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SHORT COMMUNICATION / *COMUNICAÇÃO*

Detection of 16SrII Group Phytoplasma in China Aster (*Callistephus chinensis*)

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ABSTRACT

China aster, *Callistephus chinensis,* is widely grown in Myanmar as an ornamental plant. Symptoms of flower virescence were observed in diseased China aster plants in Yezin, Myanmar. The presence of a phytoplasma was detected and identified by applying Polymerase Chain Reaction (PCR)/ Restriction Fragment Length Polymorphism (RFLP) techniques and sequencing the 16S ribosomal DNA. The phytoplasma was identified as belonging to ribosomal subgroup 16SrII-A, never reported before in China aster. It showed almost 100 % similarity with a 16S rDNA sequence of sunn hemp witches' broom phytoplasma (AB558143), which belongs to the peanut witches' broom phytoplasma group. This is the first record of China aster as a new host for a subgroup 16SrII-A phytoplasma.
Key words: China aster, Phytoplasma, 16S rDNA, RFLP.

China aster (*Callistephus chinensis*) is an annual flower which belongs to the family Asteraceae and is native to China (Navalinskienė et al., 2005). As a traditional flower in Myanmar, it has been widely grown as an ornamental plant. Recently, flower virescence symptoms in China aster plants have been frequently observed in several fields of the Yezin area. The symptoms usually start with the emergence of new yellow leaves during the vegetative growth stage, followed by the leaf petiole turning upright with the clustering of leaves, and then the affected plant stops growing and it becomes stunted. At the later stage of plant growth, some flowers show green petals instead of their normal color. Occasionally, all flowers turn green (Figure 1). The development of such symptoms is usually attributed to phytoplasma, cell wall-less plant pathogenic prokaryotes (Lee et al., 2000). Therefore, the present study was undertaken to determine the presence of the phytoplasma associated with flower virescence in China aster and to classify it.

In February 2010, the affected plants showing flower virescence symptoms were collected from several fields of the Yezin area. Healthy plants were also collected from the same fields. Total DNA was extracted from leaves of five symptomatic and three asymptomatic samples using a cetyl trimethyl ammonium bromide (CTAB)-based method as previously reported (Namba et al., 1993), and was used as a template in polymerase chain reaction (PCR) primed by the universal primer pair SN910601 (5'-GTT TGA TCC TGG CTC AGG ATT-3') and SN011119 (5'-TCG CCG TTA ATT GCG TCC TT-3') (Jung et al., 2003). Sequencing was carried out by using seven previously described primers (Jung et al*., 2*003). PCR products of 1.8 kbp, including the 16S rDNA, 16S-23S rDNA spacer region and the beginning of the 23S rDNA were digested independently with ten restriction enzymes: *Alu* I, *Bfa* I, *Sau*3A I, *Hae* III, *Hha* I, *Hinf* I, *Mse* I, *Msp* I, *Rsa* I and *Taq* I, and the RFLP patterns were compared with those of sunn hemp witches' broom phytoplasma (ShWB- subgroup 16SrII-A), previously identified (*unpublished data*) and with water dropwort witches' broom phytoplasma (WDWB - subgroup 16SrI-B) (Jung et al., 2002). In the RFLP analysis, WDWB phytoplasma was selected to compare with the present putative phytoplasma since phytoplasmas from China aster plants have been reported to be members of group I phytoplasmas. The ShWB phytoplasma was chosen for comparison with a distinct phytoplasma group. A phylogenetic tree was also constructed to study the relationship of aster virescence phytoplasma with other *Candidatus* (*Ca.*) Phytoplasma species available in Genbank, using the neighbor-joining method with bootstrap analysis of 100 replicates.

Phytoplasmas were detected in all symptomatic samples tested, as demonstrated by the amplification of DNA fragments with the expected size (1.8 kbp), not detected in asymptomatic samples. Comparison of the 16S rDNA sequences of aster virescence (AV-Mm) phytoplasma with those of other phytoplasmas revealed 99% identity with members of peanut witches' broom phytoplasma group (PnWB): crotalaria witches' broom (EU650181), sweet potato witches' broom (DQ777762) and alfalfa

FIGURE 1 - Flower virescence symptoms on naturally infected *Callistephus chinensis* (left), and healthy (right) plants in the field.

witches' broom phytoplasmas (AB259169). Restriction patterns of the AV-Mm phytoplasma were identical to those of ShWB phytoplasma, and were clearly different from those of WDWB phytoplasma (Figure 2). All RFLP patterns of the AV-Mm phytoplasma were also concordant to those of peanut witches' broom phytoplasma (L33765, subgroup 16SrII-A) (Lee et al., 1998) after trimming of the AV-Mm sequences at the sites of R16F2n/R16R2 primers from 1.8 kbp to1.2 kbp. Therefore, the AV-Mm phytoplasma was classified as a member of the peanut witches' broom phytoplasma group by sequence analysis and as a representative of the 16SrII-A subgroup by RFLP analysis. Moreover, phylogenetic analysis showed the relationship of the AV-Mm phytoplasma with 16SrII group phytoplasmas, while two German isolates from China aster plants (AV2192 and AVUT) were related to group 16SrI (Figure 3).

With the present finding, China aster represents a new host for 16SrII group phytoplasmas. To date, phytoplasmas belonging to the 16SrI group have been detected on China aster plants, including subgroups 16SrI-A (Alberta aster yellows-AY27), 16SrI-B (European aster yellows-EAY), 16SrI-L (aster yellows- AV2192: AY180957) and 16SrI-M (aster yellows-AVUT: AY265209) (Marcone et al., 2000;

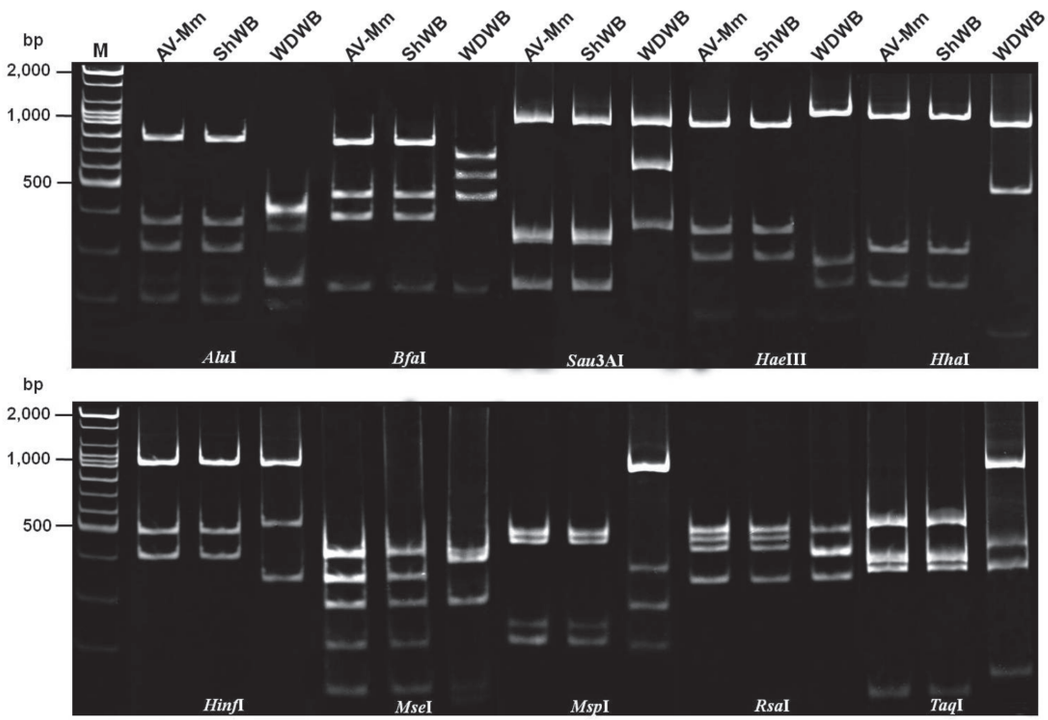

FIGURE 2 - PCR-RFLP profiles primed by SN910601 and SN011119 of aster virescence (AV-Mm) phytoplasma digested by restriction endonucleases *Alu* I, *Bfa* I, *Sau*3A I, *Hae* III, *Hha* I, *Hinf* I, *Mse* I, *Msp* I, *Rsa* I and *Taq* I compared to those of sunn hemp witches' broom (ShWB) and water dropwort witches' broom (WDWB) phytoplasmas. Lane M: 100 bp plus DNA ladder.

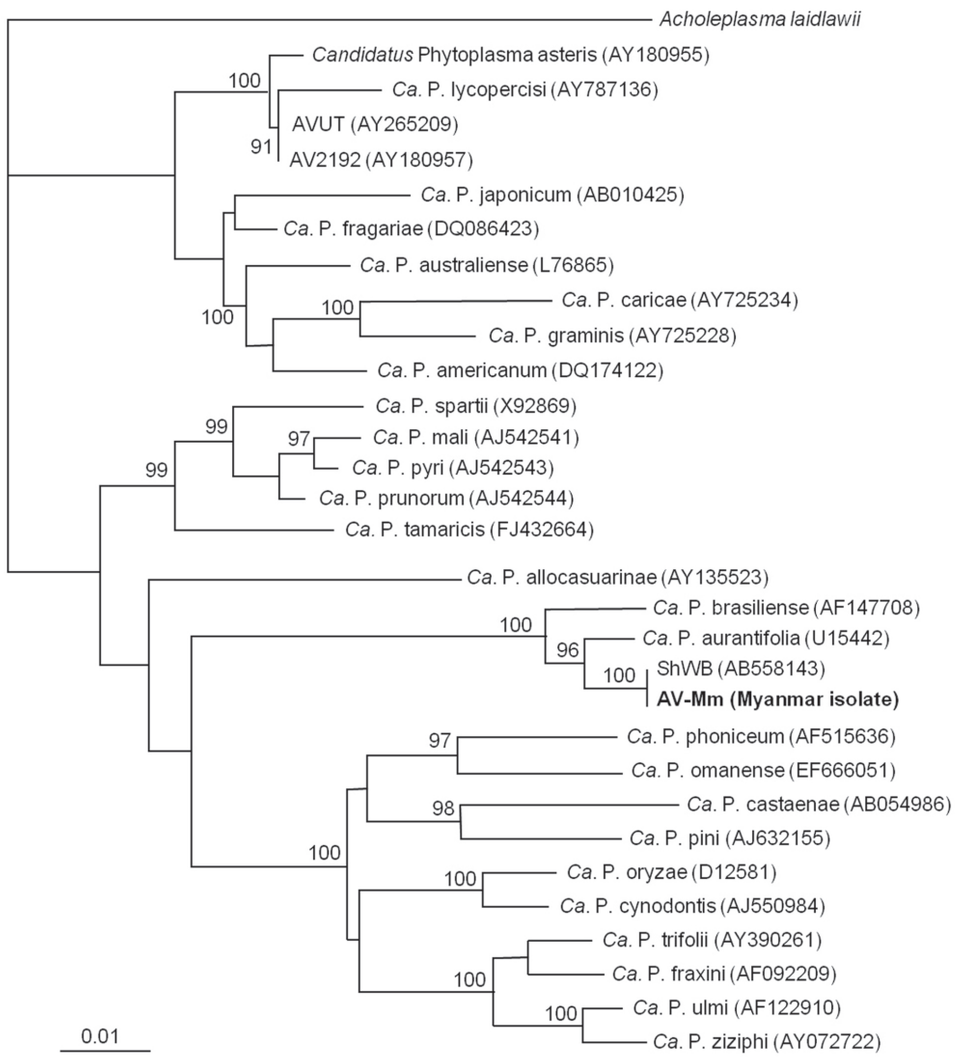

FIGURE 3 - Phylogenetic tree based on 16S rDNA sequences constructed by the neighbor-joining method showing relationships among the AV-Mm phytoplasma and reference phytoplasmas from GenBank. *Acholeplasma laidlawii* was used as an outgroup. Numbers on branches are confidence values obtained from 100 bootstrap replicates (only values above 80% are shown). AVUT: aster yellows, AV2192: aster yellows, ShWB: sunn hemp witches' broom, and AV-Mm: aster virescence phytoplasma.

Lee et al., 2004; Zhang et al., 2004). However, 16SrII group phytoplasms had not been reported on China aster until now. Recently, the identification of 16SrII group phytoplasmas on new hosts has been reported, including *Gypsophila* in Israel, chrysanthemum in Japan, and *Amaranthus* spp. in Mexico (Gera et al., 2006; Naito et al., 2007; Ochoa-Sánchez et al., 2009). The host range of 16SrII group phytoplasmas is now becoming wide not only within the family Fabaceae, but also within the Asteraceae. In addition, the 16S rDNA sequence of the AV-Mm phytoplasma was found to be identical to that of ShWB phytoplasma (AB558143) detected in the Yezin area. This result, therefore, suggests the possible involvement of insect vectors, although the potential insect vectors of witches' broom disease phytoplasmas remain unknown. The common brown leafhopper, *Orosius orientalis* (Matsumura) [= *albicinctus* (Distant)] was reported as an insect vector for the transmission of sesame phyllody phytoplasma in India, Iran, Thailand and Burkina Faso, and the associated phytoplasmas in Thailand and Iran belong to the peanut witches' broom phytoplasma group (16SrII) (Schneider et al., 1995; Esmailzadeh-Hosseini et al., 2007).

Since climatic conditions in Myanmar are favorable for the spread of insect vectors, leafhoppers could be suggested as being responsible for spreading phytoplasma diseases. Further studies need to be undertaken in order to define the relationship between phytoplasma transmissibility and putative insect vectors. Mostly, China aster plants are vegetatively propagated from cutting of young shoots rather than direct sowing of seeds. Although disease incidence was low, the use of infected propagating materials may become the main source for spreading this phytoplasma. Based on this result and previous studies, it could be concluded that China aster plants are potential reservoirs for distinct phytoplasmas.

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