

## Mollicutes and Mollicute Diseases (K168-K169)

**K-168. Genetic differentiation of phytoplasma isolates by DNA Heteroduplex Mobility Assay (HMA) and Single-Strand Conformation Polymorphism (SSCP) Analysis.** Sangsub Han, Sanghun Lee, and Byeongjin Cha. Dept. of Agricultural Biology, Chungbuk National University, Cheongju 361-763, Korea

A heteroduplex mobility assay (HMA) and single-strand conformation polymorphism (SSCP) analysis combined with PCR were developed for the analysis of genetic differentiation from various phytoplasma isolates. In HMA and SSCP analysis, both of the mobility shifts and the SSCP band patterns identified three distinct types of phytoplasmas: Type I, jujube witches-broom (JWB) and ligustrum witches-broom (LiWB); Type II, mulberry dwarf (MD) and sumac witches-broom (SuWB); and Type III, paulownia witches-broom (PaWB). The results from sequence analyses revealed that phytoplasmas of JWB and LiWB had 100% homology with MD and SuWB, respectively. Otherwise, PaWB phytoplasma had 97.8% homology compare with MD phytoplasma. The PCR-HMA and SSCP techniques were very useful to determine variations in sequence among several isolates of phytoplasma. Furthermore, the methods were very rapid, economical, easy to handle with the gels, and highly sensitive.

**K-169. Stolbur phytoplasma in *Lilium* oriental hybrids.** B. N. Chung, E. J. Lee, J. A. Jung, G. S. Choi, H. R. Kim, and J. S. Kim. Horticultural Environment Div., National Horticultural Research Institute, RDA, Suwon 440-310, Korea.

Stolbur phytoplasma (16SrXII-A) was identified from *Lilium* Oriental hybrids showing flattened stem and flower clustering. Those lilies were collected from commercial greenhouse in Korea. The presence of phytoplasma was demonstrated using polymerase chain reaction (PCR) assays with phytoplasma universal (P1/P6) and stolbur phytoplasma specific primer pairs amplifying phytoplasma 16S rDNA regions. Nucleotide sequences of the phytoplasma 16S rDNA were determined. Nucleic acid extracted from lily showing flattened stem and flower clustering amplified 1.5 kb DNA with a phytoplasma universal primer pair. In nested PCR, 1.1 kb PCR product was obtained using specific primer pair, indicating an isolate of stolbur phytoplasma. Nucleotide sequence of phytoplasma 16S rDNA reported in this study showed 99.5% and 99.1% homology with 2 stolbur phytoplasmas, subgroup A (16SrXII-A) (GenBank accession no. AF248959 and X76427), respectively. Also it exhibited a sequence homology of 98.0% with phormium yellow leaf (16SrXII-B) (GenBank accession no. U43570), and 97.9% with Australian grapevine yellows (16SrXII-B) (GenBank accession no. L76865). While, it showed 89.9% homology with an isolate of aster yellows phytoplasma, clover phyllody (16SrI-C) (GenBank accession no. L33762), and 94.7% with American aster yellows (16SrI-B) (GenBank accession no. X68373). Homology percent of the 16S rDNA nucleotide sequence suggest classification of this phytoplasma in the stolbur phytoplasma, subgroup A (16SrXII-A), as a type strain stolbur.

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