*Plant Pathol. J. 18(1) : 12-17 (2002)*

The Plant Pathology Journal


# Molecular Detection and Analysis of *Sweet potato feathery mottle virus* from Root and Leaf Tissues of Cultivated Sweet Potato Plants

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*(Received on January 10, 2002)*

**For the molecular detection of *Sweet potato feathery mottle virus* (SPFMV) from diseased sweet potato plants, reverse transcription and polymerase chain reaction (RT-PCR) was performed with the use of a set of virus-specific primers to amplify an 816 bp product. The viral coat protein gene was selected for the design of the primers. No PCR product was amplified when *Turnip mosaic virus*, *Potato virus Y*, or *Cucumber mosaic virus* were used as template in RT-PCR with the SPFMV-specific primers. The lowest concentration of template viral RNA required for detection was 10 fg. The virus was rapidly detected from total nucleic acids of leaves and roots from the virus-infected sweet potato plants as well as from the purified viral RNA by the RT-PCR. Twenty-four sweet potato samples were selected and analyzed by RT-PCR and restriction fragment length polymorphism (RFLP). RFLP analysis of the PCR products showed three restriction patterns, which resulted in some point mutations suggesting the existence of quasi-species for the virus in the infected sweet potato plants.**

***Keywords*** : detection, *Potyvirus*, RT-PCR, sweet potato, *Sweet potato feathery mottle virus.*

In Korea, sweet potato (*Ipomoea batatas* (L.) Lam.) is cultivated for industrial raw materials, such as for alcohol fermentation, and as side dish and snack. Virus disease of sweet potato has reduced the production and affected quality of the crop worldwide. More than 10 viruses have been isolated from sweet potato (Abad et al., 1992; Colinet et al., 1994; Colinet et al., 1996; Fuentes et al., 1996; Moyer and Salazar, 1989). Surveys have shown that *Sweet potato feathery mottle virus* (SPFMV) is the most prevalent and widespread pathogen in many parts of the world where sweet potato plants are cultivated (Moyer and Salazar, 1989; Nishiguchi et al., 1995; Querci et al., 1992; Usugi et al., 1991). SPFMV is a species of the genus *Potyvirus* in the family *Potyviridae* (Abad et al., 1992; Moyer and Salazar, 1989; Ward and Shukla, 1991). It is easily transmitted by some aphids in a non-persistent manner as well as through mechanical inoculation (Moyer and Salazar, 1989; Ward and Shukla, 1991). The SPFMV genome is a single-stranded, positive sense RNA, and has a poly (A) tract at its 3' terminus and a genome-linked protein (VPg) at its 5' terminus (Sakai et al., 1997). The genome is expressed initially as a large polyprotein precursor that is processed into functional proteins by viral-encoded proteases (Sakai et al., 1997; Ward and Shukla, 1991).

SPFMV generally induces systemic symptoms of irregular chlorotic patterns in infected leaves, known as feathering, associated with faint or distinct ring spots with purple-pigmented borders (Moyer and Salazar, 1989). Several strains of SPFMV have been isolated and some of their partial or complete nucleotide sequences have been reported (Abad et al., 1992; Kim et al., 1998; Kreuze et al., 2000; Nishiguchi et al., 1995; Sakai et al., 1997). In 1992, diseased sweet potato plants were found in several cultivated fields for the first time in Korea, and their causative virus was identified as SPFMV (Kim et al., 1998). Detection of SPFMV is mainly based upon symptomatology on sweet potato, particle morphology, and serological assay. However, members of the genus *Potyvirus* have similar virus particle morphology and are closely related serologically with one another (Ward and Shukla, 1991). Reverse transcription and polymerase chain reaction (RT-PCR) has been widely used in the diagnosis of plant viruses with RNA genomes. Nishiguchi et al. (1995) reported RT-PCR detection for SPFMV. However, the primers were specific only to a severe strain of the virus. Variability of SPFMV has not been investigated and useful detection method for the virus has not been studied in Korea.

In this study, RT-PCR techniques for the detection and analysis of strains of SPFMV have been developed in order to detect virus-infected sources of sweet potato for stable breeding program of the crop. RT-PCR with the primers was performed to detect the virus in harvested root tissues

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as well as leaves of cultivated sweet potato plants.

## Materials and Methods

**Virus source, samples, and viral RNA extraction.** SPFMV-K1 originally isolated from a diseased sweet potato plant (*Ipomoea batatas* cv. Kwandong 95) was obtained from the Plant Virus GenBank (PVGB) and used as a source of virus (Kim et al., 1998). The virus was propagated in sweet potato plants by mechanical inoculation and purified by polyethylene glycol precipitation and differential centrifugation methods (Kim et al., 1998). Viral RNA was extracted from purified virion particles by SDS-proteinase K/phenol extraction followed by ethanol precipitation as described previously (Ryu and Park, 1995). Leaves and roots of harvested sweet potato plants were used as samples for detection of the virus. Two potyviruses, *Turnip mosaic virus* (TuMV) and *Potato virus Y* (PVY), and a cucumovirus (*Cucumber mosaic virus*; CMV) were also obtained from the PVGB and used as negative controls.

**Extraction of total nucleic acids for RT-PCR.** Crude total nucleic acids from the leaf and root parts of sweet potato plants were extracted by using the SDS-proteinase K method (Ryu and Park, 1995). RNA was extracted from 0.1 g of each tissue and dis-

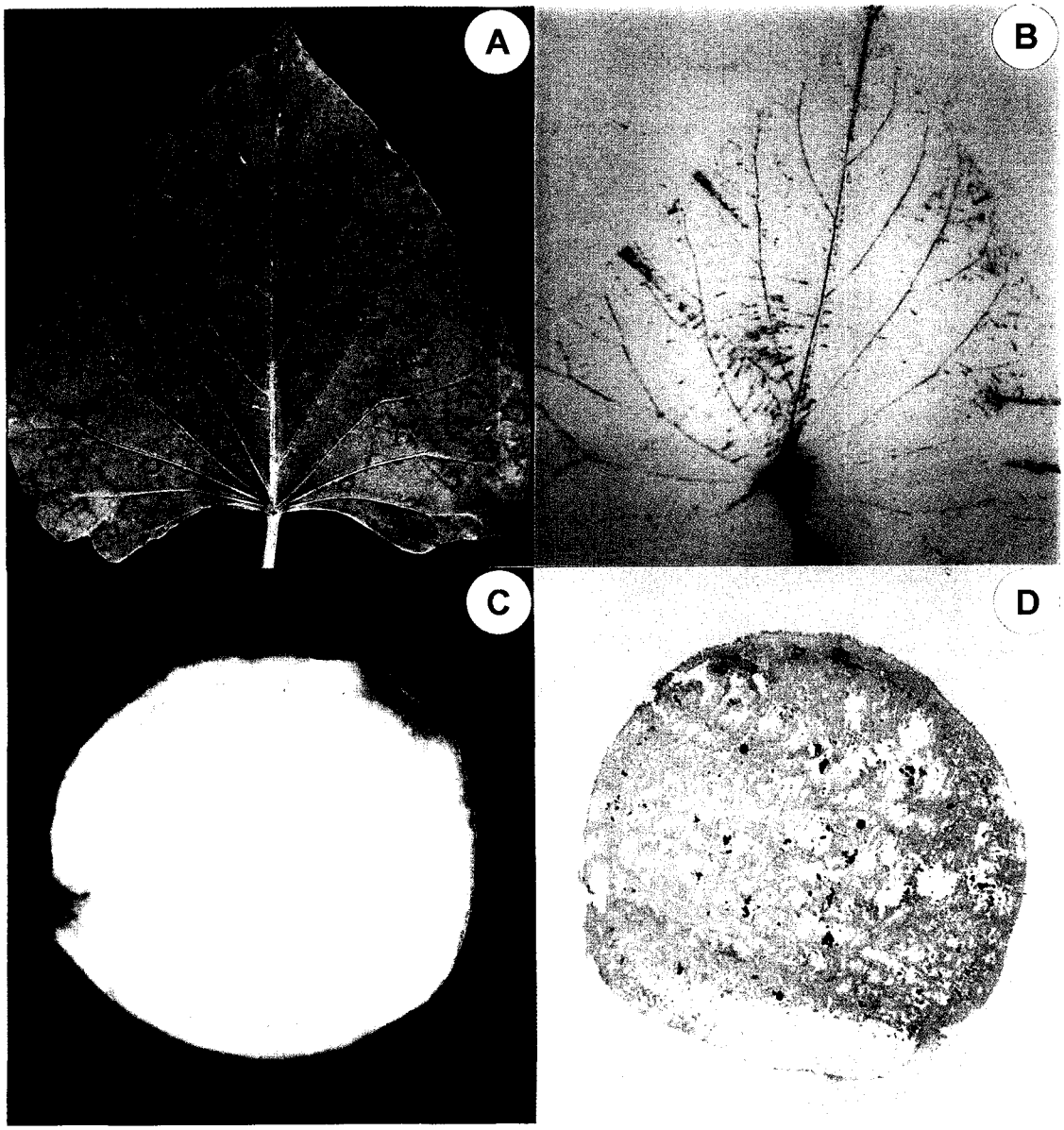


**Fig. 1.** Leaf and root symptoms of SPFMV from diseased sweet potato plant (**A**, **C**) and tissue blot assay for distribution of SPFMV in the leaf and root parts (**B**, **D**). The blots were immuno-probed with SPFMV antiserum and antibody binding was color developed using a secondary antibody conjugated to alkaline phosphatase and its substrates.

solved in 20 μl of DEPC-treated water and stored at -70°C until use.

**Tissue-press immuno-blot for detection of SPFMV.** The leaf and root were removed from sweet potato plant showing the typical symptom of SPFMV. Carborundum (350 mesh) dusted tissues were then placed onto dried nitrocellulose membrane (0.45 μm). Upper and lower surfaces of the tissues and membrane were covered with two pieces of Whatman 3 MM paper (Kim et al., 1998). This sandwich was pressed for 1 minute, and the membrane was removed and allowed to air dry. The membrane was incubated in 5% skim milk for 1 h for blocking. The primary antiserum for SPFMV was used at a final dilution of 1:1,000 (v/v) for 1 h at room temperature. The goat anti-rabbit secondary antibody conjugated with alkaline phosphatase (Promega) was used at a dilution of 1:7,500 (v/v) for 30 minutes at room temperature. The color reaction was carried out with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate.

**Design of primers for RT-PCR.** The upstream part near the C-terminal and the downstream region N-terminal parts of the viral coat protein gene were selected for designing the SPFMV-specific primers based on the nucleotide sequences of the known strains of SPFMV (Abad et al., 1992; Kim et al., 1998; Nishiguchi et al., 1995; Sakai et al., 1997). The upstream primer SPFP2 (5'-CAACCCGCAACCATTCCAG-3'; EMBL accession AJ001125) corresponded to nucleotides 145 to 163 of the SPFMV RNA (Kim et al., 1998). The downstream primer SPFCP3 (5'-GCAGAGGATGTCCTATTGC-3'; EMBL accession AJ001126) was complementary to bases 942 to 960 of the viral RNA. The set of primers was used to amplify a 816 bp PCR product (Fig. 2).

**RT-PCR detection of SPFMV.** Reverse transcription (RT) was performed at 42°C for 1 h in 10 mM Tris-HCl buffer (pH 8.3) containing sample RNA, 2.5 mM $MgCl_2$, 50 mM KCl, 1.0 mM dNTPs, 50 pmol SPFCP3 primer (EMBL AJ001126), 1 unit of RNasin (Promega), and 2.5 units of AMV reverse transcriptase. PCR volume was 50 μl containing 1.0 mM dNTPs, 50 pmol of SPFCP3 and SPFP2 primers, 0.5 unit of Taq DNA polymerase (Perkin Elmer), and 10.0 mM Tris-HCl (pH 8.3). PCR was carried out in a DNA thermal cycler (Perkin Elmer model 480). Cycle conditions were 94°C for 1 minute, 50°C for 40 seconds, and 72°C for 1 minute for 30 cycles. Serial dilutions of purified SPFMV RNA and crude total nucleic acids were applied to determine the sensitivity for detecting the virus by the RT-PCR. The PCR products were fractionated by agarose gel electrophoresis (Sambrook et al., 1989).

**Restriction digestion of RT-PCR products and electrophoresis.** Ten μl of the 50 μl RT-PCR products for SPFMV-infected sweet potato plants were directly digested with restriction enzymes (*Hae*III, *Hin*dIII, *Nde*I, *Sal*I, and *Sty*I) at 37°C for 1.5 h. The aliquots of the reactions were analyzed by electrophoresis with 1.2% agarose gel in TAE buffer (20 mM Tris-acetate, 0.5 mM EDTA, pH 8.3).

**Cloning and nucleotide sequencing.** The PCR products were cloned into the plasmid pGEM-T-Easy vector (Promega). Recombinant plasmids were introduced into *Escherichia coli* strain JM109 (Promega). Nucleotide sequence was conducted in both directions with T7 and M13 reverse primers by the dideoxy-nucleotide chain termination method (Sanger et al., 1977) using the Model 377 DNA Sequencer (ABI).

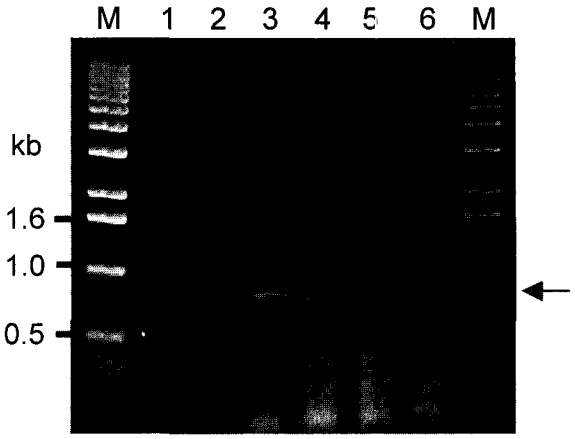


**Fig. 2.** Determination of optimum $MgCl_2$ concentration for detection of SPFMV by RT-PCR. Lane M, 1 kb DNA ladder; Lane 1, 0.0 mM; Lane 2, 1.5 mM; Lane 3, 2.5 mM; Lane 4, 5.0 mM; Lane 5, 7.5 mM; and lane 6, 10.0 mM $MgCl_2$.

## Results and Discussion

**Tissue-press immuno-blot for detection of SPFMV.** The distribution of SPFMV in the diseased sweet potato was examined by using the tissue-press blot method. The virus was detected in root as well as leaf tissues (Fig. 1). Tissues from the diseased sweet potato reacted positively with SPFMV antiserum and the virus highly accumulated in the root tissue (Figs. 1B and 1D).

**Optimization of the RT-PCR.** The designed primers specific to SPFMV successfully generated an 816 bp PCR product from the viral RNA sample. The optimum concentration of the $MgCl_2$ to detect the virus was 2.5 mM (Fig. 2). With none or higher than 2.5 mM $MgCl_2$, no visible or weak DNA product was generated.

**Sensitivity of RT-PCR detection.** The lowest concentration of template viral RNA required for the detection of SPFMV with the designed primers was 10 fg (Fig. 3A). Positive results were obtained with 1:7,500 (w/v) dilutions of the total nucleic acids from leaf samples (Fig. 3B). However, no PCR product was generated between 1/2 and 1/50 dilutions of the total nucleic acids from leaf tissue. More than 1/200 (w/v) dilution of total nucleic acids from root tissues requires successful detection of the virus (data not shown). This was repeatedly observed during independent tests. The specificity of the primers was verified using other potyviral RNAs and unrelated viral RNA. No PCR product was amplified when two potyviruses (TuMV and PVY) and CMV were used as template in RT-PCR with the SPFMV-specific primers (Fig. 4A). The two potyviruses

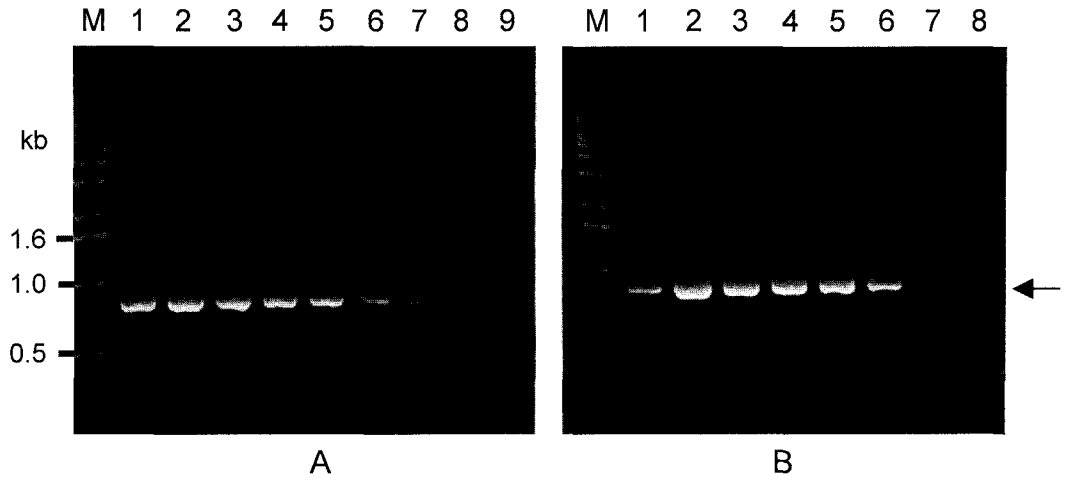


**Fig. 3.** Determinations of the detection limits of purified SPFMV RNA (**A**) and total nucleic acids of the virus-infected sweet potato leaf (**B**). Photo (A): Lane M, 1kb DNA ladder; Lane 1, 500 pg; Lane 2, 100 pg; Lane 3, 10 pg; Lane 4, 1 pg; Lane 5, 500 fg; Lane 6, 100 fg; Lane 7, 10 fg; Lane 8, 1 fg; and Lane 9, 0.1 fg of SPFMV RNA. Photo (B): Lane M, 1 kb DNA ladder; Lane 1, 1/100; Lane 2, 1/500; Lane 3, 1/1,000; Lane 4, 1/2,500; Lane 5, 1/5,000; Lane 6, 1/7,500; Lane 7, 1/10,000; and Lane 8, 1/15,000 (w/v) dilutions of the total nucleic acids. Arrow indicates the position of RT-PCR products for SPFMV detection.

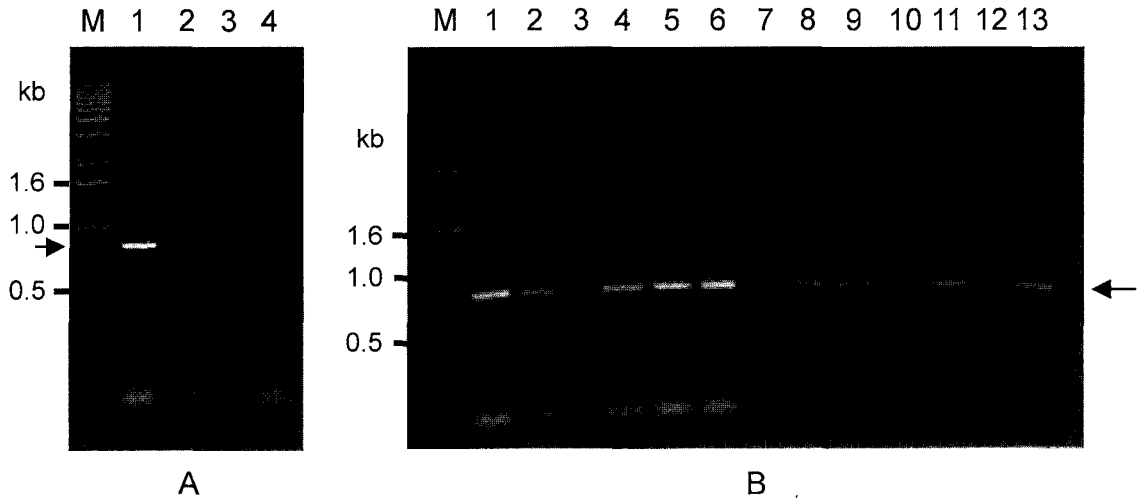


**Fig. 4.** Determination of specificity of SPFMV detection by RT-PCR (**A**) and detection of SPFMV in field-collected sweet potato plants (**B**). Photo (A); Lane M, 1kb DNA ladder; Lane 1, SPFMV RNA; Lane 2, *Potato virus Y* RNA; Lane 3, *Turnip mosaic virus* RNA; and Lane 4, *Cucumber mosaic virus* RNA. Photo (B); Lane M, 1kb DNA ladder; Lanes 1-7, total nucleic acids from roots of sweet potato plants; Lanes 8-13, total nucleic acids from leaves of sweet potato plants.

share 52.5-54.2% nucleotide sequence identities with SPFMV in the coat protein gene, and CMV was unrelated to SPFMV. This demonstrates that the primers are specific to SPFMV. Previous report also suggests that the extracts from sweet potato might contain some inhibitory compounds for RT-PCR (Nishiguchi et al., 1995). Sweet potato contains high amounts of starch. Therefore, purified starch from sweet potato was added to the RT-PCR reaction mixture, and this resulted in the significant interference for amplification of PCR product (data not shown). The virus was rapidly detected from total nucleic acids of leaves and roots from the virus-infected sweet potato plants as well as purified viral RNA by the RT-PCR (Fig. 4B). This indicates that the RT-PCR with the primers can be useful for the detection of SPFMV for evaluation of certificate and plant quarantine.

**Restriction mapping analysis of RT-PCR products.** RT-PCR products were amplified from field-collected samples of virus-infected sweet potato plants. The products were analyzed by using the restriction mapping and sequence determination. After 24 individual PCR products have been digested with 5 restriction enzymes, three types of

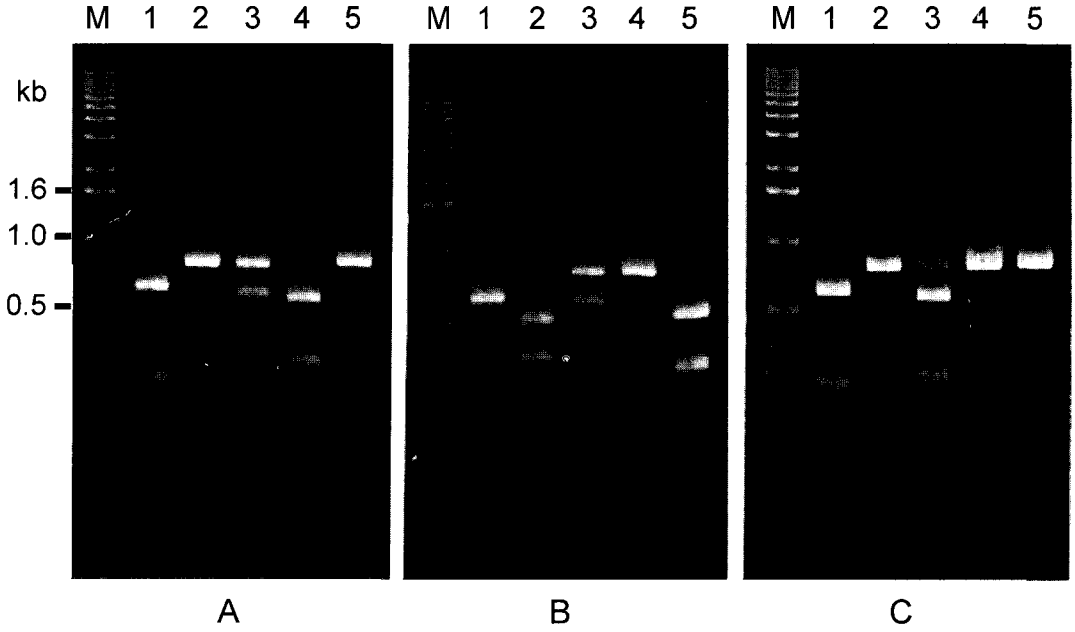


**Fig. 5.** Three types of electrophoretic patterns (**A**, **B**, **C**) of restriction fragments of RT-PCR products of SPFMV from different sweet potato sources. Lane M, 1 kb DNA ladder; Lane 1, *Hae*III; Lane 2, *Hin*dIII; Lane 3, *Nde*I; Lane 4, *Sal*I; and Lane 5, *Sty*I digestion.

restriction patterns (types A, B, and C) were detected (Fig 5A, B, C). Each PCR product was cloned and sequenced for analysis of variation among the samples, and only 1-3 nucleotide changes occurred in the 816 bp PCR production (data not shown). The restriction patterns used in this study could differentiate SPFMV mutations rapidly. Our data suggest that quasi-species of the virus exist in the field-grown sweet potato plants naturally and RFLP-RT-PCR can be useful technique for detection and assessment of variants of SPFMV. No information is available for strains of SPFMV in Korea and pathogenicity in the host plants Sweet potato is mainly propagated vegetatively and infection of virus is successively maintained from parent to progeny plant. Moreover, certification of virus-free mother plants is important for breeding program for sweet potato plant. Therefore, further analysis of variants and strains of the virus and their pathology involved in host-virus interactions should be conducted to control the virus.

## Acknowledgment

This study was supported by grants from the Natural Science Institute of Seoul Women's University to K.H. Ryu in 2000.

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