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First Report of *Sweet potato latent virus* and *Sweet potato chlorotic stunt virus* Isolated from Sweet Potato in Korea

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Infected sweet potato (*Ipomoea batatas*) showing symptoms of sunken veins, stunting, mosaic, and mottling were collected from Gimje, Gochang, Iksan, and Haenam provinces in Korea. Electron microscopic (EM) observation of the infected tissue revealed rod and filamentous rod type virus particles of various lengths. Western blot analysis of the protein samples extracted from infected sweet potato and partially purified virus identified the isolates as *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato latent virus* (SwPLV), and *Sweet potato chlorotic stunt virus* (SPCSV). Sweet potatoes were occasionally infected with more than one of these viruses. This is the first report of SwPLV and SPCSV in Korea.

Keywords : EM, sweet potato, SPCSV, SPFMV, SwPLV, Western blot.

Sweet potato (*Ipomoea batatas* (L.) Lam) is the seventh most produced food crop in the world and the fourth in the developing countries (World Bank, 1995). Since it is relatively easy to grow, has low level of disease incidences and is highly productive, sweet potato is considered as mainly a subsistence and food security crop grown for family use by millions of people, especially in sub-Saharan Africa. In addition, sweet potatos ability to grow in poor soils makes it an especially good substitute crop. China has the largest production of sweet potato in the world (World Bank, 1995). Although sweet potato cultivation and its economic impact on farmers are not very significant, many farmers still cultivate this crop when they have available fields, especially in southern provinces in Korea.

Sweet potato chlorotic stunt virus (SPCSV, genus *Crinivirus*, family *Closteroviridae*) (Wisler et al., 1998), also known as *Sweet potato sunken vein virus* (Cohen et al., 1992) and Sweet potato virus disease-associated closterovirus (Winter et al., 1992), causes severe symptoms generally referred to as Sweet potato virus disease (SPVD) in mixed infection with *Sweet potato feathery mottle virus* (SPFMV) (Gibson et al., 1998; Karyeija et al., 1998; Karyeija et al., 2000). SPVD is the main disease of sweet potato (Geddes, 1990), characterized by small, distorted leaves which are often narrow (strap-like) and crinkled with chlorotic mosaic or vein clearing and stunting (Gibson et al., 1998; Karyeija et al., 1998; Karyeija et al., 2000). The incidence of SPVD has been first reported in Congo and East Africa (Sheffield, 1953). The disease has also been reported to cause up to 80% yield reduction (Hahn, 1979). *Sweet potato latent virus* (SwPLV) is more similar morphologically to typical potyviruses than either SPFMV or *Sweet potato mild mottle virus* (SPMMV), but neither aphids nor whiteflies have been shown to transmit the virus. SwPLV has been reported in Taiwan and China and has a wider host range than SPFMV (Chung et al., 1986).

Although more than 14 virus diseases of sweet potato have been reported throughout the world (Brunt et al., 1996), SPFMV was the only one reported in Korea (Park et al., 1995; Park et al., 1994; Ryu and Choi, 2002). Nucleotide sequences of the coat protein (CP) and 3'-noncoding region were analyzed with other strains of SPFMV (Kim et al., 1998; Ryu et al., 1995; Ryu et al., 1998). This paper reports two new viruses, SwPLV and SPCSV, isolated from sweet potato in Korea. The virus isolates were collected from infected sweet potato showing sunken veins, stunting, mosaic, and mottling symptoms (Fig. 1) from Gimje, Gochang, Iksan, and Haenam provinces in Korea.

Each sweet potato sample was negatively stained using leaf dip method with 2% uranyl acetate, pH 4.3 (Hitchborn and Hills, 1965), and was examined in a JEM 1010 (JEOL, Japan) electron microscope. Rod and flexuous rod-shaped virus-like particles were consistently found in the leaf midrib tissue preparations of infected sweet potato cultivars (Fig. 2). Viruses of various lengths (700 to 1,900 nm) were observed in very low numbers ($\leq$ 1/grid opening) (Table 1).

The virus was purified from each sweet potato tissue as described previously (Cohen et al., 1988) with little modification. Apical and middle leaves were harvested and

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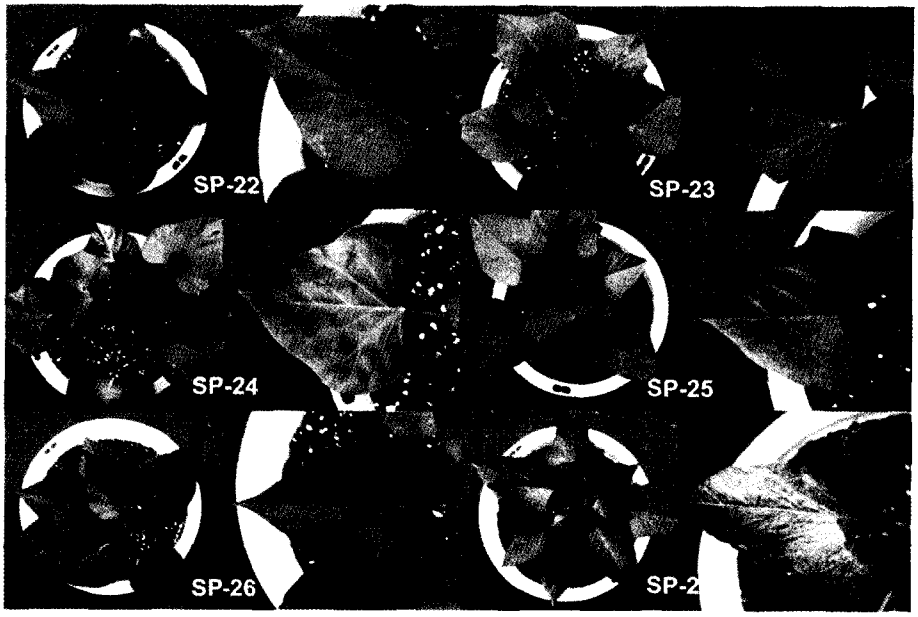

Fig. 1. Infected sweet potato leaves obtained from various fields in southern provinces in Korea. Systemic chlorotic spots on SP-22 and SP-25, ringspots on SP-23, rugose mosaic and vein chlorosis on SP-24, mild mosaic on SP-26, and severe mosaic and mottling on SP-27 were observed.

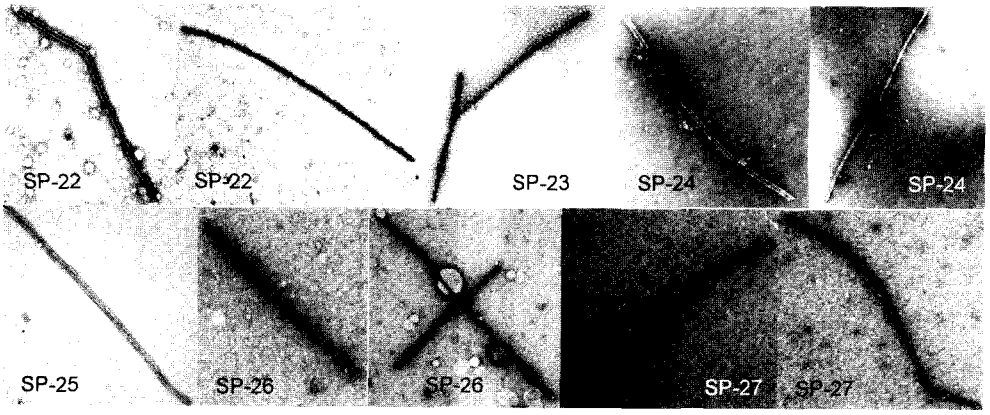

Fig. 2. EM observation of virus particles from sweet potato samples SP-22 to SP-27.

Table 1. Type of virus particles isolated from sweet potatoes using electron microscope.

Sweet potato isolate	Virus particle	
	Type	Length (nm)
SP-22	Rod, flexuous rod	700, 1000 & 1500
SP-23	Rod, flexuous rod	1200-1500
SP-24	Flexuous rod	900 & 1900
SP-25	Rod	1000
SP-26	Rod, flexuous rod	1100, 1200 & 1900
SP-27	Rod, flexuous rod	700, 900 & 1500

frozen at -80°C for at least 24 h. Leaves were homogenized with a grinder in 4 ml grinding buffer (0.5 M borate buffer, 0.01 M EDTA, pH 8.0, 1 ml chloroform, 1 ml carbon tetrachloride and 1 μl thio glycolic acid) per gram of frozen leaves and centrifuged at 12,000 rpm (SUPRA 22K, Hanil Science Industrial Co., LTD. Incheon, Korea) for 10 minutes. The upper aqueous phase was collected and centrifuged at 28,000 rpm in a TFT 70.38 rotor (CENTRIKON T-1180, KONTRON Instruments, Italy) for 3 h. Pellets were subjected overnight in re-suspension buffer (50 mM borate, 1 mM EDTA, pH 8.0, 1% Triton X-100), an equal volume of chloroform added, and were centrifuged at 12,000 rpm for 5 minutes. The upper aqueous phase was collected and separated through a cesium chloride gradient (10 to 40%) centrifuged at 35,000 rpm for 3 h. The collected fractions were then ultracentrifuged at 35,000 rpm for 3 h and subjected overnight in re-suspension buffer.

Purified viruses were separated in 12.5% polyacrylamide gel and were stained with Coomassie brilliant blue (data not

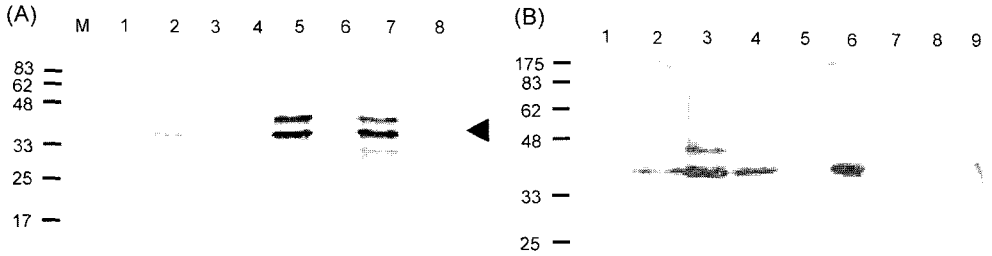

Fig. 3. Western blot analysis of total proteins or partially purified virus. Proteins were separated on 12.5% SDS-PAGE and transferred to nitrocellulose membranes. In panel A, approximately 10 g of total proteins were separated and blotted against Sweetpotato feathery mottle virus antiserum. Lanes 1 & 8, healthy; lanes 2-7; and field samples SP-22 to SP-27. Panel B shows blotting results obtained using Sweetpotato chlorotic stunt virus antiserum. Lanes 1-5 and 6-9 represents total proteins and partially purified virus, respectively. Lane 1, healthy; lanes 2 & 6, SP-22; lanes 3 & 7, SP-23; lanes 4 & 8, SP-25; lanes 5 & 9, SP-27. Sizes of standard protein molecular mass markers (lane M) are also indicated.

shown). Appropriate concentrations of the crude leaf extracts and the purified virus preparations were used in the Western blot analysis as previously described (Towbin et al., 1979). After blocking membranes with 5% skim milk for 1 h, each membrane was reacted with rabbit antisera against SPFMV, SwPLV, SPCSV, SPMMV, *Sweet potato chlorotic flecks virus* (SPCFV) (Fuentes and Salazar, 1992), *Sweet potato mild speckling virus* (SPMSV), *Sweet potato caulimo-like virus* (SPCaLV), and new flexuous virus (C-6) purchased from the International Potato Center, followed by HRP linked goat anti-rabbit IgG (New England BioLabs Inc., Beverly, USA) at a dilution of 1:5,000.

The SPFMV antiserum reacted positively with isolates SP-22, SP-25, and SP-27 (Fig. 3, panel A). The SwPLV antiserum reacted with isolates SP-25 and SP-27 (data not shown) while the SPCSV antiserum showed positive reaction with isolates SP-23 and SP-27 (Fig. 3, panel B) showing bands at similar molecular mass of CPs of SwPLV and SPCSV previously reported (Chung et al., 1986; Francki et al., 1985). The SPMSV, SPMMV, SPCFV, C-6 and SPCaLV antisera, however, did not react with any of the isolates tested in Western blots (Table 2).

Table 2. Western blot result of viruses isolated from sweet potatoes in Korea

Tested Serum	Sweet potato samples					
	SP-22	SP-23	SP-24	SP-25	SP-26	SP-27
SPFMV	+[a]	–	–	+	–	+
SPMMV	–	–	–	–	–	–
SwPLV	–	–	–	+	–	+
SPCSV	–	+	–	–	–	+
SPCFV	–	–	–	–	–	–
SPMSV	–	–	–	–	–	–
SPCaLV	–	–	–	–	–	–
C-6	–	–	–	–	–	–

[a]Symbols (+) and (–) indicate positive and negative reactions against each antiserum tested.

These results suggest that many sweet potatoes in the fields were occasionally infected with more than one virus species. Sweet potato sample SP-25 showed positive reaction to SPFMV and SwPLV antisera. Similarly, SP-27 reacted positively with SPFMV, SwPLV, and SPCSV IgG. Severe diseases occurred when the virus was mixed with SPFMV. In contrast, sweet potato samples SP-22 and SP-23 reacted positively only to SPFMV and SPCSV antisera, respectively. Although no positive reaction with any of the antisera tested for SP-24 and SP-26 in the Western blot analysis was detected in this study, these samples showed symptom developments typical of virus infection and contained virus-like particles by dip method using EM. Therefore, it is possible that these samples along with others may be infected with yet an unidentified sweet potato virus(es). However, a more detailed study on the economic impact of virus diseases caused by these viruses in Korea is necessary. In this regard, it is worthy to note that a novel transgenic approach to controlling SPVD has been conducted recently using a foreign cysteine proteinase inhibitor (Cipriani et al., 2000). Improved resistance against challenge inoculation was observed in transgenic lines expressing cysteine proteinase inhibitors.

This is the first report of natural infection of *I. batatas* by SwPLV and SPCSV in Korea. Further biological and molecular studies, however, will be required for better characterization of each virus. In addition, the virus infection of other sweet potato including SP-24 and SP-26 that did not react with any of the tested antisera has to be investigated further.

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References

Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L. and Zucher, E. J. 1996. Plant viruses online: Descriptions and lists from the VIDE database Version: 20th August 1996. *URL http://biology.anu.edu.au/Groups/MES/vide/.*

Chung, M. L., Hsu, Y. H., Chen, M. J. and Chiu, R. J. 1986. Plant virus diseases of horticultural crops in the tropics and subtropics. In: Virus diseases of sweetpotato in Taiwan. Vol. 33. Food and Fertilizer Technology Center for the Asian and Pacific Region, Taipei, Republic of China.

Cipriani, G., Fuentes, S., Bello, V., Salazar, L. F., Ghislain, M. and Zhang, D. P. 2000. Transgenic expression of rice cysteine proteinase inhibitors for the development of resistance against *Sweet potato feathery mottle virus*. CIP Program Report. International Potato Center.

Cohen, J., Frank, A., vetten, H. J., Lesemann, D. E. and Loebestein, G. 1992. Purification and properties of closterovirus-like particles associated with a whitefly transmitted disease of sweet potato. *Ann. Appl. Biol.* 121:257-268.

Cohen, J., Salomon, R. and Loebenstein, G. 1988. An improved method for purification of sweet potato feathery mottle virus directly from sweet potato. *Phytopathology* 78:809-811.

Francki, R. I. B., Milne, R. G. and Hatta, T. 1985. Atlas of Plant Viruses, Vol. 2. CRC Press Inc., Boca Raton, Florida.

Fuentes, S. and Salazar, L. F. 1992. Identification of a new sweet potato virus. *Fitopatologia* 27:50.

Geddes, A. M. W. 1990. The relative importance of crop. *Natural Res. Inst. Bull.* No. 36

Gibson, R. W., Mpembe, I., Alicai, T., Carey, E. E., Mwanga, R. O. M., Seal, S. E. and Vetten, H. J. 1998. Symptoms, etiology and serological analysis of sweet potato virus disease in Uganda. *Plant Pathol.* 47:95-102.

Hahn, S. K. 1979. Effect of virus (SPDV) on growth and yield of sweet potato. *Exp. Agric.* 15:253-256.

Hitchborn, J. H. and Hills, G. H. 1965. The use of negative staining in electron microscopic examination of plant viruses in crude estracts. *Virology* 27:528-540.

Karyeija, R. F., Gibson, R. W. and Valkonen, J. P. T. 1998. The significance of *Sweet potato feathery mottle virus* in subsistence sweet potato production in Africa. *Plant Dis.* 82:4-15.

Karyeija, R. F., Kreuze, J. F., Gibson, R. W. and Valkonen, J. P. T. 2000. Synergistic interactions of a potyvirus and a phloem-limited crinivirus in sweet potato plants. *Virology* 269:26-36.

Kim, S. J., Ryu, K. H., Choi, J. K. and Park, W. M. 1998. Sequence analysis of coat protein and 3'-noncoding region for Korean and other strains of *Sweet potato feathery mottle virus*. *Mol. Cells* 6:777-785.

Park, W. M., Kim, S. J., Ryu, K. H. and Chin, M. S. 1995. Characterization and detection of *Sweet potato feathery mottle virus* (SPFMV) from sweet potato by RT-PCR with the SPFMV specific primers and restriction analysis. *Korean J. Plant Pathol.* 11:396-397.

Park, W. M., Ryu, K. H., Kim, S. J. and Chin, M. S. 1994. Some properties of a potyvirus isolated from sweet potato in Korea. *Korean J. Plant Pathol.* 10:358-359.

Ryu, K. H. and Choi, S. H. 2002. Molecular detection and analysis of *Sweet potato feathery mottle virus* from root and leaf tissues of cultivated sweet potato plants. *Plant Pathol. J.* 18:12-17.

Ryu, K. H., Choi, J. K. and Park, W. M. 1995. Analysis of the coat protein gene of *Odontoglossum ringspot virus*-Cy (Korean isolate). *Mol. Cells* 5:181-186.

Ryu, K. H., Kim, S. J. and Park, W. M. 1998. Nucleotide sequence analysis of coat protein genes of two Korean isolates of *Sweet potato feathery mottle virus*. *Arch. Virol.* 143:557-562.

Sheffield, F. M. L. 1953. Virus diseases of sweet potato in parts of Africa. *Empire J. Exp. Agric.* 21:184-189.

Towbin, H., Staehelin, T. and Gordon, J. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76:4350-4354.

Winter, S., Purac, A., Leggett, F., Frison, E. A., Rossel, H. W. and Hamilton, R. I. 1992. Partial characterization and molecular cloning of a Closterovirus from sweet potato infected with sweet potato virus disease complex from Nigeria. *Phytopathology* 82:869-875.

Wisler, G. C., Duffus, J. E., Liu, H. Y. and Li, R. H. 1998. Ecology and epidermiology of whitefly-transmitted closteroviruses. *Plant Dis.* 82:270-280.

World Bank. 1995. World Development Report.