EVOLUTIONARY DYNAMIC OF A NEW CTV GENETIC LINEAGE: WHAT DOES THE HISTORY TELL US?

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Citrus Tristeza Virus (CTV) has been intensively studied since several decades ago, due to its capacity to cause one of the most devastating diseases in citrus industry worldwide. Nevertheless, few studies have been focused on its evolutionary history (Silva et al., 2012; Davino et al., 2013; Harper, 2013; Owen et al., 2014). In Uruguay, studies describing the presence of CTV have been done in the early 40's showing that this pathogen, along with Citrus Psorosis Virus (CPsV) and Citrus Exocortis Viroid (CEVd) are responsible of annual losses up to 30% of citrus national production. However, the prevalence and distribution of these pathogens in the country is yet not known, although studies in this matter have been already started by our group. In the past four years, we were been focused on the study of CTV genetic diversity in Uruguay based on the molecular analysis of p25, p20 and p23 genes. We describe the co-circulation of VT, T3, and T36 genotypes as well as a fourth lineage named NC, which is highly represented in Uruguayan citrus orchards (Benítez-Galeano et al., 2015). Sequences of this lineage share 99% of nucleotide identity with previously reported isolates, such as Taiwan-Pum/SP/T1 and the Hawaiian isolate HA16-5. We also described the presence of mixed infections within the same host and the presence of some recombinant genomes (Benítez-Galeano et al., 2015). Nowadays, we continue with the surveillance of citrus orchards from all the country and the presence of this new lineage is growing as well as the presence of different genotypes, such as RB not founded before. With the aim to develop a long term cross protection program, to be incorporated on the ongoing National Sanitation Program, we are trying to get a deep knowledge about these variants that circulate in Uruguay. For this purpose, we want to unravel the biological, molecular and evolutionary traits of the NC lineage, not intensely studied so far. In the present work, we studied the evolutionary history of this NC lineage based on a Bayesian coalescent approach using genomic sequences of p25 and p20 genes. To do this, a large set of heterochronous gene sequences retrieved from GenBank and also Uruguayan sequences obtained by our group during the past few years were included in the analysis. Dated sequences from Uruguay, Argentina, Brazil, Greece, Portugal, China, United States, Angola, among others, from a time period from 1979 to 2015 were used in the analysis. Recombinant sequences were discarded from the analysis in order to do not mix evolutionary histories and the best-fitting evolutionary model for each dataset was elucidated using jModel Test software. In this way, we could estimate the evolutionary rate for both genes as well as the time to the most recent common ancestor (tMRCA) of the NC lineage, showing consistent results in both cases either for p25 and p20. We also analyzed the demographic behavior of the population from its origin to the present, and we observed a consistent pattern with no growing for almost 30 years, followed by an exponential growth of the effective population size in the last 5 years. Based on a phylogeographic approach, we could determine the movement around the world of this genetic lineage. Our results pointed that the country of origin of this NC lineage is Uruguay, almost 35 years ago, and then a following radiation into two separate events leads one genetic group to Brazil and the Mediterranean region, and the other genetic group to United States, Argentina and Uruguay. Supporting our findings, Owen and co-workers (2014) described the recently introduction

towards the Mediterranean region of a new strain highly similar to the previously reported Taiwan-Pum/SP/T1 strain. Understanding the evolutionary history of CTV variants circulating in the country could be of great importance to develop strategic control plans to manage this destroying pathogen. To our knowledge, this is the first comprehensive study about the evolutionary history of a CTV genetic lineage in Uruguay.