

## Detection of Soybean Mosaic Virus Using RT-PCR

Yul-Ho Kim<sup>\*†</sup>, Ok-Sun Kim<sup>\*</sup>, Bong-Choon Lee<sup>\*\*</sup>, Jae-Hwan Roh<sup>\*</sup>, Myoung-Ki Kim<sup>\*</sup>,  
Dae-Joon Im<sup>\*</sup>, Il-Bong Hur<sup>\*</sup>, and Sang-Chul Lee<sup>\*\*\*</sup>

### ABSTRACT

Reverse transcription and polymerase chain reaction (RT-PCR) assay was used to detect SMV strains. A pair of oligonucleotide primers were designed to include the cylindrical inclusion (CI) coding region between 4,176 to 5,560 nt. Amplification from the total RNA extracted from infected plants with SMV yielded a 1,385 bp DNA fragment. RT-PCR was shown to be  $10^3$  times more sensitive than the ELISA assay and it could detect a virus in  $10^{-6}$  dilution. Restriction enzyme analysis of RT-PCR products using *EcoRI* showed that SMV isolates were classified into six groups according to the patterns of restriction fragments.

**Keywords** : SMV, RT-PCR, ELISA, restriction enzyme

Soybean mosaic virus (SMV), which belongs to the virus family potyviridae, is one of the major constraints in increasing domestic soybean production because it can cause significant reduction in yield and quality (Kim et al., 1997). SMV is seed-borne and is nonpersistently transmitted by more than 20 species of aphids. Several strains of SMV (G1 to G7 and G5H) have been identified on the basis of their pathogenicity to differential soybean varieties (Cho & Goodman, 1979; Cho et al., 1983) and transmission by aphid species. The complete nucleotide sequences of strain G2 and G7 have been determined (Jayaram et al., 1992). The genomes of G2 and G7 are 9,588 nucleotides long and encode a polyprotein of 3,066 amino acids. RT-PCR, a highly sensitive method for amplification of genomes, has become widely used in the detection of plant viruses (Langeveld et al., 1991). Yoshitaka Sano et al., (1997) demonstrated that viral genome products other than the CP are likely to be responsible for the pathogenic variation. The nucleotide sequence similarities of the five strains in the CI coding region ranged from 90 to 96% respectively (data not published).

Here we report an accuracy in RT-PCR assay to

detect SMV strains in general, and the possibility that restriction enzyme digestion could be used to classify the SMV strains.

### MATERIALS AND METHODS

#### Virus isolates and plant materials

Five SMV strains, G5H, G5, G3, G2 and G7, were used throughout the tests. Each strain was confirmed by inoculation on differential varieties of soybean. Soybean cv. Kwangankong (susceptible to all SMV strains) was used as the maintenance host. Primary leaves were mechanically inoculated with five SMV strains, respectively. Twenty days after inoculation, infected trifoliolate leaf tissue was used for total RNA extraction. Twenty four seed-borne plants with SMV were provided by Dept. of Upland Crops, National Crop Exp. Stn., Suwon, Korea.

#### ELISA

Double antibody sandwich (Test kit : Sanofi) was applied for SMV detection. Each well of microplates was coated with antibodies (IgG) and incubated for 2 hours at 37°C. After rinsing 3 times with PBS-Tween, sample homogenates (200  $\mu$ l/well) were added to each well. After the plates were incubated at 4°C for 15 hours, plates were washed by PBS-Tween buffer (pH 7.4). Conjugated antibodies (IgG-PAL) were added. Following incubation at 37°C for 3 hours, the plates were washed and substrate solution was added to each well and incubated at room temperature for 1 hour. After reaction, optical densities at 405nm were measured by the immuno reader. Samples were considered positive when the mean absorbance of triplicate wells was greater than two times the mean absorbance of the control wells (uninoculated tissue) (D'Arcy et al., 1990).

#### Total RNA extraction and RT-PCR

Total RNA was extracted from trifoliolate leaves by using the extraction kit (SV Total RNA Isolation System, Promega) as per the manufacture's instructions. A pair of oligonucleotides were designed on the basis of the sequence published by Jayaram et al (1992), amplifying a 1385 bp fragment at positions

\* National Crop Experiment Station, RDA, Suwon, Korea 441-100. \*\* National Horticultural Research Institute, RDA, Suwon, Korea 441-440. \*\*\* Kyungpook National University, Taegu, Korea 702-701. \*† Corresponding author : (E-mail) kimyuh@nces.go.kr (Phone) +82-331-290-6764. Received 15 July, 1999.

4176 to 5560 including the CI coding region(Fig 1). The upstream primer, CI5'(5'-GCATTCAACTGTG-CGCTTAAAGAAT-3'), was homologous to 4176 to 4200 nt of SMV strain G2. The downstream primer, CI3'(5'-TTGAGCTGCAAAAATTTACTCACTT-3'), was complementary to 5535 to 5560 nt. RT-PCR was carried out by using Access RT-PCR System (Promega) and by one step with cDNA synthesis and PCR amplification at the same time. RT-PCR reactions were conducted in a 50  $\mu$ l total volume(10  $\mu$ l AMV/Tfi 5 $\times$ Reaction Buffer, 1  $\mu$ l dNTP mix (10 mM each), 2  $\mu$ l MgSO<sub>4</sub> (25 mM), 1  $\mu$ l each primer (50 pmol), 1  $\mu$ l AMV Reverse Transcriptase(5 units/ $\mu$ l), 1  $\mu$ l Tfi DNA Polymerase(5 units/ $\mu$ l), 5  $\mu$ l template RNA made up to the volume with nuclease-free water. Thermocycling was performed in a PTC 200 (MJ Research, Inc.) and programmed as follows; cDNA synthesis of 1 cycle at 48 $^{\circ}$ C for 45 min, AMV RT inactivation and RNA/cDNA/primer denaturation of 1 cycle at 94 $^{\circ}$ C for 2 min; followed by 40 cycles for template denaturation at 94 $^{\circ}$ C for 30 sec, primer annealing at 60 $^{\circ}$ C for 1 min, extension at 68 $^{\circ}$ C for 2 min; followed by 1 cycle for final extension at 68 $^{\circ}$ C for 7 min. PCR products were loaded(5  $\mu$ l) on 1.2% agarose gels.

### Restriction enzyme digestion

Restriction enzyme, *Eco*R I was used to digest PCR products, which was performed as recommended by a manufacturer(Promega).

## RESULTS AND DISCUSSION

The RT-PCR amplification yielded a product of the expected size of 1385 bp for five SMV strains and necrotic symptoms(Fig. 2). No fragment was amplified from RNA extracted from uninoculated healthy soybean plants.

Using 10-fold serial dilutions of infected soybean cv. Kwangankong with SMV-G5H, a comparison of sensitivities of DAS-ELISA and RT-PCR was conducted. Based on the data in Table 1, RT-PCR was shown to be more than 10<sup>3</sup> times more sensitive than ELISA assay and it could detect a virus in a 10<sup>-6</sup> dilution. It appears that RT-PCR is a powerful method to detect viruses in such a low concentration. To know if the primers used are available to detect SMV strains in general, PCR amplifications were performed with the leaves of twenty four seed-borne plants with SMV. The expected fragments(1385 bp) were obtained in all cases(Fig. 3).

Monitoring of virulent and newly emerged strains

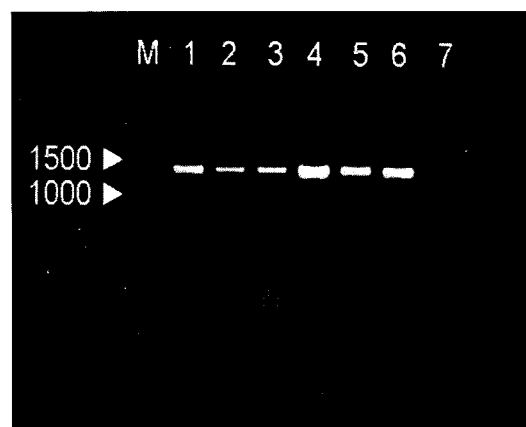


Fig. 2. Agarose gel electrophoresis of RT-PCR amplified products of SMV in total RNA extracted from infected soybean leaves. M: size marker(1kb ladder), 1~5: mosaic symptom leaves in Kwangankong infected with G5H, G3, G5, G2 and G7, respectively, 6: necrotic symptom leaves in Taekwangkong infected with G5, 7: healthy soybean.

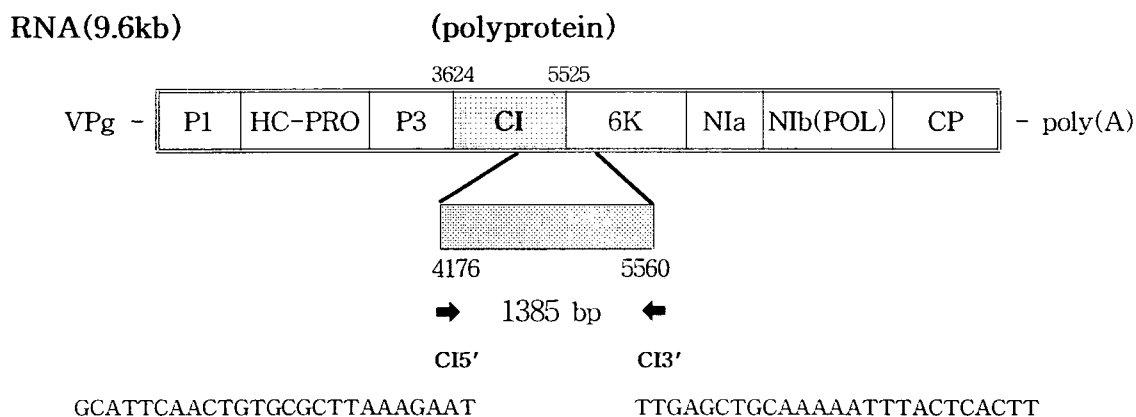


Fig.1. The genetic map of SMV polyprotein and the oligonucleotide primers used to amplify the cylindrical inclusion(CI) coding region of SMV.

**Table 1. Comparison of sensitivities between DAS-ELISA assay and RT-PCR in SMV-infected leaves.**

Sample dilution	ELISA values (OD 405nm)	ELISA	RT-PCR <sup>†</sup>
Healthy control	0.049±0.020	-	-
1	1.932	+	+
10 <sup>-1</sup>	1.333	+	+
10 <sup>-2</sup>	0.175	+	+
10 <sup>-3</sup>	0.067	-	+
10 <sup>-4</sup>	0.052	-	+
10 <sup>-5</sup>	0.050	-	+
10 <sup>-6</sup>	0.052	-	+

<sup>†</sup> - : no band detected, + : band detected

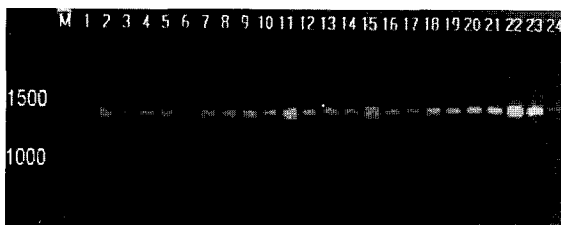
is important for the breeding of SMV resistant varieties through introduction of resistance genes. But, SMV strain differentiation based on differential cultivars demands much labor and space, and takes a long time from the inoculation of nonclassified SMV isolates to the reading of the result.

In this study, restriction enzyme analysis of RT-PCR products using *EcoR* I was employed to differentiate SMV strains. PCR products were classified into six groups according to the patterns of restriction fragments (Fig. 4). Here it was not enough to classify all SMV strains. To match the fragment patterns with SMV strains, sequencing analysis and

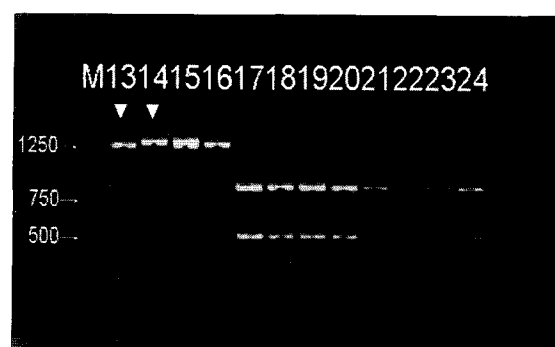
virulence tests are greatly needed.

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**Fig. 3. Detection of SMV by RT-PCR assay in twenty four seed-borne plants. M; size marker, lanes 1~24; SMV isolates.**



**Fig. 4. *EcoR* I restriction enzyme digest of PCR-amplified products from seed-borne plants. M; size marker, lanes 1~24; SMV isolates, ▽ indicates different band patterns.**