet crack strain of SPFMV and the rest into ordinary strain of SPFMV. In the present studies, a total of 28 samples were infected with 50% with SPFMV-RC, 14% with SPFMV-O, 7% with SPFMV-O+SPFMV-RC, 18% with SPGV+SPFMV-RC, 4% with SPGV+SPFMV-O and 7% with SPGV+SPFMV-O+SPFMV-RC. Therefore, similar to earlier reports (Ryu et al., 2003), most of SPFMV were clustered with SPFMV-RC. However, even though the variation in SPFMV groups was manifest, SPFMV groups and symptoms were shown not to have any relationship each other (Table 1).

In this paper, sequence analysis of the CP genes of 17 SPFMV samples were 97-99% and 91-93% identical at intra-group and inter-groups of SPFMV, respectively (Fig. 4). Additionally, we showed that the N-terminal region of the CP is highly variable and examination of the multiple alignments of amino acid sequences revealed two residues that were consistently different between SPFMV-O and

SPFMV-RC (residues 31 and 32). In addition, the variations in the aa sequences of group RC appeared from four residues (residues 13, 15, 20, and 76), whereas group O from three residues (residues 31, 32, and 71). It is also interesting to point out that mixed infection samples (209, 256, and 342) with SPFMV-O and SPFMV-RC were more diverse, but the genetic variation was not correlated with geographic regions, symptoms, or varieties. It was hypothesized that the mixed infection of SPFMV-O and SPFMV-RC might be related any specific amino acid changes (Fig. 5).

We showed that SPFMV 4 primer combinations were designed and could differentiate between SPFMV and SPGV either singly or in mixed infection. We also report that the specific primers were designed and could be used to differentiate inter-groups of SPFMV. Similar to the previous report (Ryu et al., 2003), most SPFMV isolates from major sweet potato-cultivating areas in Korea were clustered with SPFMV-RC. Additionally, the N-terminal region of the CP is highly variable and examination of the multiple alignments of amino acid sequences revealed two residues but mixed infection with SPFMV-O and SPFMV-RC were more diverse. Further experiments are needed to determine the relationship between genetic variation and the mixed infection of SPFMV-O and SPFMV-RC. This would help in developing an effective detection for differentiating the SPFMV groups by RT-PCR/RFLP analysis and the specific primers.

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