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'*Candidatus* Phytoplasma cynodontis' (16SrXIV group) affecting *Oplismenus burmannii* (Retz.) P. Beauv. and *Digitaria sanguinalis* (L.) Scop. in India

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Abstract. A phytoplasma has been detected in *Oplismenus burmannii* (Retz.) P. Beauv. and *Digitaria sanguinalis* (L.) Scop. plants with symptoms of chlorotic streaks and white leaf disease in India. By sequence and phylogenetic analyses of polymerase chain reaction-amplified rDNA sequences, the detected phytoplasma was identified as '*Candidatus* Phytoplasma cynodontis', a member of the 16SrXIV group, and the causal agent of Bermuda grass white leaf disease. This is the first report on the occurrence of '*Ca.* Phytoplasma cynodontis' in *O. burmannii* and *D. sanguinalis* grasses.

In India, several weed species have been reported to act as reservoir hosts of many phytoplasmas (Mall 2009). These species include *Cynodon dactylon*, *Parthenium hysterophorus*, *Cannabis sativa*, *Achyranthes aspera*, *Amaranthus* spp., *Datura inoxia* and *Dichanthium annulatum* (Rao *et al*. 2007, 2009; Raj *et al*. 2008). The phytoplasmas infecting the mentioned weeds, belonging mainly to 16SrI, 16SrII and 16SrVI groups, are also known to induce yellow diseases of considerable economic importance in vegetable, ornamental, medicinal, forage and fruit crop plants in India (Mall 2009). Among the most affected cultivated plants are *Daucus carota* (carrot), *Phaseolus vulgaris* (French bean), *Capsicum annuum* (pepper), *Solanum melongena* (eggplant), *Cajanus cajan* (pigeon pea), *Sesamum indicum* (sesame), *Gladiolus* spp. and *Citrus aurantifolia* (acid lime) (Ghosh *et al*. 1999; Khan and Raj 2006; Raj *et al*. 2006, 2009; Khan *et al*. 2007; Arocha *et al*. 2009). During surveys of phytoplasmal diseases of weeds in 2009, symptoms resembling those induced by phytoplasmas were observed on two grasses of the family Poaceae, namely, *Oplismenus burmannii* (Retz.) P. Beauv. and *Digitaria sanguinalis* (L.) Scop. The *O. burmannii* plants showed chlorotic streaks on the leaves when compared with healthy ones (Fig. 1*a*) at the Sugarcane Research Station campus, Gorakhpur. However, the *D. sanguinalis* plants exhibited chlorosis of leaves (Fig. 1*b*) at the Indian Agricultural Research Institute campus, New Delhi. Disease incidence ranged from 2% to 8% at both locations. Symptomatic *O. burmannii* and *D. sanguinalis* plants were examined for phytoplasma infections employing highly sensitive polymerase chain reaction (PCR) technology. The detected phytoplasma was identified and characterised using sequence and phylogenetic analyses of PCR-amplified ribosomal DNA (rDNA).

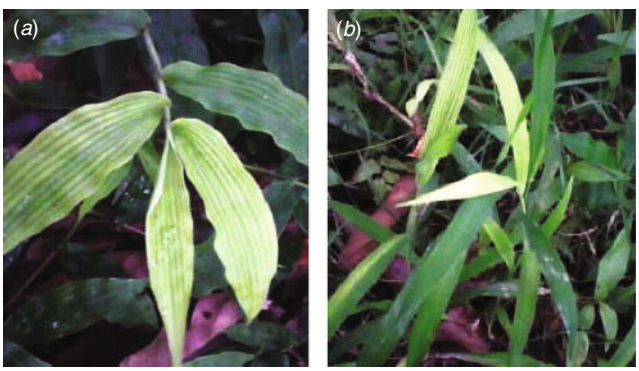

Fig. 1. *Oplismenus burmannii* (*a*) showing chlorotic streaks on the leaves and *Digitaria sanguinalis* (*b*) showing chlorotic leaves.

DNA was extracted from leaves of 15 diseased and 10 symptomless plants of each species examined, employing the phytoplasma enrichment procedure described by Ahrens and Seemüller (1992). For PCR amplification, the universal phytoplasma primer pair P1/P6 (Deng and Hiruki 1991) followed by nested primer pair R16F2n/R16R2 (Gundersen and Lee 1996), were used. These primer pairs amplified a 16S rDNA fragment of ~1500 and 1245 bp in length, respectively. PCR conditions and agarose gel electrophoresis analysis of PCR products were used as previously described (Gundersen and Lee 1996). All symptomatic plants of both the grass species tested positive for phytoplasma infections, whereas no PCR products

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were amplified from DNA extracted from symptomless plants. The R16F2n/R16R2 PCR products were separated by electrophoresis using a 1.5% agarose gel, excised from the gel and eluted using the QIAquick gel extraction kit (Qiagen, Hilden, Germany). The DNA fragments were then cloned and five recombinant clones from each grass species were sequenced. Sequences were then assembled and edited using DNASTAR's Laser Gene software (DNASTAR) and consensus sequences generated. Sequence alignments were performed by using CLUSTAL version 5, of the same software. The sequences obtained in the present work have been deposited in GenBank database (http://www.ncbi.nlm.nih.gov/Genbank) under the accession numbers GQ403690 (*O. burmannii* isolate) and GQ403689 (*D. sanguinalis* isolate).

Phylogenetic and molecular evolutionary analyses were conducted using the neighbour-joining program of the genetic analysis software Molecular Evolutionary Genetics Analysis (MEGA), version 4 (Tamura *et al*. 2007). *Acholeplasma laidlawii* was included in the analyses as an outgroup. Nucleotide sequence comparisons revealed that the phytoplasmas detected in diseased *O. burmannii* and *D. sanguinalis* plants shared 98% 16S rDNA sequence similarities to each other and 99% with members of the Bermuda grass white leaf (BGWL) phytoplasma group or 16SrXIV group which includes '*Ca.* Phytoplasma cynodontis' (GenBank accession numbers AJ550984 and EF444485), and 97% sequence similarities with sugarcane white leaf (SCWL) phytoplasma (GenBank accession numbers FM208259 and AB052874). Also, the mentioned phytoplasmas clustered together with members of the BGWL group (Fig. 2). Therefore, the phytoplasmas infecting *O. burmannii* and *D. sanguinalis* plants in India were identified as isolates of '*Ca.* Phytoplasma cynodontis' (Marcone *et al*. 2004). This taxon has previously been reported from India in Bermuda grass (Rao *et al*. 2007; Snehi *et al*. 2008) and *Dichanthium annulatum* (Rao *et al*. 2009). To our knowledge this is the first report on the occurrence of '*Ca.* Phytoplasma cynodontis' in *O. burmannii* and *D. sanguinalis* grasses. Recognition of weeds as hosts of 16SrXIV group phytoplasmas in India has epidemiological significance and therefore we suggest that emphasis should be given for weed control in and around agricultural fields to minimise phytoplasma disease incidence.

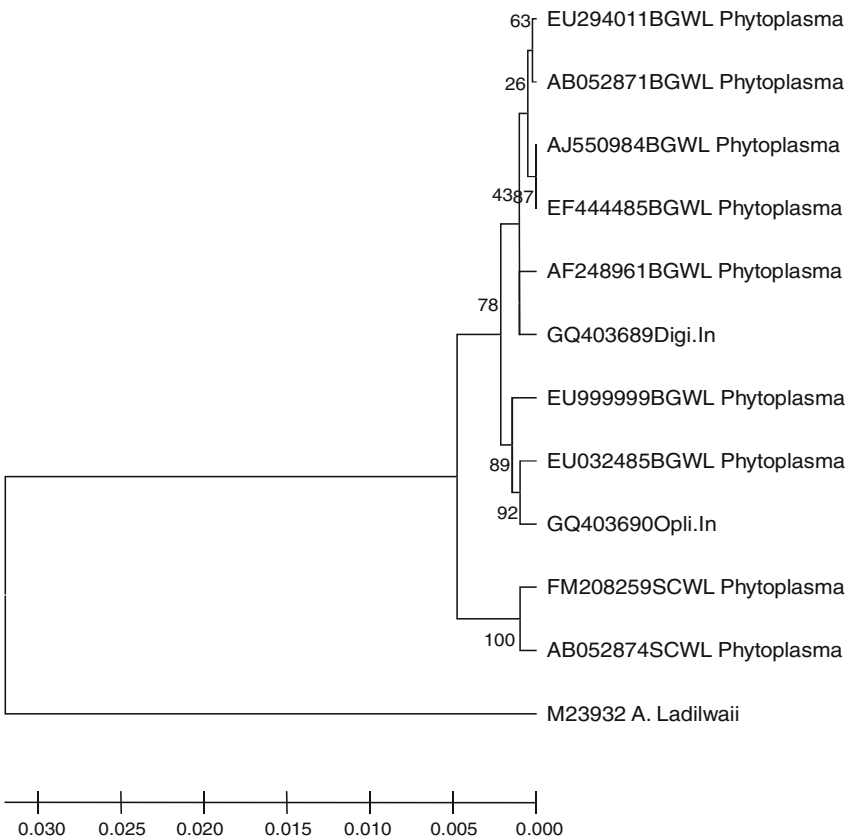

Fig. 2. Phylogenetic tree constructed using the neighbour-joining program of the genetic analysis software Molecular Evolutionary Genetics Analysis (MEGA), version 4, with the 16S rDNA sequences from phytoplasma isolates infecting *Oplismenus burmannii* and *Digitaria sanguinalis* plants in India (Opli.In and Digi.In, respectively) and Bermuda grass white leaf (BGWL) phytoplasma group isolates from around the world. The numbers above the branches indicate the bootstrap values, whereas those below the scale bar represent branch length. *Acholeplasma laidlawii* was used as the outgroup. GenBank accession numbers are shown.

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