

## A new phytoplasma associated with witches' broom of *Cassia italica* in Oman

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### Abstract

In several Oman locations plants of *Cassia italica* exhibit witches' broom symptoms. Samples were collected from four locations, and examined for phytoplasma presence. PCR amplification using ribosomal phytoplasma primers followed by RFLP analyses indicates that the phytoplasmas present in these samples were undistinguishable from each other, but differed from those reported in the literature. RFLP and phylogenetic analysis of 16S rRNA gene plus spacer sequence confirmed that the closest phytoplasma relatives were members of the pigeon pea witches' broom phytoplasma ribosomal group (16SrIX), sharing a 93-97% sequence similarity. Nested PCR experiments using primers amplifying the gene coding for ribosomal protein S3 provided amplification from phytoplasmas detected in *C. italica*, after sequencing 600 bp in this gene the higher homology showed was 78% with phytoplasmas related to '*Candidatus* Phytoplasma phoenicium' and 77% with phytoplasmas belonging to ribosomal group 16SrIX.

**Key words:** Plant disease, phytoplasma, ribosomal gene, ribosomal protein S3 gene, sequencing, PCR/RFLP analyses.

### Introduction

*Cassia italica* (Mill.) Lam. (Italian senna) is a wild plant distributed throughout the the Arabian Peninsula. It originated from Sudan (Africa) and produces yellow flowers in bunches that become black when completely open. The seeds are poisonous to animals and used for medical and cosmetic purposes, mainly for henné production.

During the spring and fall of 2006, a phytoplasma-like disease was observed in *C. italica* in several locations in Oman. The first symptoms were observed in April, and an increasing percentage of symptomatic plants were found in the following months. Reduction of leaf size, stunting, axillary bud proliferations (witches' broom), virescence, phyllody and partially or complete rosetting of flowers were observed.

### Materials and methods

*C. italica* samples from plants showing witches' broom symptoms and from asymptomatic plants were collected from Manah, Al-Hamrah, Bahla, and Nizwa in Al-Sharqiya region in Oman. For PCR detection total nucleic acid extraction using an initial enrichment step (Ahrens and Seemüller, 1992) followed by the Doyle and Doyle (1990) procedure was employed. PCR was carried out using universal primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) and products were analysed by RFLP analyses with *TruI*, *TaqI*, *RsaI*, *HaeIII*, and *HhaI*. The resulting RFLP patterns were compared with those of the most representative phytoplasma strains maintained in the reference collection (Bertaccini *et al.*, 2000), and with previously described phytoplasma strains belonging to 16SrIX ribosomal group (Abou-Jawdah *et al.*, 2003).

Further amplification was carried to amplify gene coding S3 ribosomal protein. After direct PCR using prim-

ers rpL2F3/rp(I)R1A, a first nested PCR was performed with primers rpF1C/rp(I)R1A, and then a final PCR run with primers rp(IX)F2/rp(IX)R2, specific for some of the 16S ribosomal groups, was applied (Martini *et al.*, 2007). Phytoplasma strains belonging to subgroup 16SrIX (*Pichris echinoides* yellows, PEY and Naxos virescence, NAXOS; ribosomal subgroup 16SrIX-C) and 16SrXI (Leafhopper transmitted, BVK ribosomal subgroup 16SrXI-C) were employed as positive controls. RFLP analyses and sequencing after cleaning with Qiagen kit for PCR products were then carried out on the rp amplicons from *C. italica*.

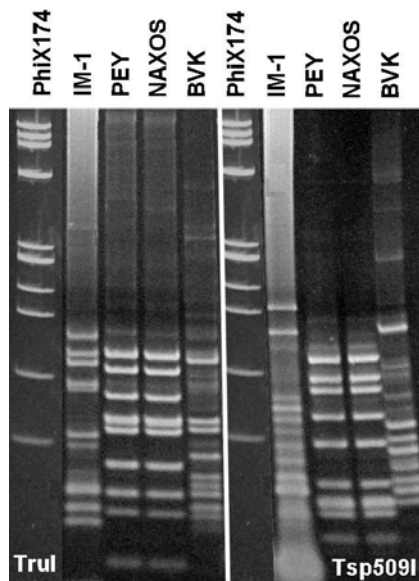
P1/P7 product from symptomatic *C. italica* sample IM-1 was purified using PCR Kleen spin columns (Bio-Rad) and cloned into *Escherichia coli* using the TOPO-TA cloning kit (Invitrogen) according to the manufacturers' instructions. After sequencing and alignments a phylogenetic tree was constructed using *Acholeplasma laidlawii* as out group taxon to root the tree.

### Results and discussion

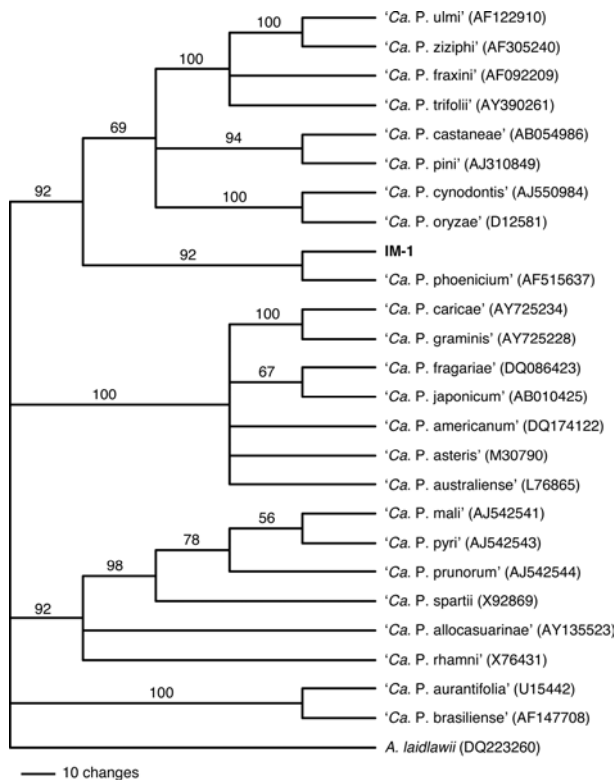
All the symptomatic samples collected from Al-Sharqiya region were positive for phytoplasma presence. RFLP analysis on P1/P7 amplicons from four representative symptomatic *C. italica* plants samples indicated that they contained phytoplasmas that could not be differentiated from each other, but that were distinguishable from all the reference strains used.

Bands of expected length (c. 800 bp) were obtained after nested PCR amplification with the rpS3 primers. RFLP analyses with *TruI* and *Tsp509I* (figure 1) and sequencing of 600 bp of obtained amplicons indicates that the phytoplasmas infecting *C. italica* are distantly related to '*Candidatus* Phytoplasma phoenicium' (Verdin *et al.*, 2003).

Comparative analysis of 16S rRNA gene plus spacer sequence (1,642 bp; EF666051) indicated that strain



**Figure 1.** RFLP patterns on polyacrylamide gels of rpS3 gene from phytoplasmas infecting *C. italica* in Oman.



**Figure 2.** Phylogenetic tree constructed by parsimony analysis of nearly full-length of 16S rRNA sequences from described 'Ca. Phytoplasma' species and the phytoplasma strain IM-1. Branch lengths are proportional to the number of inferred character state transformations. Bootstrap values > 50% are shown on branches.

IM-1 share 97% homology with phytoplasma stains VLL (AJ289195), 96% with ViLL, 95% with Iranian LSP (DQ889749) and 93% with 'Ca. P. phoenicium' (AF515637) and PnPWB (EF186825). Ribosomal protein S3 gene of the same strain (EU024406) shared 78% homology with 'Ca. P. phoenicium' (EF186803.1) and 77% with strains mainly belonging to ribosomal group 16SrIX.

Phylogenetic analysis using nearly full-length 16S rRNA gene sequence of strain IM-1, and of 24 'Ca. Phytoplasma' species is presented in figure 2. Based on RFLP data and phylogenetic analyses the phytoplasma identified in *C. italica* in Oman appears to belong to a new ribosomal group designed as 16SrXIX, and represent a new 'Candidatus' genus designed as 'Candidatus Phytoplasma omanense' (Al Saady *et al.*, 2007).

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