

First Report on *Poinsettia mosaic virus* in Korea

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Most plants of commercial poinsettia cultivars grown from cuttings develop mosaic and chlorotic dot symptoms on leaves. Reverse transcription-polymerase chain reaction (RT-PCR) test showed that they were infected with *Poinsettia mosaic virus* (PnMV). In a survey of commercially grown poinsettias conducted in Korea, PnMV was detected in ten of ten poinsettia cultivars sampled and in 100% of 178 samples tested. The virus has isometric particles and about 29 nm in diameter. Crystalline virus particles were observed in cytoplasm of cells of diseased plants by transmission electron microscopy. Nucleotide sequence of coat protein gene of PnMV-K1 showed 97.3% homology with that of a German isolate. This is the first report on PnMV in Korea.

Keywords : *Euphorbia pulcherrima*, *Poinsettia mosaic virus*, *Tymovirus*, poinsettia

Poinsettia (*Euphorbia pulcherrima*), introduced to the United States from Mexico and named after Joel Robert Poinsette in 1825, has become a major ornamental potted plant in North America. A total of 65 million pots were produced from USA with 247 million US dollars in market value (Floriculture Crops, 2002).

Poinsettia mosaic virus (PnMV) is a member of *Tymovirus* (Koenig et al., 1986). The virions of PnMV are isometric and about 26-29 nm in diameter (Koenig et al., 1986). The genome of PnMV is a single-stranded RNA about 6.1 Kb in length (Bradel et al., 2000). The genomic RNA has a poly(A) tail at its 3'-terminus, in contrast to the tRNA-like structure found in the RNA of most tymoviruses (Bradel et al., 2000). The virus was first reported in *Euphorbia pulcherrima* from USA (Fulton and Fulton, 1980), and the complete nucleotide sequence of PnMV was determined in Germany (Bradel et al., 2000).

PnMV occurs worldwide in commercial cultivars of poinsettia (Chiko, 1983). PnMV induces light mottling, chlorotic spots, angular mosaic, bract and leaf distortion,

and vein clearing (Bartkowski and Kahsnitz, 1990; Chiko, 1983; Fulton and Fulton, 1980). When poinsettia plants are infected with PnMV, most of the bracts do not grow, remain small and chlorotic, and do not turn red. In some cases, the reproductive structures are deformed (Bertaccini et al., 1996).

Until the early 1990s, only a few poinsettia cultivars, such as 'V-10 Amy' and others, were marketed in Seoul and the metropolitan areas in small scale, usually for decoration. Poinsettias were highly in demand since the mid 1990s, especially in the months of November and December. Today, about 80 commercial growers, mostly in Seoul and Gyeonggi Province, produce poinsettias with an estimated production area of approximately more than 10 hectares (Lee, 2003).

However, production of high quality poinsettia is the key factor to cope with the increasing competition with other floricultural plants in the coming years.

Here we report the identification of PnMV-K1 in poinsettia, grown as a pot plant in Korea, based on symptom of diseased plants, biological assay, particle morphology, and the nucleotide sequence analysis of coat protein gene. This is the first report on PnMV in Korea.

Materials and Methods

Source of virus. Poinsettia cultivar 'V-10', showing mosaic symptom (Fig. 1), yellowing, and vein clearing symptoms, (Fig. 2) was collected from a glass house at National Horticultural Research Institute in Gyeonggi Province. The poinsettia cultivar 'V-10' plant, showing mosaic symptom, was designated as PnMV-K1 isolate in this study. The isolate was deposited in the NHRI and Plant Virus GenBank (<http://www.virusbank.org>).

Host range test. One of the seven indicator plants, *Nicotiana benthamiana*, was inoculated with crude sap of leaf samples from a source plant of PnMV-K1 in 0.01 M phosphate buffer, pH 7.2. Test was conducted in November through December in a glass house.

Survey of virus infection rate. Virus infection rate was investigated by using PnMV antibody and alkaline phosphatase enzyme-conjugated PnMV antibody (Agdia, Indiana, USA). Total of 178 samples were randomly collected from 10 commercial cultivars in commercial fields in Goyang and Paju, Gyeonggi

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Fig. 1. Mosaic symptom in *Euphorbia pulcherrima* naturally infected with *Poinsettia mosaic virus*.



Fig. 2. Vein clearing and yellowing in *Euphorbia pulcherrima* naturally infected with *Poinsettia mosaic virus*.

Province.

Electron microscopy. Using Carl Zeiss LEO 906 transmission electron microscope researchers observed virions of PnMV in crude sap of a source plant of PnMV-K1 which was negatively stained with 0.5% phosphotungstic acids, and with a pH of 7.0. To observe the ultrastructure of diseased poinsettia, leaves of a source plant of PnMV-K1 were prefixed in 1% Karnovsky's fixative solution for 4 hr. The leaves were then washed three times for 20 min in each wash in 0.05 M Cacodylate buffer, pH 7.2. The specimens were postfixed for 2 hr in 1% Osmium tetroxide in 0.1 M Cacodylate buffer, pH 7.2. Fixed specimens were dehydrated in an ethanol series of 50, 75, 90, 95 and 100% for 30 min in each step. The tissue was then embedded in Spurr resin (Electron Microscopy Science, Washington, PA). Ultrathin sections were stained with 2% uranyl acetate and 0.08 M lead citrate buffer, pH 12.0, and examined with Carl Zeiss LEO 906.

Reverse transcription-polymerase chain reaction (RT-PCR). To amplify coat protein gene of PnMV-K1, researchers synthesized specific primer based on the nucleotide sequences reported previously (Bradel et al., 2000; GenBank accession no. PM0271595).

The sequence of the forward primer was 5'-GCTACGAGGT-GCAGACATGTCA-3', and that of the reverse primer was 5'-AAAAGGTGGAGGCTAGAGGGCT-3'.

Total RNA was extracted from a source plant of PnMV-K1 by using RNeasy Plant Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instruction. RT-PCR was conducted by using GeneAmp RNA PCR kit (PerKin Elmer, NJ, USA) according to manufacturer's instruction. PCR was conducted with the following cycles: Denaturation at 94°C for 30 sec, primer annealing at 66°C for 1 min, and extension at 72°C for 1 min for 40 cycles with a final extension at 72°C for 7 min. Amplified RT-PCR products were analyzed on 1.5% agarose gel in 0.5 × TBE buffer (Sambrook et al., 1989).

Sequence analysis of CP gene. PCR products of 634 bp DNA was gel-purified with the GENECLEAN III KIT (Q-BIO gene, USA) and ligated into the pGEM-T easy vector (Promega, USA) according to manufacturer's instruction. The ligation mixture was used to transform competent cells of *Escherichia coli* JM 109. Recombinants were screened as described previously (Chung et al., 2001). The nucleotide sequence was determined from three independent clones, and was aligned with a CVMV isolate derived from GenBank by using CLUSTAL Method of DNASTAR software version 5.1 (DNASTAR, Madison, WI, USA).

Results

Bioassay. Reactions of indicator plants by inoculation of PnMV-K1 are shown in Table 1. PnMV-K1 systemically infected *Nicotiana benthamiana* and *N. clevelandii* with mosaic symptom. Meanwhile it did not infect *Nicotiana tabacum*, *N. rustica*, *Gomphorena globosa*, and *Chenopodium* sp.

Survey of virus infection rate. PnMV-K1 was detected in all of the ten commercial poinsettia cultivars sampled and 100 percent of 178 samples tested by using ELISA (Table 2).

Virus morphology. Virions of PnMV-K1 were isometric particles of 29 nm in diameter (Fig. 3).

Electron microscopy. Crystalline arrays of spherical virus particles were shown in the cytoplasm (Fig. 4).

RT-PCR. A DNA product of 634 bp was detected after

Table 1. Symptomatological responses of indicator plants to PnMV-K1 infection

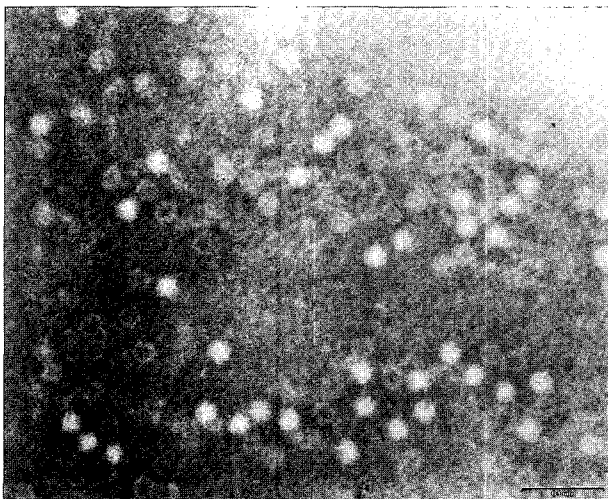
Indicator plants	Reaction ^a
<i>Nicotiana tabacum</i> cv. Samsun	NI
<i>N. rustica</i>	NI
<i>N. clevelandii</i>	M
<i>N. benthamiana</i>	M, VC
<i>Gomphorena globosa</i>	NI
<i>Chenopodium quinoa</i>	NI
<i>C. amaranticolor</i>	NI

^aNI, not infected; M, mosaic; VC, vein clearing.

Table 2. Detection of *Poinsettia mosaic virus* in commercial poinsettia cultivars in Korea

Cultivar	No. samples tested	No. samples infected ^z	Percent samples infected
Freedom Red	30	30	100.0
Freedom White	16	16	100.0
Lemon Snow	12	12	100.0
Lemon Drop	15	15	100.0
Millenium	32	32	100.0
Red Max	17	17	100.0
Sonora	19	19	100.0
Prestage	6	6	100.0
Cortez	16	16	100.0
Winter Rose	15	15	100.0
Average	-	-	100.0

^zVirus was detected by using PnMV ELISA kit (Agdia, Indiana, USA).

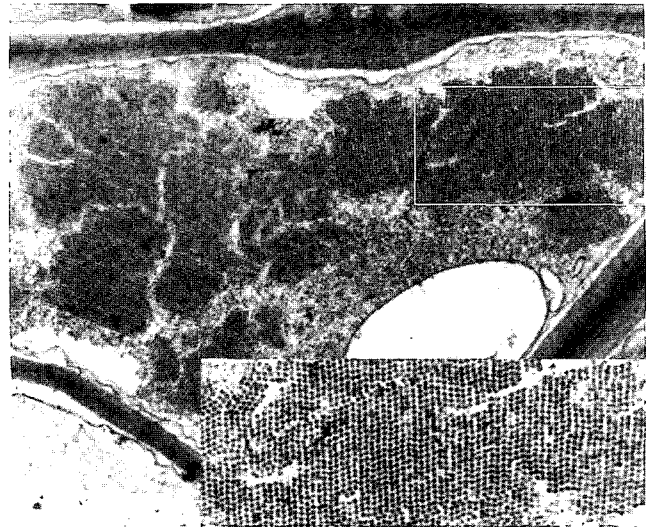
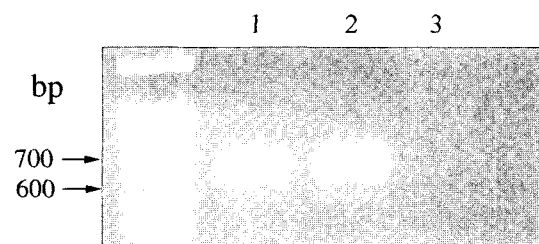
**Fig. 3.** Particles of *Poinsettia mosaic virus*-K1 (PnMV-K1) from a crude sap negatively stained with phosphotungstate.

amplification of nucleic acids from a source poinsettia plant of PnMV-K1 (Fig. 5). This product was not detected from healthy chrysanthemum plant (Fig. 5).

Sequence analysis of CP gene. The nucleotide sequences of CP gene of PnMV-K1, amplified from a source plant of PnMV-K1, were determined and deposited in the GenBank database under the accession no. AY464943. Nucleotide sequence of coat protein gene of PnMV-K1 showed 97.3% homology with that of a German isolate. The region encoding the CP gene is 603 nucleotide long and the deduced protein consists of 200 amino acids.

Discussion

Most of poinsettia cultivars usually develop mosaic

**Fig. 4.** Ultrathin section of leaf tissue of poinsettia infected with PnMV-K1. Crystalline arrays of spherical virus particles are shown in the cytoplasm.**Fig. 5.** Amplification of coat protein gene from poinsettia infected with PnMV-K1. Lanes 1 and 2, diseased plants; lane 3, healthy plant.

symptom in summer. The mosaic symptom is masked when the bracts reach to their coloring stage during the winter season. After bracts turn red, mosaic symptoms are observed in all green leaves and chlorotic dots in lower green leaves. This observation agrees with that of Fulton and Fulton (1980).

Nicotiana benthamiana, *N. glutinosa*, *N. rustica*, *N. clevelandii* or *N. tabacum* are reported as host plants of PnMV (Koenig et al., 1986), but PnMV-K1 did not infect *N. glutinosa*, *N. rustica*, and *N. tabacum* in spite of the three trials with 5 replications made by the researchers.

Meanwhile, PnMV-K1 infected *N. benthamiana* and *N. clevelandii*, although, not all of the three test plants were fully infected. From this result, we assume that PnMV can infect those host plants with different degrees of success.

In this study, all samples of poinsettia cultivars tested were infected with PnMV. Most of these cultivars were bred in America and imported recently by Korea. Floral industries have grafted restricted branching cultivars onto free branching ones to induce free branching poinsettia cultivars, since

Gutbier first began exploring the idea of grafting as a means of developing better poinsettias (Hartley, 1995; Lee et al., 1997; Preil, 1994).

Accordingly, researchers have assumed that PnMV has been transmitted from commercial PnMV-infected cultivars to new cultivars through grafting. PnMV is transmitted by mechanical inoculation and grafting, and the vector is unknown (Koenig, 1986).

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