Transmission of 16SrIII-J Phytoplasmas to Grapevine by *Bergallia valdiviana* Leafhopper

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***Abstract*— *Bergallia valdiviana* has been previously described as a vector of the phytoplasma 16SrIII-J in periwinkle. The present work shows that *B. valdiviana* can transmit 16SrIII-J phytoplasmas also to grapevine. This information helps to a better understanding about the epidemiology of 16SrIII-J phytoplasmas in Chile.**

***Key words*— Auchenorrhyncha, transmission trials, nested-PCR, *tuf* gene, 16S rRNA gene, RFLP.**

I. INTRODUCTION

Several phytoplasmas were associated to grapevine yellows in Chile. Among them, 16SrIII-J is the prevalent in the vineyards of the central zone of the country (Gajardo et al. 2009; Gonzalez et al. 2010). This phytoplasma has been reported infecting various crops and spontaneous plants in Chile, suggesting the necessity of epidemiological studies to identify its insect vectors (Gonzalez et al. 2011; Quiroga et al. 2017). Several leafhoppers (Hemiptera, Auchenorryncha, Cicadellidae) were found positive to phytoplasma presence, including *Bergallia valdiviana* Berg 1881 among the most common species present in infected vineyards. Transmission trials carried out using periwinkle [(*Cantharanthus roseus* L. (G. Don.)] as indicator plant, show that *B. valdiviana* is vector of 16SrIII-J phytoplasmas (Quiroga et al. 2015). The purpose of this work was to demonstrate that B. valdiviana transmits the 16SrIII-J phytoplasma also to the grapevine.

II. MATERIAL AND METHODS

During 2014 (November-December) and 2015 (January-May) surveys for *B. valdiviana* presence were carried out in a phytoplasma-infected vineyard variety Pinot noir in the Valparaiso Region (Casablanca, Chile). The insects were captured by means of an entomological sweeping net. During the sampling period, adults of *B. valdiviana* captured were released into entomological cages (Table 1) to let them feed on three grapevine plants, grown from seed obtained by Cabernet Sauvignon fruits and previously PCR-tested to ascertain the absence of phytoplasmas (Table 1). A total of 21 plants were used, including three healthy plants. Grapevine plants were tested every 6 months from the start of transmission trials, and dead leafhoppers (in batches of four to six individuals) were also tested after maintenance in 70% ethanol, in order to detect phytoplasma presence. Total nucleic acids from plants and insects were extracted with a chloroform/phenol method, dissolved in Tris-EDTA pH 8.0 buffer, and maintained at 4°C; 20 ng/µl of nucleic acid were used as template for amplification. Direct and nested PCR of tuf gene were carried out according to the protocol of Makarova et al. (2012). Further, direct PCR with primer pair P1/P7 and nested PCR with R16F2n/R2 primers on the 16S rRNA gene (Gundersen and Lee 1996) were performed following a published protocol (Schaff et al. 1992). Amplicons from nested PCRs for both genes were purified using Concert Rapid PCR Purification System and DNA fragments were cloned. Putative recombinant clones were analyzed by colony PCR. Selected fragments from cloned DNAs were sequenced in both directions in MacrogenUSA Corp (Rockville, MD, USA). The sequences were then aligned with those of reference strains deposited in GenBank and Q-Bank, using BLAST engine for local alignment (version Blast N 2.2.12). Phytoplasma identification was done using *in silico* restriction fragment length polymorphism (RFLP) with *Mse*I, *Nde*II, *Hha*I, *BstU*I, and *Rsa*I restriction enzymes (Wei et al. 2007; Zhao et al. 2009). The first two enzymes were used for *tuf* gene and the last three for 16S rRNA gene sequences.

Table 1. Number of individuals of B. valdiviana, captured in the Casablanca vineyard, released per month in cages on grapevine plants for transmission trials.

Month	Number of insects
December	17
January	20
February	28
March	24
April	29
May	26

III. RESULTS

B. valdiviana survived 4-5 days on grapevine plants. Two out of 21 plants used for transmission trials were positive to phytoplasmas in nested PCR using primers for tuf and 16S rRNA genes. The positive plants (V85a and V86c) corresponded to the transmission trials carried out with insects captured in two different months (January and February 2015, respectively), and 16SrIII-J phytoplasma was detected for the first time two year after the start of transmission trials.

The transmission rates were 9.5%. Cloned nested PCR fragments from both genes were sequenced and there were no sequence differences among the cloned fragments from the phytoplasmas detected in the two grapevines in tuf gene sequence (438 bp), while sequence identity was 99.9 to 100% in 16S rDNA sequences (1,250 bp). In 16S rDNA the identity percentages of phytoplasmas detected in the two grapevines, showed a close correlation (99.8%) with the strain Ch10 (GenBank accession number AF147706), corresponding to

chayote witches' broom phytoplasmas (16SrIII-J) from Brazil (Montano et al. 2000). In *tuf* gene the highest nucleotide identity was 100%, with the isolate Hort72 (Fiore et al. 2015) that belongs to a 16SrIII-J phytoplasma from Chile (GenBank accession number KM658259). The *tuf* and 16S rDNA sequences were also analyzed *in silico* RFLP confirming their assignment to phytoplasmas in the ribosomal subgroups 16SrIII-J. The phytoplasma 16SrIII-J was also detected in *B. valdiviana* specimens used for transmission assays. The two grapevines plants infected with 16SrIII-J showed symptoms of short internodes, and leaves with downward rolling, deformation, yellowing and necrosis two year after the beginning of transmission trials.

IV. Discussion

The described trials demonstrated that *B. valdiviana* transmits 16SrIII-J phytoplasmas not only to periwinkle (Quiroga et al., 2015), but also to grapevines. *B. valdiviana* lives on weeds and only occasionally feed on grapevine or other crops. The main differences between the results shown by the transmission in periwinkle and those in grapevine concerns the period of transmission (autumn and summer, respectively), the days that the insect survives in contact with the test plants (6-7 and 4-5, respectively) and the time in which it was possible to detect positive grapevines from transmission tests (latent period) (1 and 2 years, respectively).

The phytoplasma 16SrIII-J and its vector are widely distributed in Chile, on different weed species and also in crops of agronomic interest (Castro et al. 2000; Hepp and Vargas 2002; González et al. 2010; 2011; Longone et al. 2011; Fiore et al. 2012). Considering the *B. valdiviana* transmission rates observed, if environmental conditions are favorable, there is a high likelihood to expect an outbreak of grapevine yellows in Chile due to 16SrIII-J phytoplasma transmission by this and other insect vectors such as *Paratanus exitiosus* (Fiore et al. 2012).

ACKNOWLEDGMENT

This work was carried out in the frame of European Union's Horizon 2020 research and innovation programme, project TROPICSAFE under grant agreement No 727459.

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