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 objetive 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

removed from the cards after 1,2,3,4,5,6 and 7 days of exposure. The DNA quality was tested by PCR using universal primers for begomoviruses and for whiteflies. It was concluded that the Proteinase-K method was the best method for DNA extraction for the whiteflies. When evaluating the incubation time in the traps, in a one-day exposure, the virus was detected in 93.3% of whiteflies, 86.6% in a 2-day exposure, 80.0% in a 3-day and 4-day exposure, in a 5-day exposure, 33.3 for 6-days and 20% for 7-days. The decrease in the virus detection upon longer incubation times is possibly related to the desiccation of the insects in prolonged exposure to the sun, thus causing degradation of the virus DNA. Monitoring of viruliferous whiteflies can be done using the yellow sticky cards, and the exposure time of the traps must be planned taking into consideration the virus degradation in the trapped insects. Financial Support: Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq e Fundação de Apoio a Pesquisa do Distrito Federal – FAP-DF.

PIV25 - VIRAL METAGENOMIC ANALYSIS OF SWEET POTATO GENOTYPES

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The sweet potato (*Ipomoea batatas* L.) presents significant global importance being currently the sixth most consumed food in the world. In Brazil, sweet potato is the most relevant crop in the Northeast, which is one of the most appreciated vegetables by the population. This culture can be affected by several pathogens. Among these, viruses are considered the main problem reducing drastically crop yield due to the vegetative propagation of sweet potatoes. The co-infection of sweet potato by Sweet potato feathery mottle virus – SPFMV (genera *Potyvirus*, family *Potyviridae*) and Sweet potato chlorotic stunt virus – SPCSV (genera *Crinivirus*, family *Closteroviridae*) cause the devastating disease known as sweet potato virus disease. In order to check the viral diversity in sweet potato samples from producing areas of the Northeast, branches of 40 plants were grafted on Ipomoea setosa, widely used in sweet potato indexing tests. Sixty days after grafting, one leaf of each grafted plant was collected, viral particles were semi-purified and RNA extracted using Trizol reagent according to the manufacturer`s instructions. The nucleic acids were sequenced by Illumina plaform. As a result, it was possible to assemble 1.249 contigs where the largest, with 10.517 nucleotides, showed 99% coverage and 99% identity with Sweet potato virus G - SPVG (genera *Potyvirus*, family *Potyviridae*) when compared to sequences from GenBank. It was also possible to detect the SPCSV to two contigs of 8.475 nucleotides (with 91% coverage and 91% identity to RNA-1) and 7.534 nucleotides (86% coverage and 86% identity to RNA 2), respectively. And finally, one contig related to SPFMV with 2.668 nucleotides with 95% coverage and 95% identity when compared to GenBank sequences. Further research will focus on the designing of specific primers to detect the viruses in each plant separately. Financial Support: CAPES.

PIV30 - INTERCEPTION OF WHEAT MOSAIC VIRUS (WMOV) IN MAIZE SEEDS FROM USA

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High Plains disease (HPD) was first described in wheat (*Triticum aestivum*) and maize (*Zea mays*) crops in Nebraska and other High Plains States in United States since 1993. The causal agent is a negative sense RNA virus in the genus *Emaravirus*, referred to as High Plains virus (HPV), or Wheat mosaic virus (WMoV). The virus has since been found in Israel, Chile, Argentina, Australia and has a host range that includes economically important plants such as wheat, maize, barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*) and some weeds. HPD symptoms and severity vary considerably from mild to severe and include mosaic, chlorosis and/or necrosis. The virus is transmitted by the eriophyd wheat curl mite *Aceria tosichella* which is also the vector of *Wheat streak mosaic virus* (WSMV), often found in mixed infections with WMoV. There is no report of WMoV in Brazil until the moment and the recent detection of the pathosystem