Plant Pathol. J. 23(2) : 106-108 (2007)

The Plant Pathology Journal

Note

Candidatus Phytoplasma trifolii Associated with Witches' broom of *Lespedeza cyrtobotrya* M.

Young-Hwan Kim and Hee-Young Jung*

Division of Applied Biology and Chemistry, Kyungpook National University, Daegu 702-701, Korea

(Received on March 28, 2007; Accepted on May 11, 2007)

The Symptoms of witches' broom disease caused by phytoplasma including general stunting and yellowing, were observed in leafy lespedeza (*Lespedeza cyrtobotrya* M.) on Doam-myeon, Pyeongchang-gun, in 2006. Based on the sequence analysis of PCR-amplified 16S ribosomal DNA and 16S-23S spacer region DNA products using universal phytoplasma primers, the phytoplasma associated with leafy lespedeza witches' broom (LLWB) disease was identified as a member of *Candidatus* Pytoplasma trifolii. It was most closely related to alsike clover proliferation phytoplasma (99.8% similarity, accession no. AY390261), *Candidatus* Pytoplasma trifolii strain. RFLP patterns generated with *Alu*I, *Hpa*II clearly differentiated LLWB phytoplasma from the referenced phytoplasma strains, water dropwort witches' broom, mulberry dwarf, glehni aster yellow dwarf and jujube witches' broom. This paper is the first report on *Candidatus* Phytoplasma trifolii in leafy lespedeza identified at a molecular level.

Keywords : Phytoplasma, *Lespedeza cyrtobotrya*, Phylogeny

In the autumn of 2006, a vegetative growth disorder was observed on several leafy lespedeza (*Lespedeza cyrtobotrya* M.) in a forest on Doam-myeon, Pyeongchang-gun, Korea. The most evident symptoms were typical of witches' broom disease, including general stunting, yellowing and proliferation of auxillary shoots; it was considered to be caused by phytoplasma (Fig. 1).

The objectives of this study were to identify the presumed causal agent and, if it was a phytoplasma, to determine the phylogenetic position of the leafy lespedeza witches' broom (LLWB) phytoplasma. A direct PCR with universal primers SN910601 (5'-GTT TGA TCC TGG CTC AGG ATT-3') and SN011119 (5'-TCG CCG TTA ATT GCG TCC TT-3') designed by Jung et al. (2003a) was used in attempts to amplify the phytoplasma 16S rDNA and 16S-23S rDNA spacer region (SR). From all of diseased leafy lespedeza samples examined, PCR amplified approximately 1.8-kbp fragments, which is the same size as that expected for the phytoplasmal 16S rDNA plus 16S-23S rDNA SR (data not shown). Under the same conditions, however, no amplification products were obtained from nonsymptomatic plants collected in the same area (data not shown). From the observed symptoms and the PCR studies of diseased plants, we concluded that the witches' broom disease was indeed caused by phytoplasma infection.

The PCR products of LLWB phytoplasmas were sequenced using 8 primers (350F, 520R, 788F, 920R, 1099F, 1100R, 1505F, 1840R) that have been used to sequence phytoplasma 16S rDNA and 16S-23S rDNA SR (Jung et al., 2003b). All of the 16S rDNA and SR sequences of LLWB isolated from several independent plants were identical; the sequence has been deposited in the GenBank database library (accession no. AB279597).

The LLWB 16S rDNA sequence was aligned with those of most phytoplasmas reported to date, and sequence similarities were evaluated using the GENETYX-WIN package, version 3 (Software Development, Tokyo, Japan). Direct pairwise comparison of the 16S rDNA sequences showed that the LLWB sequence was most similar to those of alsike clover proliferation (CP) phytoplasmas (99.8%, accession no. AY390261), potato witches' broom (PWB-K) phytoplasma (99.7%, accession no. AB076404), Fragaria

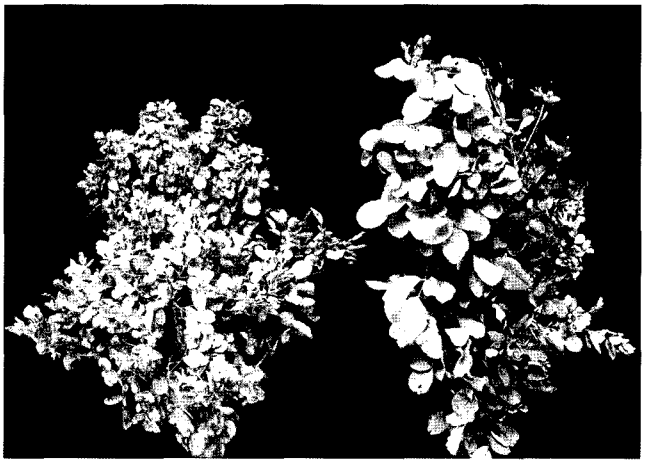

Fig. 1. Witches' broom symptoms in an infected *Lespedeza cyrtobotrya* (left) compared to a healthy plant (right).

*Corresponding author.
Phone) +82-53-950-5760, FAX) +82-53-950-6758
E-mail) heeyoung@knu.ac.kr

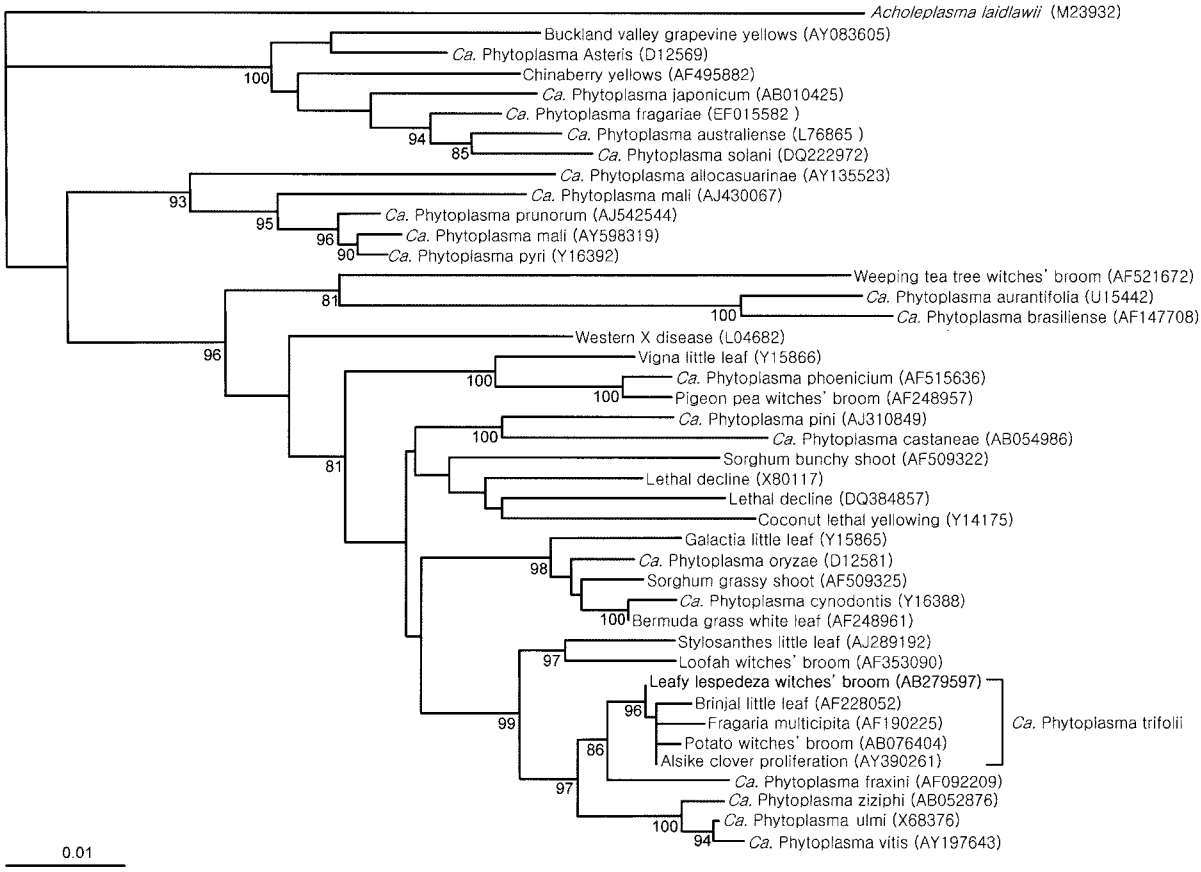

Fig. 2. Phylogenetic distance tree constructed by the neighbor-joining method, comparing the 16S rDNA sequences of LLWB phytoplasma with those of other phytoplasmas from GenBank. *Acholeplasma laidlawii* was used as the outgroup. Accession numbers are shown in parentheses. Numbers on the branches are confidence values obtained for 100 replicates (only values above 80% are shown). The bar represents a phylogenetic distance of 1%.

multicipita (FM) phytoplasma (99.2%, accession no. AF190224), and brinjal little leaf (BLL) phytoplasmas (98.9%, accession no. AF228052). For phylogenetic analysis, a single neighbor-joining tree was obtained using the 16S rDNA sequences of 41 phytoplasmas with *Acholeplasma laidlawii* as outgroup (Fig. 2). The LLWB isolate was most closely related to the CP, BLL, PWB-K, and FM phytoplasmas, which belong to *Candidatus* Phytoplasma trifolii (Hiruki and Wang, 2004). A bootstrap value of 96% supports the LLWB / PWB / FM / BLL / CP clade as being distinct from the *Candidatus* Phytoplasma fraxini.

For the studied LLWB phytoplasma isolate, the RFLP analysis of the 1.8-kbp PCR product (SN910601/ SN011119) compared with the reference phytoplasma water dropwort witches' broom (WDWB), mulberry dwarf (MD), glehni aster yellow dwarf (GAYD), and jujube witches' broom (JWB). No pattern differences were observed among the 2 isolates of each studied LLWB phytoplasma, and RFLP patterns coincided with the putative restriction sites calculated from the 16S rDNA sequences (data not shown). In this comparison, similar patterns were found throughout the strains when restriction enzymes *Sca*I was used for analysis of the amplified phytoplasma sequences, indicating conservation of the restriction sites of these enzymes among the phytoplasmas examined. LLWB and JWB phytoplasma had identical *Kpn*I, *Hae*III and *Rsa*I patterns, which were different from the WDWB, MD, and GAYD phytoplasmas. However, *Alu*I and *Hpa*II produced RFLP patterns distinguishable between LLWB and JWB phytoplasmas (Fig. 3). The comparison of the collective RFLP patterns obtained in this work with others previously reported (Lee et al., 1998a) showed that the LLWB had the same profile as the CP phytoplasma.

LLWB symptoms were first reported in 1987 (La, 1987) and a phytoplasma was implicated as the etiological agent of the disease. The disease was referred to as bush clover witches' broom disease. However, there was no adequate information on the incidence, characterization, transmission, and particularly, classification of this disease. In this

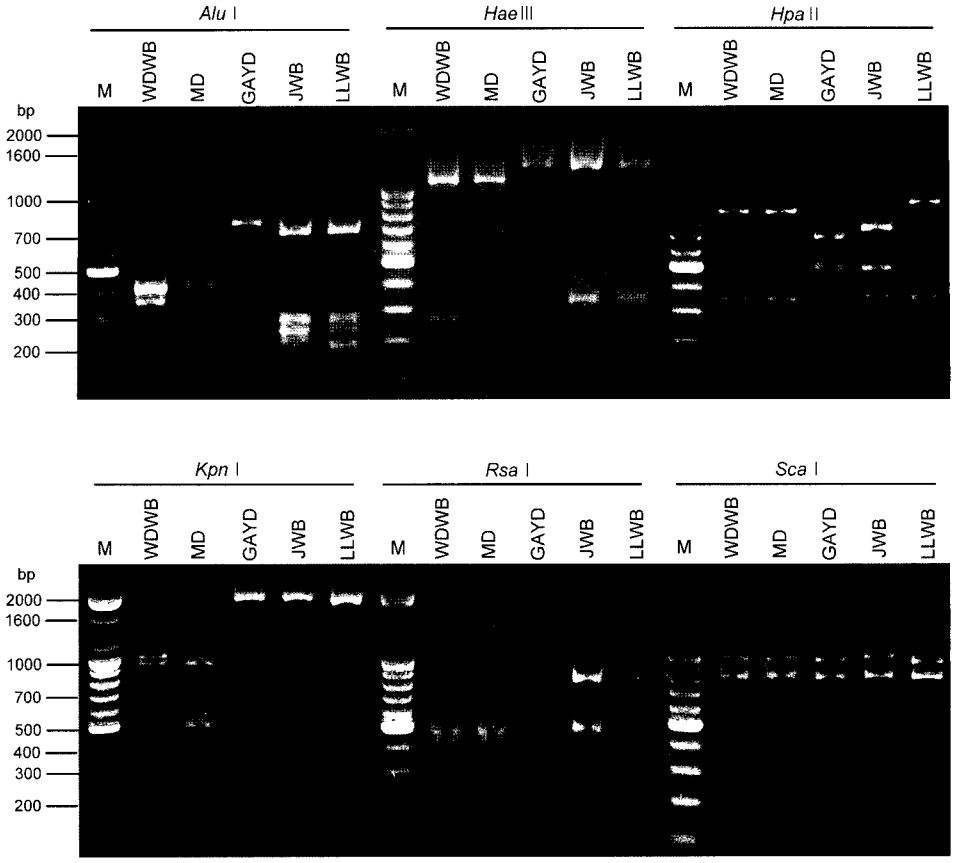

Fig. 3. Restriction enzyme analysis of 16S rDNA and 16S-23S spacer region DNA products after PCR amplification with primer pair (SN910601/SN011119), using endonuclease *Alu*I, *Hae*III, *Hpa*II, *Kpn*I, *Rsa*I and *Sca*I. Lane M, 100 bp DNA marker. Lanes WDWB to LLWB represent WDWB (Water dropwort witches' broom), MD (Mulberry dwarf), GAYD (Glehni aster yellow dwarf), JWB (Jujube witches' broom) and LLWB (Leafy lespedeza witches' broom) phytoplasma, respectively.

study, we demonstrated that the pathogen causing LLWB is indeed a phytoplasma. We then determined the phylogenetic placement of LLWB phytoplasma by amplifying and sequencing its 16S rDNA. The Sequencing analysis indicates the placement of this plant pathogenic phytoplasma within the class Mollicutes, specifically *Candidatus* Phytoplasma trifolii.

Acknowledgement

We thank Dr. Young-Il Hahm for kindly providing reference samples. This research was supported by Kyungpook National University Research Fund, 2006.

References

Hiruki, C. and Wang, K. 2004. Clover proliferation phytoplasma: "*Candidatus* Phytoplasma trifolii". *Int. J. Syst. Evol. Microbiol.* 54:1349-1353.

Jung, H. Y., Sawayanagi, T., Kakizawa, S., Nishigawa, H., Wei, W., Oshima, K., Miyata, S., Ugaki, M., Hibi, T. and Namba, S. 2003a. "*Candidatus* Phytoplasma ziziphi", a novel phytoplasma taxon associated with jujube witches' broom disease. *Int. J. Syst. Evol. Microbiol.* 53:1037-1041.

Jung, H. Y., Hahm, Y. I., Lee, J. T., Hibi, T. and Namba, S. 2003b. Characterization of a phytoplasma associated with potato witches' broom disease in Korea. *J. Gen. Plant Pathol.* 69:87-89.

La, Y. J. 1987. Current status of researches on mycoplasmal diseases of plants in Korea. *Korean J. Plant Pathol.* 3:22-27.

Lee, I. M., Dawn, E., Gundersen, D. E., Davis, R. E. and Bartoszyk, I. M. 1998a. Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences. *Int. J. Syst. Bacteriol.* 48:1153-1169.

Lee, I. M., Gundersen, D. E. and Bertaccini, A. 1998b. Phytoplasma: Ecology and genomic diversity. *Phytopathology* 88:1359-1366.