

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 (Na_2SO_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

30,000 was observed after sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Indicator plant test showed mosaic, necrotic local lesion and sunken areas in leaves of *Nicotiana clevelandii* and *Tetragonia expansa*, while the others of indicator plants did not infect. An enzyme-aided purification protocol was used, which eliminated a highly viscous mucilage from extracts of the *Ornithogalum*. Total RNA extracted from infected *Ornithogalum* leaves were amplified of 411bp fragment in reverse transcription (RT)-PCR when primers specific for the coat protein gene. An isolate of *Ornithogalum mosaic virus* (OrMV) of the genus Potyvirus was identified as the causal agent of the disease on the basis of electron microscopic, biological and serological reaction.

4-33. Characterization of an Isometric virus Infecting Paprika (*Capsicum annuum* var. *glossum*) in Korea

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An Isometric virus was isolated from Paprika (*Capsicum annuum* var. *glossum*) showing necrosis spot and malformation on the fruit and the leaves, respectively, at yecheon in Korea. The virus could infect locally on *Chenopodium amaranticolor*, *C. quinoa*, *Petunia × hybrida* and *Nicotiana glutinosa*, but could not infect on *Gomphrena globosa* and *Physalis floridana*. The virus could infect systemically on red pepper and *Lycopersicon esculentum*. *Datura stramonium*, *N. clevarandii*, *N. rustica* and *N. tabacum* cvs. were produced necrosis or necrotic ring spot lesions on the inoculated leaves and mosaic, vein necrosis or lethal death on the upper leaves. The virus was not related serologically to *cucumber mosaic virus* (CMV). In RT-PCR assay, it could not be detected with specific primers of CMV and BBWV-II. The virions contain one molecule of genomic RNA, which was approximately 3.8Kb and the coat protein (CP) of the purified virion migrated as a single band with molecular weight of about 29KDa in SDS-PAGE.

4-34. Population of Rice Stripe Virus-Viruliferous Insect and Natural Weed Host of Rice Stripe Virus.

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Among over-wintering small brown planthoppers, population of the rice stripe virus (RSV)-viruliferous insects was surveyed throughout the country in late April of 2003 by using