

'Cheongyang' and 'Wangshilgun'. However, in cultivars of 'Manitta' and 'Bugang', symptoms were not occurred. In single infection of PepMoV, symptoms of mottle and malformation were produced on the tested cultivars of 'Manitta', 'Bugang', 'Cheongyang' and 'Wangshilgun'. In the cultivars of 'Cheongyang' and 'Wangshilgun', synergistic symptoms of stunt and lethal death were induced by mixed infections in the two combinations of TMV+PepMoV and PMMoV+PepMoV. However, in cultivars of 'Manitta' and 'Bugang', synergistic symptom was not occurred as mottle which was milder than that of single infection. Cells were single infected with TMV and PMMoV the cultivars of 'Cheongyang' and 'Wangshilgun', respectively, had typical ultrastructures of tobamovirus as the stacked-band structure and multiple spiral aggregate (SA). Ultrastructures of cell and tissues infected with PepMoV on the cultivars of 'Cheongyang', 'Wangshilgun', 'Manitta' and 'Bugang', the potyvirus inclusions of pinwhills, scrolls, lamminated aggregates and amorphous inclusion were observed. Infected cells with a combination of TMV+PepMoV and PMMoV+PepMoV, the virus particles and inclusions of the two different viruses were found only mixed infection in the same cytoplasm and the amounts of viruses in mixed infections were abundant than in single infection. The angled-layer aggregates (ALA) was observed in the cells infected mixedly with TMV and PepMoV.

**4-29. Identification of *Potato mop-top virus* from *Solanum tuberosum* cv. Gawon in Korea.**

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*Potato mop-top virus*(PMTV) was identified from *Solanum tuberosum* cv. Gawon showing bright chlorotic mottle symptom in Namwon, Korea. Samples were collected green-house in February, 2003. Electron microscopic examination of negatively stained preparation revealed that PMTV were rigid-rod shaped particles about 100-150, 250-300 nm × 18-20 nm in length. In ultrathin sections of leaf tissue from diseased potato plants, cluster of viruses particles were observed in the cytoplasm. TAS-ELISA determined that the virus was serologically related to PMTV. PMTV produced double ring necrotic local lesion in inoculated leaf of *Chenopodium amaranticolor* in incubated at 15°C. The PMTV could be detected with RT-PCR using PMTV detectable primer set designed to amplify about 540 bp of the partial CP gene of PMTV.

**4-30. Characterization of *Grapevine leafroll-associated virus 1* and *Grapevine leafroll-associated virus 3* isolated from *Vitaceae* in Korea.**

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*Grapevine leafroll-associated 1 virus* (GLRaV-1) and *Grapevine leafroll-associated 3 virus* (GLRaV-3), member of the genus *Ampelovirus*, are important viral disease of grapevine in the world. these viruses transmitted only dicotyledonous host by vectors such as mealybugs and there

is no suitable herbaceous host for virus. The diseased leaves turn yellowish or reddish depending on cultivars and viruses. Viruses are existed at low concentration and ununiformly distribution in grapevine. Using small-scale double-stranded RNA (dsRNA) extraction method, reverse transcription and polymerase chain reaction (RT-PCR) product of 1Kb long which encoded of coat protein (CP) gene for both viruses was successfully amplified with a specific primers. The RT-PCR product was cloned into the plasmid vector and its nucleotide sequences were determined from selected recombinant cDNA clones. Sequence analysis revealed that the CP of GLRaV-1 consisted of 969 nucleotide, which encoded 323 amino acid residues and CP of GLRaV-3 consisted of 942 nucleotide, which encoded 314 amino acid residues. The CP of GLRaV-1 and GLRaV-3 has 93.8% and 98.7% amino acid sequence identities, respectively.

#### 4-31. Improved RNA extraction for fruit tree viruses in RT-PCR assay

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Tissues from woody plant contain higher amount of phenolic compounds and polysaccharides, which give inhibitory effects on reverse transcriptase and/or *Taq* polymerase. The common multiple-step protocols using several additives to inhibit polyphenolic compounds during nucleic acid extraction are time consuming and laborious. Sodium sulfite ( $\text{Na}_2\text{SO}_3$ ) was used as inhibitor of polyphenolic oxidases in extraction buffer and compare it's effect between commercial RNA extraction kit and small-scale double-stranded RNA (dsRNA) extraction by RT-PCR. During nucleic acid extraction procedure, addition of 0.5%-1.5% (w/v) sodium sulfite to lysis buffer or STE buffer resulted in lighter color change than extracts without sodium sulfite and improve the RT-PCR detection. When commercial RNA extraction kit used, optimal concentration of sodium sulfite were variable according to the host plant. However, using dsRNA as RT-PCR template, 1.5% sodium sulfite in STE buffer improves the detection of both viruses and unspecific amplifications were reduced significantly. Furthermore, when viruses existed at low titers in host plant, small-scale dsRNA extractions were very reliable.

#### 4-32. Identification of *Ornithogalum mosaic virus* isolated from ornithogalum.

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*Ornithogalum* showing mosaic symptoms were collected from the isolated field of National Plant Quarantine Service in Sengrimmyon of Kyungnam province. Electron microscopic examination of negatively strained preparation was filamentous particle of 740nm in length. Indirect-ELISA determined that the virus was serologically related to potyvirus. A single major protein band of Mr