Phytopathogenic Mollicutes
Vol. 9 (1), June 2019, 189-190

doi: 10.5958/2249-4677.2019.00095.1

TROPICSAFE

New contribution about the epidemiology of grapevine yellows-associated phytoplasmas in Chile

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Abstract

Three 16SrIII-J phytoplasma-positive vineyards were visited each 15 days from September 2017 to August 2018 to capture insects using entomological sweep nets and yellow sticky traps. Additionally weeds showing typical symptoms associated with the presence of phytoplasmas were also collected. The phytoplasma 16SrIII-J was detected for the first time in the leaf hoppers *Amplicephalus ornatus*, *A. pallidus* and *Bergallia* sp., and in the weed *Brassica campestris*. Another phytoplasma classified as 16SrXI, was detected in one leaf hopper belonging to the genus *Paratanus*. The identification of the 16SrXI ribosomal subgroup and the species of *Bergallia* sp. and *Paratanus* sp. are in progress, as well as the transmission trials with these two insects plus *A. pallidus* and *A. ornatus*.

Keywords: insects vector, GY, nested-PCR, RFLP, sequencing, weeds

Introduction

In Chile, grapevine yellows (GY) are associated with phytoplasmas belonging to diverse ribosomal subgroups as 16SrI-B and 16SrI-C ('*Candidatus* Phytoplasma asteris'-related), 16SrIII-J ('*Ca.* P. pruni'-related), 16SrV-A ('*Ca.* P. ulmi'-related), 16SrVII-A ('*Ca.* P. fraxini'-related), and 16SrXII-A ('*Ca.* P. solani'-related or "stolbur") (Gajardo *et al*., 2009; Fiore *et al*., 2015). The phytoplasma 16SrIII-J is the prevalent in the vineyards located in the central zone of the country, and so far it has been reported in various crops and spontaneous plant species in Chile (Quiroga *et al*., 2017). Several alternative host plants, present in or closer to some infected vineyards, have been found infected by phytoplasmas, and two insect species, *Paratanus exitiousus* (Beamer) and *Bergallia valdiviana* Berg 1881, have been proved as vector of the phytoplasma 16SrIII-J (Longone *et al*., 2011; Quiroga *et al*., 2019). This work describes new potential phytoplasma insect vectors, as well as a new reservoir plant species of the phytoplasma 16SrIII-J in Chilean vineyards.

Materials and Methods

From September 2017 to August 2018, three 16SrIII-J phytoplasma-positive vineyards were visited each 15 days to capture insects using entomological nets and yellow sticky traps. The vineyards planted with cultivar Pinot Noir are located in Casablanca (Valparaiso region), with Carménère in San Javier (Maule region) and with Cabernet Sauvignon in Marchigue (O'Higgins region). In these vineyards, weeds with typical symptoms associated with the phytoplasma presence, were also collected. The insects were sorted out by their external morphological features and analysed in batches of 5 individuals for phytoplasma presence. Male specimens were preserved in ethanol 70% and send to the University of Turin, Italy, to be identified by their genitalia. Weeds were identified and 150 mg of plant samples were separated and frozen for phytoplasma detection. The extraction of nucleic acids was carried out according to a silica capture method (Rott and Jelkmann, 2001). The PCR amplification was carried out using 20 ng/µl of nucleic acid; direct PCR with primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) and nested PCR with R16F2n/R2 primers on the 16SrRNA gene (Gundersen and Lee, 1996) were performed following Schaff *et al*. (1992). Amplicons from nested PCRs were purified using Concert Rapid PCR Purification System (Life Technologies, Gaithersburg, MD, USA). DNA fragments were ligated into T/A cloning vector pTZ57R/T following the instructions of the InsT/Aclone PCR Product Cloning Kit (Fermentas, Vilnius, Lithuania). Putative recombinant clones were analysed by colony PCR and

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Table 1. Phytoplasmas detected in weed and insect species.

Vineyard locality	Insect vectors	Phytoplasma ribosomal group	Spontaneous plants	Spontaneous plants
Casablanca	*Amplicephalus ornatus*	16SrIII-J	*Brassica campestris*	16SrIII-J
Casablanca	*Amplicephalus pallidus*	16SrIII-J	*Brassica campestris*	16SrIII-J
Casablanca	*Bergallia* sp.	16SrIII-J	*Brassica campestris*	16SrIII-J
Marchigue	*Paratanus* sp.	16SrXI	No plants collected	-
San Javier	*Paratanus exitiosus*	16SrIII-J	*Brassica campestris*	16SrIII-J
San Javier	*Amplicephalus curtulus*	16SrIII-J	*Brassica campestris*	16SrIII-J

selected fragments were sequenced in both directions in MacrogenUSA Corp (Rockville, MD, USA). The sequences were then aligned with those of strains deposited in GenBank using BLAST engine for local alignment (version Blast N 2.2.12). The 16SrIII-J phytoplasma identification was performed using *in silico* restriction fragment length polymorphism (RFLP) analysis with *Hha*I, *BstU*I and *Rsa*I restriction enzymes.

Results

The 16SrIII-J phytoplasma was detected in two samples of *Brassica campestris*, from San Javier and Casablanca, which showed yellowing, dwarfism and corky leaves. The analyzed insects were 450. *Amplicephalus ornatus* Linnavuori, *Amplicephalus pallidus* Linnavuori and *Bergallia* sp. from Casablanca, were positive to the phytoplasma 16SrIII-J. *A. curtulus* and *P. exitiosus* captured in San Javier were also positive to the same phytoplasma. In one group of five individuals belonging to the genus *Paratanus* from Marchigue, was detected a phytoplasma classified as 16SrXI (Table 1). The nucleotide sequences of the phytoplasma 16SrIII-J showed a close correlation (99.8%) with the strain Ch10 (GenBank accession number, acc. no., AF147706), corresponding to chayote witches' broom phytoplasmas (16SrIII-J) from Brazil. The fragment obtained from the *Paratanus* sp., (1,155 bp) showed 99.5% of nucleotide identity with strains of sugarcane grassy shoot phytoplasma from India (acc. no. MG744609).

Discussion

Based on these results, it seems that new insect species within the *taxon* Auchenorrhyncha could be involved in the transmission of phytoplasmas in the Chilean vineyards. Furthermore, *B. campestris* is an herbaceous species that begins its life cycle after the first autumn rain and stays green until the end of the spring. This means that it may play a relevant role as reservoir of the phytoplasma 16SrIII-J. The identification of a 16SrXI phytoplasma in *Paratanus* sp., is the first report in the Americas. It is important to emphasize that the 16SrXI phytoplasma finding in Chile was never reported, thus an intense sampling of vegetal material, grapevines and weeds, is currently taking place in the O'Higgins region where it was detected.. The identification of the species of *Bergallia* sp. and *Paratanus* sp. are in progress, as well as the transmission trials with these two insects along with *A. pallidus* and *A. ornatus*.

Acknowledgements

This work was funded by the European Union's Horizon 2020 research and innovation programme under grant agreement No. 727459, project "Insect-borne prokaryote-associated diseases in tropical and subtropical perennial crops" TROPICSAFE. The authors thank the Chilean National Commission for Scientific and Technological Research (CONICYT) for Ph.D. Scholarship N° 21171998 to N. Quiroga.

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