**Pauta de informe muestras otorgadas por Profesores Nicola Fiore y Alan Zamorano**

***Introducción***: Indicar antecedentes generales de la muestra colectada, de lo más general a lo más específico.

***Diagnóstico visual***: Indicar cuales son los síntomas y signos observados y, según bibliografía (a indicar en “Referencias bibliográficas”), indicar cuales podrían ser los agentes causales y porque.

***Diagnóstico definitivo***: Indicar que cuales han sido los pasos realizados para completar el diagnóstico.

***Resultados y consideraciones finales***: Indicar cuales han sido los resultados del diagnóstico definitivo. Hacer correlaciones con el diagnóstico visual. Escribir un breve análisis crítico acerca del trabajo realizado y los resultados obtenidos.

***Referencias bibliográficas***: Listado de todos los artículos revisados para la realización del informe. Ordenar las citas en orden alfabético. Cada cita bibliográfica debe ser indicada en el texto, en el lugar correspondiente (seguir ejemplo).

Ejemplo a usar solo para ordenar las citas bibliográficas:

Texto

Genetic variability and divergence of Chilean isolates of *Grapevine rupestris stem pitting-associated virus*

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INTRODUCTION

*Grapevine rupestris stem pitting-associated virus* (GRSPaV) is known to be a widespread virus and has been proposed to co-evolve together with grapevine (Gambino et al., 2012). It belongs to the “rugose wood complex”, a group of viruses and/or syndromes associated with alterations of trunk in vines. Additionally, due to the high genetic variability associated to this virus, different diseases have been reported depending on the lineage and the grapevine cultivar infected (Bouyahia et al., 2005; Morelli et al., 2011). Several studies have derived in a four-group genetic classification (Meng et al., 2006; Nolasco et al., 2006). In Chile, GRSPaV was first detected in 2008 and since then, as expected, several detections have been done in different grapevine cultivars (Fiore et al., 2008). However, no information of genetic variability has been established associated with Chilean isolates of GRSPaV. Therefore, the objective of this research is to establish phylogenetic distribution of Chilean isolates of GRSPaV.

MATERIALS AND METHODS

One hundred and ten samples were collected from table and wine grape varieties between the regions of Atacama and Maule. Phloem scrapings from mature canes were used for virus testing. Total nucleic acid extraction was performed using silica capture method (MacKenzie et al., 1997). Specific detection of GRSPaV was performed according previously described primers (Boscia et al., 2001). Molecular characterization was done using PCR primers reported by Lima et al. (2006), which partially amplified (776-bp) the helicase subunit of RdRp coding region (Hel). PCR fragments were purified and cloned in pGEM-T Easy kit (Promega). Five clones per isolate were sequenced to determine the eventual presence of more variants of the virus infecting the same sample. Molecular analyses were performed using MEGA6.0 tools (Tamura et al., 2013)

RESULTS AND DISCUSSION

GRSPaV was detected in 65 out of the 110 samples tested (59.1%). Nucleotide identity comparison and neighbor joining analyses carried out with the detection amplicons, gave a distribution of Chilean isolates in three groups (data not shown). Thereafter, 15 representative isolates from the three groups were randomly selected for a more exhaustive genetic analysis using Hel coding sequence. Figure 1 shows the phylogenetic distribution obtained in maximum parsimony analysis. Reference isolates used correspond to complete genome sequences available in GenBank and were edited to perform alignments and phylogenetic trees. In all cases, there was no sequence difference between the five cloned fragments of GRSPaV from each sample. Topologies of trees obtained with reference isolates using complete genome and Hel region sequences, were homologous (data not shown). Thus, Hel sequence oriented analysis appears to be robust enough to consider this sequence as a good indicator for phylogenetic analyses. Due to the high genetic distance observed among Syrah and PN isolates and unlike with previously reports about phylogeny classification of GRSPaV, we propose five groups: GRSPaV-1 (I), BS (II), SG1 (III), PN (IV), and Syrah (V). Chilean isolates were distributed in the groups GRSPaV-1 (most of them), SG1 and BS. Two samples, RSP-HEL 7056 and RSP HEL 6584, were divergent isolates inside GRSPaV-1 group (Figure 1). In addition, deduced aminoacid phylogenetic analyses showed a change of group of RSP HEL 6584 isolate, which shifts from GRSPaV-1 to BS and grouped with HEL RSP 6582 (data not shown). This trend must be confirmed by using, in the phylogenetic analysis, of the complete amino acid sequence of Hel. Finally, it is important note the common geographic and cultivar origin of the samples RSP HEL 6584 and RSP HEL 6582, the last one markedly associated with BS group. The results showed a high genetic variability among Chilean isolates and a genetic divergence for two of them. To our knowledge, this is the first molecular characterization study about GRSPaV from South America.

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