

Grapevine yellow speckle viroid 1 detected in symptomatic grapevines in Brazil

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In Brazil, about fifteen viruses have been identified infecting grapevines, causing leafroll, rugose wood (corky bark, stem pitting, stem grooving), degeneration, leaf flecking and vein necrosis. So far, *Hop stunt viroid* (HSVd) and *Citrus exocortis viroid* (CEVd) were the only viroids infecting grapevines in Brazil. All these diseases are graft-transmissible and usually induce losses. Viroids are non-protein-coding, small (their genome size varies between 246 to 401 nucleotides) and circular single-stranded RNA pathogens. The aim of this work was to confirm infections by *Grapevine yellow speckle viroid 1* (GYSVd-1, genus *Apscaviroid*) in *Vitis labrusca* 'Niagara Rosada' and *V. vinifera* 'Semillon' plants exhibiting yellow speckle symptoms in the leaves and cultivated in experimental fields in Bento Gonçalves-RS, Brazil. Total RNAs were extracted using RNeasy Plant Mini kit (Qiagen) from symptomatic leaves. The primers used for RT-PCR amplification were: 5' CAACTCGAGCCTCGCTGCTCTGGGC 3' (sense) and 5' TTCGTCGACGACGAGGCTC 3' (reverse) - enzyme sites underlined. RT-PCR reactions were performed using the OneStep RT-PCR kit (Qiagen). The DNA amplified fragments were ligated into pGEM-T Easy vector and the recombinant plasmids were used to transform *Escherichia coli*. Sequencing was performed from recombinant plasmids or directly from DNA amplified fragments. The obtained nucleotide sequences were analyzed using available bioinformatic tools. The amplification of a 318 bp of the GYSVd-1 partial genome from two symptomatic sources was obtained. Comparisons of the obtained sequences showed high nucleotide identities (98%) with other seven symptomatic and asymptomatic isolates of GYSVd-1 from Australia (GenBank JX892929-JX892935). Nucleotide sequence identity between 'Niagara Rosada' and 'Semillon' Brazilian isolates of GYSVd-1 was 99%. Although, we have detected GYSVd-1 in plants exhibiting yellow speckles, the symptoms observed in the experimental vineyards can not be attributed to viroid infection until further investigations are completed. Apparently, GYSVd-1-infected plants did not exhibit any other kind of symptoms besides those we have already observed in the leaves. To our knowledge, this is the first report of GYSVd-1 infecting grapevines in Brazil. The presence of this viroid infecting Brazilian grapevines can be attributed to the introduction of infected propagative material from other countries since this viroid has no vectors associated to its transmission.

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