Next Generation Sequencing applied for identification of three new viruses infecting grapevine in Brazil

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The application of high-throughput sequencing technologies, known as next generation sequencing (NGS), enables the recovery of hundred of thousand sequence fragments from diseased plants and may help in the identification of unknown pathogens. This study was designed to identify viruses infecting two grapevine samples: *Vitis vinifera* cv. Cabernet Sauvignon exhibiting leafroll (s1) from experimental field and asymptomatic *V. flexuosa*, a wild grapevine (s2) from Embrapa Grape and Wine germplasm collection. The viral-enriched double-stranded RNAs were extracted from 30 g of bark scrapings using CF11 cellulose. The RNA was sequenced at Macrogen (South Korea) using an Illumina HiSeq 2000 platform. The paired-end reads were trimmed and assembled using CLC Genomics Workbench v. 6.0.3. The contigs related to viruses were selected using blastx against the RefSeq virus database. About twenty and seventeen million reads were generated for each library, and after quality trimming and *de novo* assembly 1,374 and 1,589 contigs were obtained of which 184 (s1) and 51 (s2) produced hits to viral sequences. The deduced amino acid (daa) identities between obtained contigs and homologous grapevine virus sequences in the GenBank were high (usually over to 90%). We were able to identify assembled contigs of several viruses covering large extensions of their viral genomes. It has found in mixed infections the following viruses: GCSV (Grapevine Cabernet Sauvignon virus), GVCV (*Grapevine vein-clearing virus),* GRGV (*Grapevine Red Globe virus*), GSyV-1 (*Grapevine Syrah virus 1*), GLRaV-2 and -3 (*Grapevine leafroll-associated virus*), GRSPaV (*Grapevine rupestris stem pitting-associated virus*), GVB (*Grapevine virus B*) and GFkV (*Grapevine fleck virus*). We observed viruses already described infecting grapevine in Brazil (GLRaV-2 and -3, GRSPaV, GVB, GFkV and GCSV, a *Reoviridae* recently report in Brazil) as well as others not previously reported in this country (GVCV, *Badnavirus* genus; GRGV, *Maculavirus* and GSyV-1, *Marafivirus*). The daa identities of new identified Brazilian isolates with other homologous viruses in the GenBank were 97% (153/157) to GVCV, s1; 96% (101/105) to GRGV, s1 and 98% (2025/2064) to GSyV-1, s2. These nucleotide sequences have been deposited in the GenBank: GVCV (accession KR107537), GRGV (KR107538) and GSyV-1 (KR153306). Further (RT-) PCR specific analysis should be undertaken to confirm the identifications of these new viral species infecting grapevine in Brazil.

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