

Molecular identification of 'Candidatus Phytoplasma cynodontis' associated with Bermuda grass white leaf disease in India

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Bermuda grass white leaf (BGWL) is a destructive disease of Bermuda grass (*Cynodon dactylon*), which is known to occur in several countries. BGWL was noticed on Bermuda grass during spring 2007 at several locations in Gorakhpur, UP, India. The diseased plants showed typical white leaf symptoms, proliferation of axillary shoots, bushy growing habit, small leaves and shortened stolons.

Samples from plants with symptoms were collected and total DNA was extracted from approximately 100 mg of leaf tissue employing a phytoplasma enrichment procedure (Ahrens & Seemüller, 1992). The amplicons of ~1500 bp and 1250 bp were amplified by PCR using P1/P6 universal primers (Deng & Hiruki, 1991) followed by nested PCR with R16F2n/R16R2 primers (Gundersen & Lee, 1996), respectively. The ~1250 bp amplicon was cloned, sequenced and data submitted to GenBank (Acc. No. EU032485) which shared highest identity 99% with isolates of 'Candidatus Phytoplasma cynodontis': Juyom Bermuda grass white leaf phytoplasma (EF444486) and Firoozabad Bermuda grass white leaf phytoplasma (EF444485) from Iran; Bermuda grass white leaf phytoplasma (AF248961), 'Ca. Phytoplasma cynodontis' (AB052871) and Brachiaria grass white leaf phytoplasma (AB052872) from Thailand; and Bermuda grass white leaf phytoplasma (AJ550984) from Italy.

A white leaf disease on *C. dactylon* had previously been reported to occur in India on the basis of symptomatology, remission of symptoms

after tetracycline treatment of affected plants, and electron microscopic observations (Singh *et al.*, 1978). However, molecular identification of the causal agent and its phylogenetic relatedness to other phytoplasmas has not previously been reported. This is the first molecular identification of 'Ca. Phytoplasma cynodontis' (16SrXIV group) associated with Bermuda grass white leaf disease in India.

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First report of the presence of the lethal yellowing group (16Sr IV) of phytoplasmas in the weeds *Emilia fosbergii* and *Synedrella nodiflora* in Jamaica

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Coconut lethal yellowing (CLY) disease is the single most important disease affecting coconuts in Jamaica. A study was conducted in 2005 to investigate alternative hosts of the CLY phytoplasma affecting coconuts. Weeds have been known to act as reservoir hosts of many plant pathogens. As a result, weeds growing in areas where coconuts are planted were tested for the presence of the CLY phytoplasma. The weeds *Emilia fosbergii* and *Synedrella nodiflora* (Asteraceae) were collected at random from coconut farms in areas that had high as well as low incidences of CLY. None of the weeds showed symptoms. DNA from weed samples was extracted by the method of Dellaporta *et al.* (1993), while nucleic acid extraction from the reference coconut trunk sample (coconut plant infected with CLY) was done using the method of Doyle & Doyle (1990). Nested PCR was conducted using the primer pairs P1/P7 and LY16Sf/LY16S23r (Harrison *et al.*, 2002a,b).

A 1.6 kb amplicon was obtained from 9/51 *E. fosbergii* plants tested and 4/36 *S. nodiflora* plants. The amplicons were compared by RFLP with the restriction endonucleases *RsaI*, *MspI*, *MseI*, *TaqI*, *HinfI*, *AluI* and *HbaI* to that of the CLY reference sample. Each pattern was identical to that of the reference sample (data not shown). Amplicons from *E. fosbergii* and *S. nodiflora* were cloned into pGEM@-T Easy cloning vector (Promega) and sequenced using the primers LY16Sf/LY16S23r at the Center for Functional Genomics (University of Albany, NY, USA). Sequences were deposited in the GenBank (Accession No. EU026214 *E. fosbergii* and EU026213 *S. nodiflora*). Blast analysis of the rDNA sequences showed a 99% similarity to that of coconut lethal yellowing phytoplasma in Jamaica, Nevis and Florida (GenBank Accession No. AF498301, DQ378279 and AF498309), members of the 16Sr IV group of phytoplasmas.

This is the first report of the lethal yellowing (16Sr IV) group of phytoplasmas affecting the weeds *E. fosbergii* and *S. nodiflora* in Jamaica. Recognition of weeds as hosts of 16Sr IV group of phytoplasmas in Jamaica has epidemiological significance and suggests that weed control in and around coconuts may benefit disease management over the long term. It also lends credence to the possibility of the existence of another CLY vector other than *Myndus crudus* which has been known to feed only on monocotyledonous plants.

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