



First report of a '*Candidatus Phytoplasma asteris*' isolate associated with *Macroptilium lathyroides* yellow leaf disease in Brazil

M.S. Alves^{1,2*}, A.N. de Souza¹, G.M. Ribeiro¹ and C.M. Carvalho¹

¹ Instituto Nacional de Ciência e Tecnologia em Interações Planta-Praga / Departamento de Fitopatologia, Universidade Federal de Viçosa, Avenida PH Rolfs s/n, 36570-900, Viçosa, Minas Gerais, Brazil; ² Department of Biochemistry and Molecular Biology, Universidade Federal do Ceará, Campus do Pici, Avenida Humberto Monte s/n, 60440-900, Fortaleza, Ceará, Brazil

*E-mail: murilobqi@gmail.com

Received: 06 Feb 2018. **Published:** 27 Feb 2018. **Keywords:** '*Candidatus Phytoplasma asteris*'; Fabaceae, *Macroptilium lathyroides*, 16S rRNA

Macroptilium lathyroides, known as phasey bean is a member of the Fabaceae native to the Americas (Bianco *et al.*, 2014). It is commonly cultivated for fodder and green manure, and is considered an invasive weed in crop orchards and public areas in Brazil. Three *M. lathyroides* vines located in the coastal area of the municipality of Vila Velha (S20°23'28.0572", W40°19'18.5016", Espírito Santo, Brazil), exhibited little leaves, yellowing and stunting symptoms, commonly associated with a phytoplasma infection (Fig. 1). None of the symptoms were observed in the axillary shoots (Fig. 1).

In order to verify whether such symptoms were associated with a phytoplasma, leaves were collected from three diseased and three symptomless plants, these latter ones being used as negative controls. Total nucleic acid was extracted from 200 mg of each sample (NucleoSpin II Plant Mini Kit, Macherey-Nagel, Germany), and subjected to nested PCR with universal primers P1/P7 followed by R16F2n/R2 that amplify the phytoplasma 16S rRNA (Gundersen *et al.*, 1996). Nested PCR amplicons of the expected size (1.2 kb) were obtained from all the diseased plants but not from the negative controls. The 16S rDNA amplicons were purified (Wizard[®]; SV Gel and PCR Clean-Up System, Promega, USA), cloned in pGEM-T Easy vector (pGEM[®]-T Easy Vector System, Promega, USA) and sequenced (Macrogen Inc., Korea). BLASTn analysis showed that the 16S rDNA sequence of the *Macroptilium lathyroides* yellow leaf disease (MLYL) phytoplasma shared 99.3% sequence identity with that of the 16SrI group, '*Candidatus Phytoplasma asteris*'. The 16S rDNA nucleotide of the MLYL phytoplasma was deposited in GenBank (Accession No. KY270560). The virtual RFLP patterns generated by *rPhyClassifier* (Zhao *et al.*, 2013) on the MLYL phytoplasma 16S rDNA sequence with all the 17 enzymes except for *HhaI*, *HinfI* and *MseI* were similar to those shown by phytoplasma members of the 16SrI-S subgroup (HM067755). Actual RFLP was performed on nested PCR amplicons of the MLYL phytoplasma with *HhaI*, *HinfI* and *MseI* restriction endonucleases and exhibited the exact RFLP profiles of the 16SrI-S phytoplasma subgroup. Both actual and virtual RFLPs indicated that the MLYL phytoplasma was a member of the 16SrI-S subgroup.

Previous studies reported phytoplasmas infecting *M. lathyroides* in the

Caribbean (Arocha *et al.*, 2005; Brown & McLaughlin 2011). To our knowledge, this is the first report of a 16SrI group phytoplasma and a member of the 16SrI-S subgroup infecting *M. lathyroides* in Brazil. Since the 16SrI phytoplasma group has the largest host range among all phytoplasmas, this finding may pose a phytosanitary threat for other species in the family Fabaceae in Brazil, such as soybean and common bean.

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Figure 1

To cite this report: Alves MS, de Souza AN, Ribeiro GM, Carvalho CM, 2018. First report of a '*Candidatus Phytoplasma asteris*' isolate associated with *Macroptilium lathyroides* yellow leaf disease in Brazil. *New Disease Reports* **37**, 11.

<http://dx.doi.org/10.5197/j.2044-0588.2018.037.011>

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