Disease Note

FIRST REPORT OF A '*CANDIDATUS* PHYTOPLASMA ASTERIS' RELATED PHYTOPLASMA ASSOCIATED WITH EUCALYPTUS LITTLE LEAF DISEASE IN IRAN

M. Salehi[1], S.A. Esmailzadeh Hosseini[2] and E. Salehi[1]

[1] *Plant Protection Research Department, Fars Agricultural and Natural Resources Research and Education Centre, AREEO, Zarghan, P. O. Box. 1151-71955, Iran*
[2] *Plant Protection Research Department, Yazd Agricultural and Natural Resources Research and Education Centre, AREEO, Yazd, Iran*

During 2008-13 surveys, *Eucalyptus camaldulensis* little leaf (ELL) disease was observed in Firuzabad, Shiraz and Darab (Fars province, Iran), consisting of proliferation of auxiliary buds, formation of brooms on branches and trunk, yellowing, reddening and reduction of the leaf size, tree decline and death within 3-4 years from the onset of symptoms. Total DNA extracted from 15 ELL-affected and five symptomless plants was tested by direct and nested PCR using the phytoplasma primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al.,* 1995) followed by R16F2n/R16R2 (Gundersen and Lee, 1996). Amplicons of *ca*. 1.25 kbp were obtained from 9 of 15 symptomatic but not from all symptomless and six symptomatic trees, probably due to the low titres and unevenly distribution of phytoplasma or the presence of PCR inhibitors in the latter. Nine R16F2n/R16R2 amplicons from infected samples (three from each sampled area) were directly sequenced in both directions, one of which from Firuzabad ELL (FELL) was deposited in GenBank (Accession No. KT992689). BLAST search showed that the FELL phytoplasma had 100% sequence identity with four 16SrI ('*Candidatus* Phytoplasma asteris') group-related phytoplasmas including mulberry dwarf, aster yellows, onion yellows, sasa witches' broom and epilobium phyllody phytoplasmas (AB693124, FJ824597, AP006628, AB293421, AY101386). Computer-simulated restriction analysis using *i*PhyClassifier revealed that the virtual RFLP pattern derived from the query 16S rDNA F2nR2 fragment of FELL is identical (similarity coefficient 1.00) to onion yellows phytoplasma (NC_005303) representative of subgroup B of the 16SrI group. Phylogenetic analysis revealed that FELL phytoplasma clustered with 16SrI- B members closer to a phytoplasma associated with Eucalyptus yellows and witches' broom (AY685054). To our knowledge, this is the first report of the association of a 16SrI phytoplasma with ELL in Iran.

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Corresponding author: M. Salehi
E-mail: salehi_abarkoohi@yahoo.com

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Disease Note

FIRST REPORT OF FRECKLE DISEASE OF BANANA CAUSED BY *PHYLLOSTICTA CAPITALENSIS* IN GUANGXI, SOUTHWEST CHINA

J.M. Sun[1], Y. Zhang[2], J.Z. Zhang[3], X. Lan[1] and J. Lu[1]

[1] *Guangxi Crop Genetic Improvement and Biotechnology Key Lab, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, 530007, China*
[2] *Institute of Plant Protection, Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, 530007, China*
[3] *Institute of Biotechnology of Guangxi Academy of Agricultural Sciences, Nanning, Guangxi, 530007, China*

In December 2013, irregular spots with gray centers, dark brown edges and chlorotic halos were observed on the old leaves of bananas of cv. Williams (AAA, Cavendish) in Guangxi province (China), with an incidence of 15% to 30%. Small pieces of diseased leaf tissues were surface-sterilized in 75% ethanol for 30 s followed by 2 min in 0.1% $HgCl_2$, rinsed in sterile water, plated on potato dextrose agar (PDA) and incubated at 28°C in darkness. A single-spore culture had initially an olive or dark green mycelium with granular surface and white edges which, over time, turned almost black. Asci containing 8 ascospores, were clavate or cylindrical, stipitate, and measured 35.6-43.6 × 8.5-10.6 µm. Conidia measured 7.2-11.8 × 4.0-6.3 µm, and were obpyriform or nearly elliptic. Single spore cultures of the isolate were obtained and used for molecular identification. The internal transcribed spacer (ITS) region of rDNA was amplified and sequenced by utilizing the primers ITS4/ITS5 (2). The ITS region of the isolate (Accession No. LM994823) was 629 bp in length. BLAST analysis of the sequences showed a 99% homology with two *Phyllosticta capitalensis* isolates (accession Nos KM979840 and JQ743587). Pathogenicity was conducted using healthy banana plants with five or six leaves, approximately 30 cm in height. Nine plants were used for each treatment and three leaves of each plant were artificially inoculated. Mycelial plugs were placed on the surface of the leaves, whereas controls were treated only with agar plugs. All the treated plants were covered with plastic bags for 2 days and incubated at 28°C, 75% relative humidity (RH) and with a 12-h photoperiod. After 7 days, typical lesions identical to those observed on the field-grown plants appeared on the inoculated plants, whereas control plants remained healthy. The re-isolated fungus was identified as *P. capitalensis* by morphology and molecular analysis. *P. capitalensis* is generally considered an endophyte or saprophyte, within a wide host range and distributed in different plants (Wulandari *et al.*, 2010; Wong *et al.,* 2012). However, it is a weak plant pathogen which was the causal agent of plant diseases in few cases (Wikee *et al.*, 2013). To our knowledge, this is the first report of *P. capitalensis* causing freckle disease of banana in China.

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Corresponding author: J. Lu
E-mail: jiang.lu.cau@gmail.com

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