



First report of the association of a 'Candidatus Phytoplasma ulmi' isolate with a witches' broom disease of reed in China

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Phragmites australis (Poaceae) is widely distributed around the world. It is not only a perennial weed, but also a raw material for the paper industry, a medicinal plant, animal fodder, an ornamental in landscaping and a building material. It now covers approximately two million ha in China, including ten thousand ha in Xinjiang Province. During September 2012, symptoms of witches' broom, stunting, and smaller leaves (Fig. 1A) were observed in over 57% of *P. australis* plants at the Shihezi University Experimental Station (Shihezi, Xinjiang Province).

Leaf samples were collected from both symptomless plants and plants showing symptoms. Total DNA was extracted from 0.5 g fresh leaf midribs from *P. australis* plants with and without symptoms, using the CTAB method (Angelini *et al.*, 2001). The DNA extracts were tested for phytoplasma infection by a nested polymerase chain reaction (PCR) assay with phytoplasma universal 16S rDNA primer pairs P1/P7 (Deng & Hiruki, 1991) and R16F2n/R16R2 (Gundersen & Lee, 1996). The two primer pairs produced PCR products of 1.8 and 1.2 kb, respectively, from most of the plants with symptoms (4/5), but not from the symptomless plants (2/2).

R16F2n/R16R2 amplicons were cloned (pMD18-T simple vector, TaKaRa, China) and sequenced. All of the phytoplasma R16F2n/R16R2 sequences were 100% identical to each other. BLAST comparisons showed that the *P. australis* witches' broom phytoplasma 16S rDNA consensus sequence (GenBank Accession No. KC331052) had 99% sequence identity with those of members of group 16SrV ('Candidatus *Phytoplasma ulmi*'). Similarity coefficients and *in silico* restriction fragment length polymorphism (RFLP) analyses of the R16F2n/R16R2 sequence with *AluI*, *BamHI*, *BfaI*, *BstUI*, *DraI*, *EcoRI*, *HaeIII*, *HhaI*, *HinfI*, *HpaI*, *HpaII*, *KpnI*, *Sau3AI*, *MseI*, *RsaI*, *I*, and *TaqI* restriction endonucleases (iPhyClassifier, <http://plantpathology.ba.ars.usda.gov/cgi-bin/resource/iphyclassifier.cgi>) (Fig. 2), indicated that *P. australis* contained a phytoplasma strain identical to those of the reference strain AB052876, 'Ca. *Phytoplasma ulmi*', subgroup 16SrV-B. A neighbour-joining dendrogram (MEGA 5.0, USA) based on the 16S rDNA sequences of the *P. australis* witches' broom phytoplasma and twenty-five reference phytoplasmas in NCBI database supported the PCR and RFLP results since the *P. australis*

witches' broom phytoplasma grouped within the 16SrV-B phytoplasma clade (Fig. 3). The group 16SrV was previously associated with witches' broom diseases of several plant species in China including jujube (Zhu *et al.*, 1997), paper mulberry (Liu *et al.*, 2004), and Chinese honeylocust (Min *et al.*, 2009). This is the first report of a 'Ca. *Phytoplasma ulmi*'-related strain affecting *P. australis* in China.

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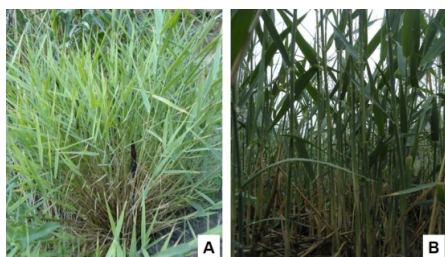


Figure 1

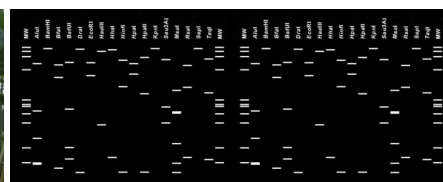


Figure 2

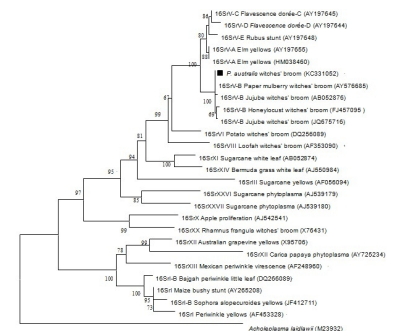


Figure 3

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