Received: 28 May 2022 | Accepted: 28 June 2022

DOI: 10.1002/ndr2.12103

New Disease Reports Open Access BSPP

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NEW DISEASE REPORT

First report of a 16SrIII-X phytoplasma associated with *Lactuca sativa* witches'-broom in Argentina

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Funding information

Instituto Nacional de Tecnología Agropecuaria, Grant/Award Number: INTA/2019-PD-E4-I090-001; Fondo para la Investigación Científica y Tecnológica, Grant/Award Number: PICT 2016-0862

KEYWORDS

lettuce, X-disease group, phylogeny, RFLP

During the summer of 2022, symptoms of witches'-broom, flowering malformation and redness were observed in lettuce plants (*Lactuca sativa*) in fields in Cordoba Province, Argentina (Fig. 1a). In the same fields, garlic (*Allium sativum*) plants exhibiting symptoms of phyllody (Fig. 1b-c) were also observed. To confirm the presence of phytoplasma, leaf samples from two diseased lettuce and three garlic plants were collected. As negative controls, leaf samples from symptomless plants (one lettuce and two garlic) were collected.

Total DNA was extracted from each sample using the CTAB method and quantified by spectrophotometry using a NanoDrop-1000 (Thermo Scientific, USA). The DNA was used as a template for PCR reactions using the 16Sr RNA universal phytoplasmas primers, P1/P7 (Deng & Hiruki, 1991; Schneider et al., 1995) and R16F2n/R16R2 (Gundersen & Lee, 1996) in direct and nested (P1/P7, dilution 1:20) reactions, respectively. Amplicons of the expected size (c. 1.8 and 1.2 kb, respectively) were obtained in all diseased samples but not from symptomless samples. Nested amplicons (R16F2n/R16R2) were subjected to restriction using *Mse*I, *Hha*I and *Rsa*I enzymes (NEB, USA) according to the manufacturer's instructions. The RFLP profile of the lettuce samples was indistinguishable from the 16SrIII-X subgroup, while for the garlic samples the profile was indistinguishable from that of the 16SrIII-J subgroup (Galdeano et al., 2013). Nested amplicons from two samples named LWB-Arg1 (Lettuce Witches' Broom) and GarPhy-Arg1 (Garlic Phyllody) were cloned and sequenced.

New Dis Rep. 2022;46:e12103.
https://doi.org/10.1002/ndr2.12103

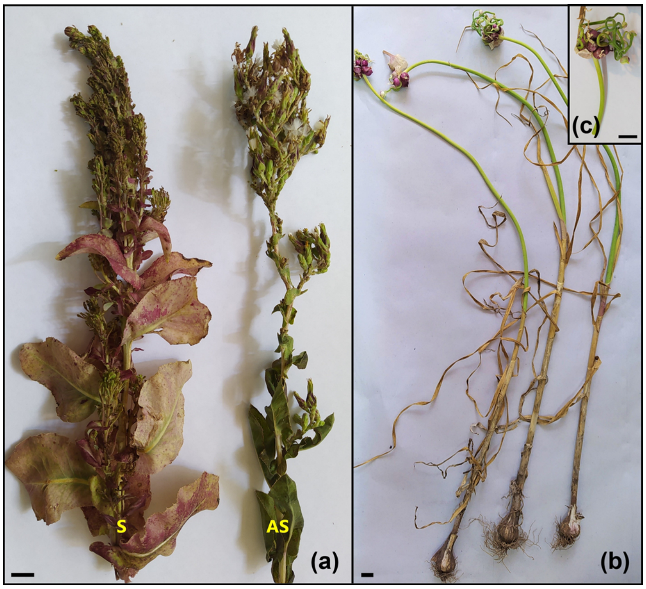

FIGURE 1 (a) Symptomatic lettuce plant showing witchest' broom, flower malformation and leaf redness (S) compared with an asymptomatic plant (AS); (b) symptomatic garlic plants showing development of vegetative structures in floral pieces (phyllody); (c) detail of phyllody. Scale bars = 1 cm

Consensus sequences were deposited in GenBank with Accession Nos. ON623076 (GarPhy-Arg1) and ON623077 (LWB-Arg1). *In silico* classification with *i*PhyClassifier (https://plantpathology.ba.ars.usda.gov/) showed that LWB-Arg1 has an identical RFLP profile to the reference strain of subgroup 16SrIII-X (KC412026) and GarPhy-Arg1 has an identical RFLP profile to the reference strain of subgroup 16SrIII-J (AF147706). Phylogenetic inference was determined using the partial 16S rRNA sequences obtained in this work, representative sequences from X-disease group and selected '*Ca*. Phytoplasma species' employing IQ-TREE software (http://www.iqtree.org/) under the maximum likelihood method (1000 bootstraps). The tree showed that LWB-Arg1 branches with representative sequences from subgroup 16SrIII-X and GarPhy-Arg1 representative sequences from subgroups 16SrIII-J (Fig. 2) confirming the affiliations obtained in the virtual RFLP.

Garlic decline phytoplasma (subgroup 16SrIII-J) was reported previously in association with Garlic decline disease in Argentina (Conci et al., 1998). However, this is the first time that symptoms of phyllody in garlic associated with this phytoplasma have been reported. To our knowledge, this is the first report of phytoplasmas from subgroup 16SrIII-X affecting lettuce in Argentina and worldwide. Previous work reported the presence of phytoplasmas from subgroup 16SrIII-X affecting different species of weeds in the same region (Galdeano et al., 2013; Fernández et al., 2020) which may suggest the presence of a common insect vector with polyphagous feeding behaviour.

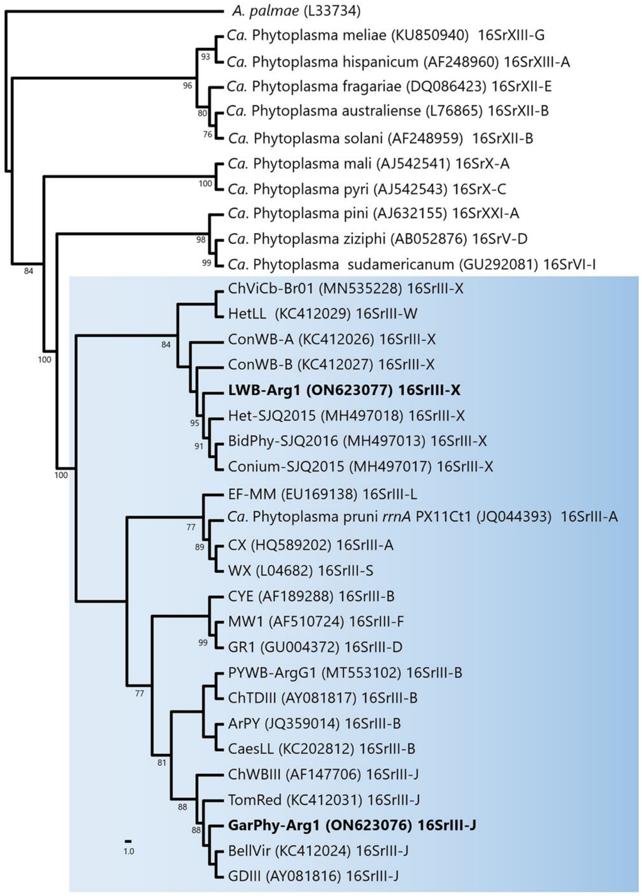

FIGURE 2 Phylogenetic tree based on the partial 16S rRNA gene sequences of Garlic phyllody phytoplasma (GarPhy-Arg1), Lettuce witches'-broom phytoplasma (LWB-Arg1), phytoplasmas from X-disease group (16SrIII) and selected '*Candidatus* (*Ca.*) Phytoplasma' reference strains. *Acholeplasma palmae* was used as an outgroup. GenBank accession numbers, group and subgroup classifications are provided. Sequences obtained in this work are in bold. The X-disease phytoplasma clade (16SrIII) is highlighted in light blue. The scale bar represents the number of nucleotide substitutions per site

ACKNOWLEDGEMENTS

This work was supported by a grant of the Instituto Nacional de Tecnología Agropecuaria (INTA): INTA/2019-PD-E4-I090-001 and FONCyT PICT 2016-0862.

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How to cite this article: Fernandez, F.D., Carloni, E., Alessio, F., Bongiorno, V. & Conci, L.R. (2022) First report of a 16SrIII-X phytoplasma associated with *Lactuca sativa* witches'-broom in Argentina. *New Disease Reports*, 46, e12103. https://doi.org/10.1002/ndr2.12103