

7. Genetic Relationship among Phytoplasmas of Woody Plants and Localities. Han, Mu-Seok.
Biotechnology Division, Forestry Research Institute, Suwon 441-350, Korea

A dozen of plant species growing in Korea have been reported to be infected by phytoplasmas. Among them, three economically important tree species including *Zizyphus jujuba*, *Paulownia coreana*, and *Morus alba* have been devastated by the phytoplasma diseases. Therefore, we need to identify the characteristics and genetic relationship of phytoplasmas occurring in Korea. Plants showing typical symptoms of phytoplasma infection were collected throughout the country. These include *Ligustrum obtusifolium*, *L. ovalifolium*, *Rhus japonica*, *Paulownia coreana*, *Morus alba*, *Forsythia koreana*, and *Zizyphus jujuba*. The presence of phytoplasma in the plants and the genetic relationship among them were studied by transmission electron microscopy, PCR (polymerase chain reaction) amplification of 16S *rRNA* gene, restriction digestion, and sequencing.

The following results were obtained;

Phytoplasmas of diverse shapes were observed in the phloem sieve elements of the infected plants by transmission electron microscopy.

PCR amplification of total DNA extracted from infected plants with a primer set specific to 16S *rRNA* gene of phytoplasma resulted in a 1.4kb band. The amplified bands strongly reacted with DIG-labeled probe prepared with phytoplasma 16S *rRNA* gene.

The amplification products were cloned into pGEM-T vector for further analysis by restriction digestion and sequencing. In some cases, two different kinds of inserts were observed in isolates originated from a single plant. It turned out that they were due not to double infection by two different strains of phytoplasmas but to the amplification of 16S *rRNA* gene of both host plant chloroplasts and phytoplasma.

It was possible to differentiate phytoplasma DNA from chloroplast DNA by restriction digestion of the recombinant plasmids. Only the recombinant plasmids carrying phytoplasma 16S *rRNA* gene produced a 1.4kb band when digested with the enzyme *Ban* II. Of 52 recombinant plasmids analyzed, 42 appeared to be the 16S *rRNA* gene of host plant chloroplasts.

No variation was detected in 16S *rRNA* gene among nine *Phytoplasma zizyphi* strains examined. However, it appeared to be distantly related to *Phytoplasma paulownia*.

DNA sequence comparison of 16S *rRNA* genes revealed that while *P. zizyphi* showed 90.2% homology to *Oenothera hookeri* phytoplasma (O. phytoplasma), *P. paulownia* was 99.5% homologous to O. phytoplasma. The homology between *P. zizyphi* and *P. paulownia* was 89.8%.