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Genetic Structure and Molecular Variability of *Grapevine fanleaf virus* populations within three naturally infected California vineyards

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*Grapevine fanleaf virus* (GFLV) from the genus *Nepovirus*, family *Secoviridae* causes fanleaf degeneration, one of the most important viral diseases of grapevines worldwide. It is specifically transmitted from grapevine to grapevine by the ectoparasitic nematode *Xiphinema index*. GFLV has a bipartite (+)ssRNA genome, which is expressed into two polyproteins that are cleaved into at least eight individual proteins. Due to its error-prone replication and quasi-species nature, GFLV possesses great potential for genetic variation. While the variability within the coat protein and movement protein genes is well characterized for numerous GFLV isolates, information on the variability of other genomic regions - particularly within the RNA1 - is scarce. Similarly, little is known on the diversity of GFLV isolates from the U.S. To gain insights into the evolutionary mechanisms of GFLV, the sequences of the RNA2-encoded genes 2A<sup>HP</sup>, 2B<sup>MP</sup> and 2C<sup>CP</sup>, and partial sequence information from the RNA1-encoded gene 1E<sup>Pol</sup> of 14 GFLV isolates from three naturally infected California vineyards were characterized. Phylogenetic analyses suggested two evolutionarily divergent lineages that did not reflect the vineyard origin of the isolates or an association with rootstock genotype or scion cultivar. Examination of the genetic variability of the California isolates alongside isolates worldwide revealed similar patterns of molecular evolution for the different regions within the GFLV genome, but distinct selection constraints with the strongest pressure exerted on genes 2C<sup>CP</sup> and 2B<sup>MP</sup>, an intermediate level of pressure exerted on gene 1E<sup>Pol</sup>, and the weakest pressure exerted on gene 2A<sup>HP</sup>. Some of the California isolates resulted from interspecies recombination events between GFLV and *Arabis mosaic virus* with crossover sites suspected in gene 1E<sup>Pol</sup> and identified in genes 2A<sup>HP</sup> and 2B<sup>MP</sup>; and intraspecies recombination events most frequently observed within gene 2C<sup>CP</sup>. Our study suggested that purifying selection and recombination are important evolutionary mechanisms in the genetic diversification of GFLV.