Plant Pathol. J. 22(3) : 239-247 (2006)

The Plant Pathology Journal

Virus Disease Incidences of Sweet Potatoes in Korea

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(Received on July 31, 2006; Accepted on August 7, 2006)

In 2003, a survey of sweet potato virus disease was carried out in seed boxes as well as in various sweet potato fields. Virus infection rate was 5~100% and 100% at seed boxes and fields, respectively. No relationship of the disease incidence and severity was observed between sweet potato cultivating areas and cultivars. A total of 179 samples were collected and analyzed based on serological, electron microscopic and molecular properties. Field-grown sweet potatoes were examined to inspect 8 different viruses using NCM-ELISA, resulting that 30% of sweet potato was infected by one virus, whereas 70% was by more than 2 viruses. However, RT-PCR using primers selected for seven viruses, such as *Sweet potato feathery mottle virus* (SPFMV) revealed that of one-hundred seventy-nine tested; 71 of SPFMV, 29 of SPGV, 19 of SPFMV+SPGV, 1 of SPFMV+SwPLV, 1 of SPFMV+SPLCV, 2 of SPFMV+SPGV+SwPLV, 6 of SPFMV+SPGV+SPLCV, 2 of SPFMV+SPGV+SwPLV+SPLCV and 48 of unknown viruses were identified from the field samples. In root, viral diseases were severer in Yeoju than in Mokpo Experiment Station and infection rate was much different depending on sweet potato cultivars.

Keywords : disease incidence, sweet potato, SPFMV, SPGV, SPLCV, SwPLV

Viral diseases of sweet potato (*Ipomoea batatas* L.) have become widespread, causing serious losses around the world (Brunt et al., 1996; Moyer et al., 1989; Sheffield, 1953). At least 19 different viruses have been described in sweet potatoes to date, but only 11 have currently a formal taxonomic position in the viral classification system (Brunt et al., 1996). The most severe symptoms in sweet potatoes are caused by co-infection 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 (Karyeija et al., 2000). Synergism has also been observed between SPCSV and the possibly whitefly-transmitted *Sweet potato mild mottle virus* (SPMMV) (Gutiérrez et al., 2003; Hahn, 1979). In Africa, sweet potato is grown in many countries, but viruses infecting the crop have been studied mostly in sub-Saharan Africa where they cause yield reductions of 56-98% (Gibson et al., 1998; Sheffield, 1953; Winter et al., 1992).

It has been reported that sweet potato can be infected by at least five members of the family *Potyviridae* (Moyer and Salazar, 1989, 1990). The *Potyvirus* genus, SPFMV, *Sweet potato G virus* (SPVG), *Sweet potato mild speckling virus* (SPMSV), and *Sweet potato latent virus* (SwPLV) is transmitted mechanically by aphid mouthparts in a non-persistent, non-circulative, stylet borne manner (Colinet et al., 1994, 1996; Fuentes et al., 1996; Karyeija et al., 1998). However, SPMMV is characterized by its flexious filamentous particles, inclusion bodies in infected plant cells and by having a polyprotein genome strategy. It differs from most of the other genera in having white fly transmission (Wisler et al., 1998). Like all the genera except the genus *Bymovirus*, it has a monopartite genome (Alvarez et al., 1988). SPCSV previously also known as sweet potato sunken vein virus belongs to the genus *Crinivirus* in the family *Closteroviridae* and is a pathogen of sweet potato. It is phloem-limited and transmitted in a semi-persistent manner by *B. tabaci* and *T. abutilonea* (Cohen et al., 1992; IsHak et al., 2003). And, *Sweet potato leaf curling virus* (SPLCV) is a type member of the genus *Badnavirus*, family *Geminiviridae* (Banks et al., 1999; Chung et al., 1985; Lotrakul et al., 1998; Lotrakul et al., 2001; Osaki et al., 1991).

In Korea, SPFMV, SwPLV and SPCSV have been detected in sweet potato plants (Kim et al., 1998; Park et al., 1994, 1995; Ryu and Choi, 2002; Yun et al., 2002). The two Korean isolates, SPFMV-K1 and SPFMV-K2, cause systemic foliar symptoms of typical chlorotic spots associated with strong dense purple pigmented boundaries, and their internal

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cork symptoms in fleshy roots, and they are not distinguishable visually on infected sweet potato plants. The coat protein (CP) gene of the genomic RNA of two Korean isolates of SPFMV, SPFMV-K1 and SPFMV-K2, was cloned and sequenced (Kim et al., 1998). Western blot analysis of the protein samples extracted from infected sweet potato and partial purification of viruses revealed that three different viruses including SPFMV, SwPLV, and SPCSV occasionally singly or mixed infected sweet potatoes (Yun et al., 2002). In this paper we report the results of a survey of sweet potato virus in seed boxes as well as field.

Materials and Methods

Survey and sample collection. In 2003, a survey of sweet potato virus was carried out in 14 different main sweet potato growing areas in 8 provinces of Korea including Gyeonggi, Gyeongsangnam, Gyeongsangbuk, Jeollanam, Jeollabuk, Chungcheongnam, Chungcheongbuk and Jeju (Table 1). The survey was conducted by walking through sweet potato seed boxes in the greenhouses and fields while inspecting the crops for sweet potato disease symptoms. Investigation was conducted visually in 3 replications. Disease incidence was calculated as the number of plants showing sweet potato virus symptoms relative to the total number of plants observed in an area. Cuttings collected from the field were planted in pots and kept in an insect-free glasshouse at 20-25°C with a 12-15 h light period. For disease incidence and symptom of root, 100 seeds of each of 15 cultivars using for foundation seed were sown in Yeoju and Mokpo Experiment Station (MES) fields (Table 3) and cultivated according to farmer's practice. The symptoms of roots were investigated by visual inspection and ten plants were randomly selected. And then, seedlings from the selected foundation seeds were inspected for disease symptoms for 30 days after emergence and analyzed for virus infection by RT-PCR.

Host ranges. To determine the infectivity of virus isolates and the symptoms induced on test plants, 5-10 seedlings from each of the species, *Chenopodium amaranticolor*, *C. quinoa*, *N. benthamiana*, *N. tabacum* cv. 'Xanthi-nc', *Physalis floridana*, *Petunia* spp., *Datura stramonium*, *Tetragonia expansa*, *I. setosa* at the 3-5 leaf stage were inoculated by sap prepared by homogenizing infected leaf samples in 0.01M phosphate buffer, pH 7.0. The test plants were put in an insect-free greenhouse maintained at 20-25°C with 12-15 h light period. Disease symptoms were recorded three times a week for 30 days. Both symptomatic and non-symptomatic plants were verified for the virus infection by electron microscopy, NCM-ELISA and RT-PCR.

NCM-ELISA. Nitrocellulose membrane ELISA (NCM-ELISA) was carried out as described by Abad and Moyer (1992). Leaf samples were collected by excising discs with a cork borer, weighing them, and grinding the discs in sample buffer at 1 g/10 ml. The sap was allowed to clarify for 30 min after which aliquots of 10 or 100 ml were dotted on NCM membrane (Bio-Rad, USA) or transferred to a microtitre plate (Greiner Laborteknik, Germany), respectively.

Table 1. Occurrence of virus diseases on sweet potato in the fields

Area		Seed boxes		Fields	
		Diseased fields (%)	Disease incidence (%)	Diseased fields (%)	Disease incidence (%)
Gyeonggi	Yeoju	100	60~90	100	100
	Icheon	NI[a]	NI	100	100
Chungnam	Nonsan	NI	NI	100	100
Chungbuk	Chungju	NI	NI	100	100
	Jecheon	NI	NI	100	100
Jeonbuk	Iksan	100	30~100	100	100
Jeonnam	Haenam	100	5~100	100	100
	Muan	100	30~100	100	100
	Yeongam	100	10~100	100	100
Gyeongbuk	Yeongju	NI	NI	100	100
Gyeongnam	Sancheong	NI	NI	100	100
	Jinju	NI	NI	100	100
	Yokjido	NI	NI	100	100
Jeju		NI	NI	100	100

[a]Not investigated.

Table 2. Infection rates and symptoms of root on different sweet potato cultivars

Area	Cultivars	No. of root		Disease incidence (%)	No. of symptoms	
		Investigated	Infected		String	Russet crack
Yeoju	Jinhongmi	64	33	51.6	33	0
	Sinyulmi	58	34	58.6	34	0
	Geonmi	83	70	84.3	0	70
	Hayanmi	71	45	63.4	45	0
	Yeonmi	26	0	0.0	0	0
	Sigeonmi	36	0	0.0	0	0
	Sincheonmi	33	9	27.3	0	9
	Gogeonmi	36	18	50.0	18	0
	Geumsi	24	0	0.0	0	0
	Total	431	209	48.5	130	79
Mokpo Experiment Station	Jinhongmi	58	8	13.5	8	0
	Sinyulmi	44	0	0.0	0	0
	Geonmi	56	10	17.9	0	10
	Hayanmi	53	3	5.7	0	3
	Gogeonmi	58	0	0.0	0	0
	Sincheonmi	72	0	0.0	0	0
	Juhwangmi	50	0	0.0	0	0
	Yulmi	77	11	14.3	0	11
	Borami	82	5	6.1	5	0
	Sinhwangmi	82	3	3.7	3	0
	Jeungmi	41	0	0.0	0	0
	SinJeungmi	50	0	0.0	0	0
	Sinjami	62	0	0.0	0	0
	Jami	61	0	0.0	0	0
	Hayanmi	79	3	3.8	3	0
	Total	925	43	4.7	19	24

Table 3. The new sense and antisense primer pairs used for specific sweet potato viruses in RT-PCR

Virus	Sequence	polarity	Size (bp)
SPFMV	5' GGA CCA AGC CCC ATA CAA T 3'	sense	347
	5' GGA ATG GTT GCG GGT TGC 3'	antisense	
SPGV	5' TGG CGC ATC AAG GAA AAG 3'	sense	313
	5' ACC TGG TGG TAA TGG TCC 3'	antisense	
SPMSV	5' GCC AAA ACC AAC AAG CAT CA 3'	sense	275
	5' ATT CGC ATT TCC TCA TCA TCT 3'	antisense	
SwPLV	5' GGG TGA TGA TGG ACG GAG ACA 3'	sense	298
	5' CCG ATG ATG TGT ATT TGT GAG C 3'	antisense	
SPMMV	5' CCG CGC CAA CAA AGG AAC TA 3'	sense	298
	5' TTG ATG GGG TAA TAA AGC ACT 3'	antisense	
SPCSV	5' GTA GGT TTC GGG GAC AGG 3'	sense	302
	5' TTG GAA TAG AGA TGC GTA GAA A 3'	antisense	
SPLCV	5' GGA CCC TTT GCA GAA CCC ACT AC 3'	sense	574
	5' TCT GTC ACG AAT CAA CCA ATA C 3'	antisense	

[a] SPFMV, *Sweet potato feathery mottle virus*; SPGV, *Sweet potato G virus*; SPMSV, *Sweet potato mild speckling virus*; SwPLV, *Sweet potato latent virus*; SPMMV, *Sweet potato mild mottle virus*; SPCSV, *Sweet potato chlorotic stunt virus*; SPLCV, *Sweet potato leaf curl virus*.

Table 4. Detection of viral diseases by NCM-ELISA in sweet potato samples

Collected samples	No. of plants		No. of infected virus (%)							
	Investi-gated	Infected	1	2	3	4	5	6	7	8
Seed boxes	87	58	18 (31.0)	7 (12.1)	7 (12.1)	4 (6.9)	2 (3.5)	2 (3.5)	5 (8.6)	13 (22.4)
Fields	50	35	11 (31.4)	4 (11.4)	11 (31.4)	4 (11.4)	4 (11.4)	0 (0.0)	1 (2.9)	0 (0.0)
Total (%)	137	93 (67.9)	29 (21.2)	11 (8.0)	18 (13.1)	8 (5.8)	6 (4.4)	2 (1.5)	6 (4.4)	13 (9.5)

Electron microscopy. Dip preparations were prepared by grinding a small piece of infected *N. benthamiana* in 2-3 drops of 2% phosphotungstic acid, pH 7.0. Ultrathin sections were conducted as described by Choi et al. (2005). For interpretation of results, the sections were viewed under electron microscope LEO 912AB (Carl Zeiss, Germany) at 80 kV.

Total RNA extraction and RT-PCR. Total RNA was extracted from infected leaf samples as described by Choi et al. (2005). Published sequences were obtained from genomic databases at GenBank and aligned by using the multiple sequence alignment application of DNAMAN version 4.0 (Lynnon Biosoft, Canada) full optimal sequence alignments and neighbor-joining method options of Saitou and Nei (1987) with 100 bootstrap (Felstein, 1985) replications. Depending on the virus to be detected, primer combinations shown in Table 4 were used. Reverse transcription (RT) was carried out in a final 5 μl volume obtained by adding 1 μL total RNA (2.5 ng/μl), 10 pmole (1 μl) of the downstream primer, 1×RT buffer, 2 mM dNTP, and 0.5 U AMV reverse transcriptase (Promega Co., USA) and made up to volume with DEPC-dH_2O. RT was carried out at 42°C for 30 min, and was denatured by heating at 95°C for 5 min. When RT was completed, total 25 μl of 10 pmole (1 μl) of the upstream primer, 1 U Taq DNA polymerase (Promega Co., USA), 1×PCR buffer and 1.5 mM $MgCl_2$, were added. Mixtures were then amplified in a PTC-0200 Peltier Thermal Cycler (MJ research, USA) for a total 35 cycles. Each cycle included a denaturing step at 94°C for 30 sec, an annealing step at 50°C for 30 sec, and extension step at 72°C for 90 sec, and finally kept at 72°C for 10 min. PCR products were analyzed by electrophoresis in 1.0% agarose gel, staining with ethidium bromide, and DNA bands were visualized using a UV transilluminator.

Table 5. Detection of viral diseases by RT-PCR in seed boxes depending on areas

Area	No. of samples tested	F	G	F+G	Unknown
Iksan	4	2		1	1
Yeongam	2	2			
Haenam	2	1		1	
Jinju	6	3	2		1
Total (%)	14	8 (57.1)	2 (14.3)	2 (14.3)	2 (14.3)

[a]F, *Sweet potato feathery mottle virus*; G, *Sweet potato G virus*.

Results

Disease incidence and symptoms. In 2003, a survey of sweet potato virus disease was carried out in seed boxes as well as field, and virus infection rate was 5-100% and 100% at seed boxes and field, respectively. The incidence and severity of the diseases were shown not to have any relationship in cultivars and area grown (Table 1). The most common symptom observed on the leaves was chlorotic spot, necrotic spot, and vein banding, which was attributed to the depression of interveinal tissues causing the veins to be more pronounced (Fig. 1). In addition to mosaic, fern leaves and leaf rolling were rarely observed.

Disease incidence of root was much different depending on sweet potato cultivars and areas, whereas internal symptoms of roots were shown not to have any relationship in cultivars and area grown as the same leaf samples. In Yeoju, disease incidence of sweet potato roots depending on cultivars ranged from 0 to 70% with Geonmi having the highest incidence while Yeonmi, Sigeonmi and Geumsi had the lowest (Table 3). In cultivars Jinhongmi, Sinyulmi, Hayanmi and Gogeonmi, only string symptom was observed, but russet crack symptom alone was observed in cultivars Hayanmi and Sincheonmi. On the other hand, in Mokpo Experiment Station, there was an average disease incidence of 4.7% viral diseases and infection rate depending on sweet potato cultivars isolates induced similar symptoms in Yeoju. It is interesting to note that although Hayanmi

cultivar samples using the same foundation seed, one showed string symptoms while the other showing russet crack symptoms. Disease incidence of roots according to cultivars was different, i.e., 17.9% in Geonmi, 14.3% in Yulmi, 13.5% in Jinhongmi, 6.1% in Borami, 5.7% in Yeonmi, 3.8% in Hayanmi and 3.7% in Sinhwangmi. Host range studies showed that SPFMV caused chlorotic spot symptoms on *C. amaranticolor*, *C. quinoa* and *I. setosa* but the isolates could not infect *N. glutinosa*, *N. benthamiana*, *Phyalis floridana*, *Datura stramonium* and *G globosa*. However, all the test plants were found to be immune to the other isolates as no virus was detected in the plants by RT-PCR.

Electron microscopy and NCM-ELISA. Based on electron microscopy examination of crude sap extracts, sixty-seven out of 135 samples revealed long flexious particles 700-800 nm in length. Thin section from sweet potato examination revealed typical cytoplasmic inclusions bodies like pinwheels and scrolls (Fig. 1). NCM-ELISA revealed that 31.0% of the 87 samples were infected a single infection with SPFMV, SwPLV, SPCFV, C-6, and SPCSV in seed boxes, whereas in fields 31.4% of the 50 samples were a single infection with SPMSV and SPCSV. There was no single infection of SPFMV and SwPLV. A striking feature was that 22.4% had a mixed infection of 8 viruses in seed boxes but no virus was detected in fields. However, most of the 137 samples were not consistent with either electron microscopy and/ or RT-PCR (data not shown).

Distribution of virus diseases by RT- PCR. Of the 669 plants that collected 87 leaf samples seed boxes, 368 leaf samples from fields and 214 root seedlings, 179 samples were analyzed by RT-PCR. In seed boxes, all fourteen samples collected from 4 areas were infected with viruses including 8 with SPFMV, 2 with SPGV, 2 with SPFMV+ SPGV and 2 unknown viruses all detected in samples, whereas 42.7% were infected with SPFMV, 18.3% with SPGV, 13.4% with SPFMV and SPGV, 1.2% with SPFMV, SPGV and SwPLV, 3.7% with SPFMV, SPGV and SPLCV and 20.7% unknown virus of the 82 samples tested were infected with different viruses and varied depending on

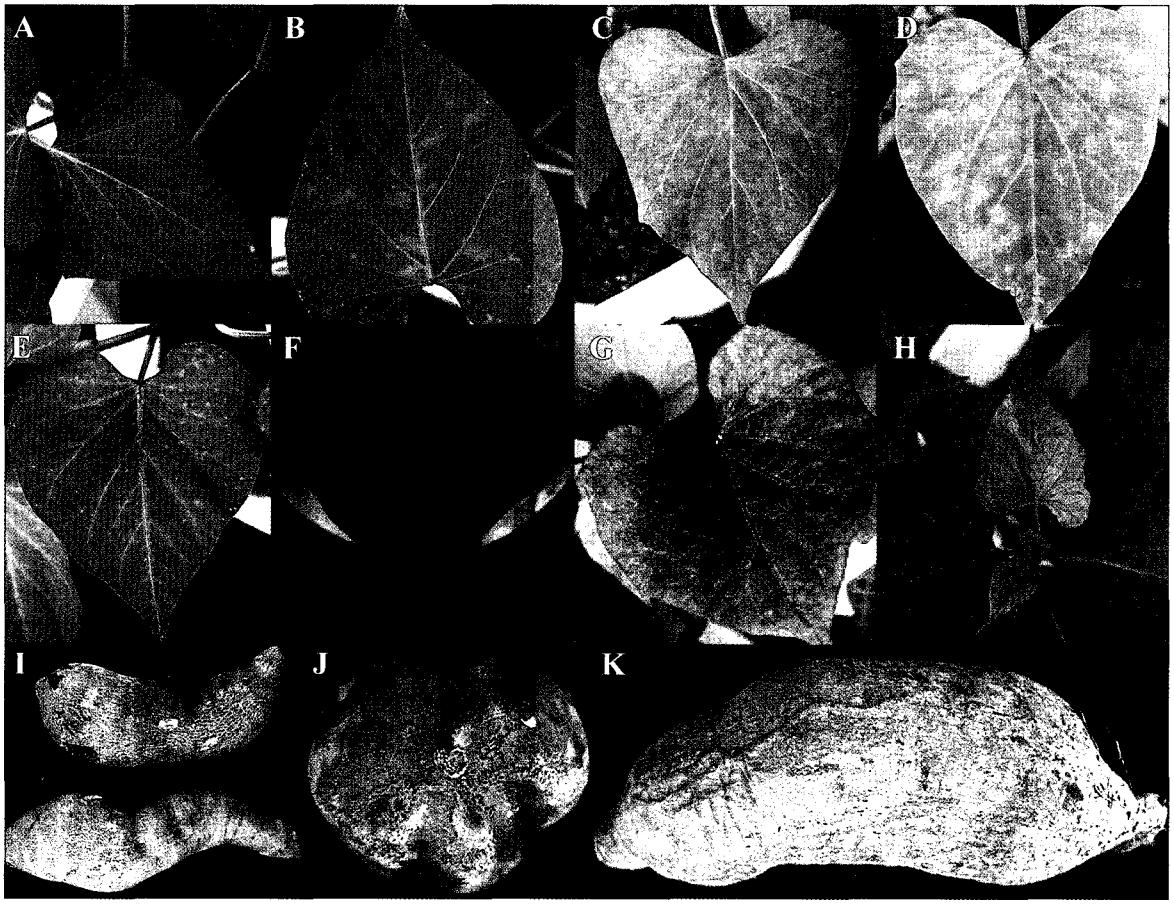

Fig. 1. Virus-infected sweet potato plants showing chlorotic local lesion (A and B), mosaic and mottle (C and D), necrotic local lesion (E, F and G), mosaic and fern leaf symptoms in leaves (H), string (I and G) and russet crack symptoms in roots (H).

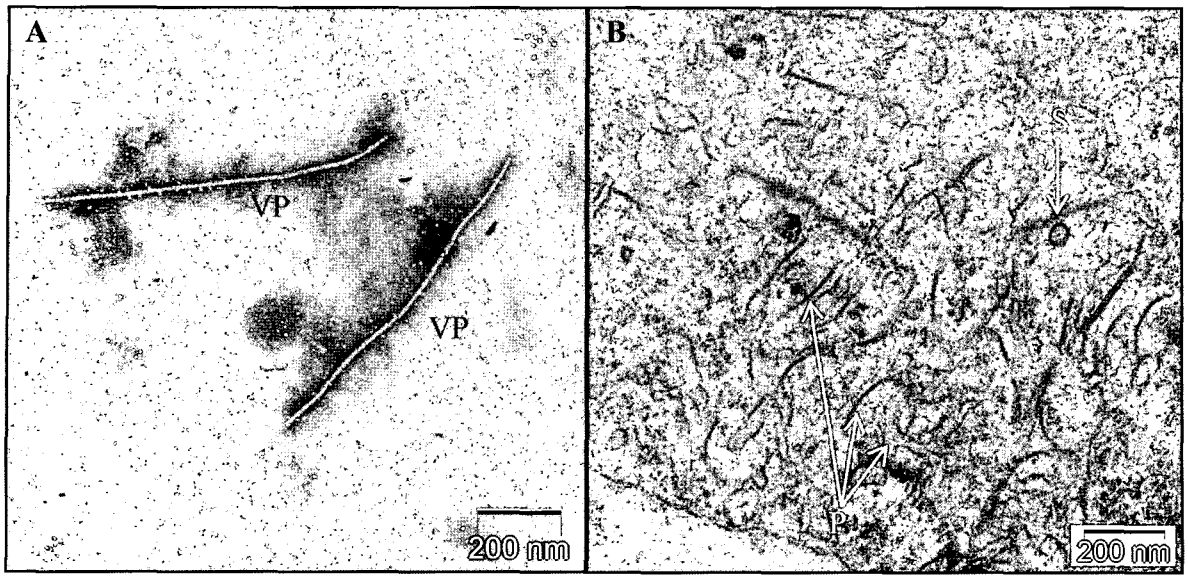

Fig. 2. Electron micrograph (A and B) of virus particles in crude sap and of typical cytoplasmic inclusion bodies like pinwheels and scrolls aggregates in cells of *Ipomoea batatas* L., respectively. VP, S, and P represent virus particles, scrolls, and pinwheels, respectively.

Table 6. Detection of viral diseases by RT-PCR in sweet potato samples depending on areas

Area	No. of samples tested	F	G	F+G	F+G+L	F+G+LC	Unknown
Yeoju	19	5	4	2		1	7
Iksan	11	7	1		1		2
Nonsan	8	2	2	1		1	2
Chungju	6	2	1	2		1	
Jecheon	5	3	1				1
Yeongam	2			1			1
Haenam	5	3	1	1			
Mokpo	4	1	1	1			1
Muan	8	6	1				1
Naju	1	1					
Jinju	1	1					
Sancheong	1	1					
Yokjido	1	1					
Yeongju	9	2	2	3			2
Jeju	1		1				
Total (%)	82	35 (42.7)	15 (18.3)	11 (13.4)	1 (1.2)	3 (3.7)	17 (20.7)

[a]F, *Sweet potato feathery mottle virus*; G, *Sweet potato G virus*; L, *Sweet potato latent virus*; LC, *Sweet potato leaf curl virus*.

areas. Additionally, total of 83 root seedling samples from different cultivars were collected and analyzed. Thirty-four % of the samples were infected with SPFMV, SPGV with 14%, SPFMV and SPGV with 7%, SPFMV and SwPLV with 1%, SPFMV and SPLCV with 1%, SPFMV, SPGV and SwPLV with 1%, SPFMV, SPGV and SPLCV with 4%, SPFMV, SPGV, SwPLV and SPLCV with 2% while 29% of the samples were infected with unknown viruses. In conclusion, 40.0% of the 179 samples were infected with SPFMV, 16% SPGV, 11% SPFMV and SPGV, 0.6% SPFMV and SwPLV, 0.6% SPFMV and SPLCV, 1.1% SPFMV, SPGV and SwPLV, 3.4% SPFMV, SPGV and SPLCV, 1.1% SPFMV, SPGV, SwPLV and SPLCV and 27% unknown viruses. Altogether these results indicated that the major viruses infecting sweet potato in Korea were SPFMV and SPGV.

Table 7. RT-PCR detection of different sweet potato cultivars in the field

Cultivar	No. of samples tested	F	G	F+G	F+L	F+LC	F+G+L	F+G+LC	F+G+L+LC	Unknown
Sincheonmi	3	1		1						1
Geumsi	2	2								
Geonmi	19	4	5	3				1	1	5
Yeonmi	6	2		1						3
Hayanmi	7	3	2			1				1
Jinhongmi	9	3	3	1				2		
Sinjami	12	2								10
Borami	4	3			1					
Gogeonmi	2									2
Juhwangmi	2	1								1
Yulmi	5	1	1				1		1	1
Sinhwangmi	6	2								4
Jami	4	3	1							
Singeonmi	1									1
Sinyulmi	1	1								
Total (%)	83	28 (34)	12 (14)	6 (7)	1 (1)	1 (1)	1 (1)	3 (4)	2 (2)	29 (35)

[a] F, *Sweet potato feathery mottle virus*; G, *Sweet potato G virus*; L, *Sweet potato latent virus*; LC, *Sweet potato leaf curl virus*.

Discussion

A survey of sweet potato virus disease incidence was carried out in sweet potato seed boxes as well as in samples obtained from various fields. Virus infection rates were ranged 5-100% and 100% at seed boxes and in field, respectively. Our results indicated that the virus transmission is probably initiated from seed boxes. Once introduced onto each sweet potato field, each virus might be further transmitted by aphids or whitefly in the field. The incidence and severity of the diseases were shown not to have any relationship in cultivars and area grown (Table 1). Despite the high incidence, severity was very low as there was no marked reduction in yield. This implies that even when infection pressure is high, farmers could still get high yields and that the sweet potato cultivars cultivated in most fields were probably tolerant to the virus. When sweet potato plants were co-infected with SPCSV and SPFMV, it causes the synergistic response on sweet potato and thus cause yield reduction, which also defined as sweet potato virus disease (SPVD).

In seed boxes, disease incidence of root was much different depending on sweet potato cultivars and areas, whereas internal symptoms of roots did not show any relationship among cultivars and area grown as the same leaf samples. Viral diseases were severer in Yeoju than in Mokpo Experiment Station and infection rate was much different depending on sweet potato cultivars. This indicates that as the relationships between soil condition and virus a lower quality could be produced. Kim et al. (1998) reported that SPFMV-Korean isolates cause systemic foliar symptoms of their internal cork symptoms in fleshy roots, but we could not observe the specific internal symptoms of roots. In only cultiva Hayanmi, string symptoms were observed in Yeoju while russet crack in Mokpo Experiment Station. To further characterize the relationship between symptoms and time of infection, if any, more experiments are needed.

We could isolate SPFMV from virus-infected samples of sweet potato, but other viruses were not infected by sap inoculation. Although Colinet et al. (1997) and Hollings et al. (1976b) reported that SwPLV and SPMMV could easily cause chlorotic spot symptoms on *C. quinoa*; SwPLV confirmed by EM and RT-PCR did not infect the indicator plants. This could possibly be due to differences in genetic diversities. Hence further studies involving additional SwPLV isolates representing different agro-climatic regions of the country are required in order to map the existence of various distinct SwPLV isolates. Based on electron microscopy, NCM-ELISA and RT-PCR, most of the 137 samples were consistent with both electron microscopy and RT-PCR, whereas the samples could not be consistent by NCM-ELISA. This indicates that false positive or negative could be possibly involved this method (data not shown).

In this paper, as the results of a survey of sweet potato virus, we showed that the major viruses of sweet potato virus in Korea were SPFMV and SPGV. Additionally, we report the infection of sweet potato by SPGV and SPLCV that hitherto had never been found in Korea. This has been

confirmed by results of electron microscopy and RT-PCR in which many sweet potatoes in the fields were occasionally infected with more than one virus species. However, contrary to earlier reports (Jeong et al. 2001), SPCSV was not detected in samples from Korea. The absence of SPCSV in these samples could have been due to the fact that samples were not representative of all growing sweet potato areas. Further experiments are needed to determine the relationship, if any, between severity of infection and the possible yield reduction, and to explain the reason of false positive or negative by the tested antisera. This would help in developing an effective strategy for managing the viruses.

Acknowledgements

We are sincerely grateful to Mr. J.S. Lee for assistance with the electron microscope work. This research was supported in part by grants from the Rural Development Administration and from the BioGreen21 Program, Rural Development Administration.

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