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PIV14 - FIRST REPORT OF GRAPEVINE REOVIRUS INFECTING CABERNET SAUVIGNON GRAPEVINE IN BRAZIL

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Grapevine Cabernet Sauvignon reovirus (GCSV) was first described on grapevine cv. Cabernet Sauvignon in California by deep sequencing analysis in 2015 (Al Rwahnih et al., 2015). Subsequently, a Brazilian GCSV isolate was discovered as a member of a mixed viral infection. The infection was in a *Vitis vinifera* cv. Cabernet Sauvignon vine in an experimental field in the municipality of Bento Gonçalves, State of Rio Grande do Sul, Brazil. The symptoms in this host were those of severe grapevine leafroll disease. The Brazilian GCSV isolate was characterized from a total nucleic acid extract of 30g of bark scrapings that had been enriched for double-stranded RNA. Sequencing data were generated from a complementary DNA library that was constructed by Macrogen Inc. (Seoul, Korea) from that extract. The Illumina HiSeq 2000 platform was used to generate about 20 million reads. CLC Genomics Workbench software (CLC Bio, Qiagen, USA) was used for quality trimming and de novo contig assembly from the reads. All contigs were analyzed using NCBI BLASTX program against the viral RefSeq database. About 0.8% (166,800) of the reads, assembled into twenty five contigs with lengths from 289 to 3849 bp, were identified as homologous to GCSV. The sequence information in those contigs was sufficient to cover 96% of the sequences from the ten genomic components (accession numbers KM236567 and KM378720 through KM378728) reported by Al Rwahnih et al. (2015). The nucleotide sequence identities of the Brazilian sequences compared with those of the Californian isolate ranged from 94-98%. The genomic sequences for the Brazilian strain of GCSV have been deposited in the GenBank under accession numbers KR107527 through KR107536. To confirm the NGS identification, dsRNA was extracted from fresh plant material from the original source and was analyzed by RT-PCR using the specific PCR primer pair Ctg468F (5'ACGTTGGATCAACTAGCCGAAG3') and Ctg468R (5'TATTCACGAGGCTCAGACGACT3'). Primers had been designed from the sequence of viral genomic

component 4. The resulting 386 bp amplicon was cloned and sequenced (accession no. KR074408) and found to share 98% nucleotide identity with component 4 of the GCSV isolate from Brazil (KR107530). To our knowledge Brazil is the second country, after the U.S.A., where GCSV has been reported in grapevine. Further RT-PCR analyses have been undertaken to better establish the prevalence of GCSV and to evaluate its potential effects on grape yield and on wine quality. Financial Support: EMBRAPA (Project 02.13.14.002).

PIV19 - EVALUATION OF THE USE OF YELLOW STICHY TRAPS IN MONITORING VIRULIFEROUS WHITEFLIES TO BEGOMOVIRUS

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Viruses of the genus *Begomovirus* (Family *Geminiviridae*) represent a large group of plant viruses that cause considerable losses to agriculture worldwide. The natural spread of begomoviruses is based exclusively on their transmission by whiteflies, belonging to the *Bemisia tabaci* (Hemiptera: Aleyrodidae) species complex. It is essential that epidemiological studies on begomovirus diseases are carried out considering the insect vector monitoring. The most used strategy for whitefly monitoring is the use of traps (yellow sticky cards) exposed to field conditions for a specific period of time. These trapped insects are therefore prone to degradation caused by the influence of light, wind, temperature and water. As currently it is not known if the use of these insects is adequate for virus detection purposes, the objective of this study was to develop a protocol for begomovirus detection in card trapped insects. Three DNA extraction methods were tested, as well as the length of time that the whitefly can be left in the card for a reliable virus detection. Virus-free whiteflies were allowed to feed on a begomovirus infected tomato leaf for 48 hours. After the feeding period, the viruliferous whiteflies were adhered in a yellow sticky card (BioControle) and exposed to the sun in a glass cage for one to seven days, in two repetitions. For DNA extraction, the following methods were compared on their efficiency, cost and processing time: Proteinase-K, CTAB and CHELEX DNA was extracted from whiteflies