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Grapevine viruses induce reduction of productivity and quality of grapes. Grapevine leafroll-associated virus 4, GLRaV-4 (Closteroviridae, Ampelovirus) causes leaf roll in grapevine. Absolute quantification determines the absolute amount of a target (expressed as a copy number or concentration). The objective of this study was to generate a standard curve for GLRaV-4 absolute quantification in infected grapevines. Reagents and reaction set up for GLRaV-4 amplification were previously described. To generate a standard curve, 5 or 6 different amounts (tenfold diluted) of the standard were quantified by TaqMan real time RT-PCR. Reactions were carried out in triplicates and standard curves were generated by two independent experiments. For quantification of RNA molecules as standard, a fragment containing part of the GLRaV-4 genome (300 bp covering 94 bp hHSP70 DNA fragment amplified by real time RT-PCR) was transcribed in vitro from a previously obtained transcriptional recombinant vector. This clone carries partial sequences of 14 viruses, fused in tandem, including GLRaV-4. After in vitro transcription, plasmid DNA template was removed with DNase and transcribed RNA concentration was measured by spectrophotometry. The use of RNA standard takes the variable efficiency of the reverse transcription reaction into account. The copy number of standard GLRaV-4 RNA molecules was calculated using the formula: Y molecules/µl = (X g/µl RNA / [transcript length in nucleotides x 340]) x 6.022 x 10^23 (Qiagen Handbook, 2011). After a standard curve was generated, 76 infected grapevine samples were evaluated to determine GLRaV-4 titre. The standard curve (plot of CT value, threshold cycle, against log of amount of standard) was generated: y = -1.509ln(x) + 41.202; in which R2 = 0.9999, y = CT value and x = RNA molecules/µl. The CT value of the target was compared with the standard curve (used as a reference in all subsequent reactions), allowing calculation of the GLRaV-4 amount in the samples. The absolute amount of GLRaV-4 nucleic acid in analyzed samples was determined and ranged from ca. 1000 to 150,000 copies of GLRaV-4/µl. This result can improve virus diagnosis by accurately quantifying virus titre variations in grapevines. Financial support: Embrapa

PIV56 - DETECTION AND COAT PROTEIN GENE CHARACTERIZATION OF GRAPEVINE VIRUS B ISOLATES FROM DIFFERENT GRAPEVINE SPECIES

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Corky bark, a component of the grapevine rugose wood complex, caused by Grapevine virus B, GVB (Betaflexiviridae, Vitivirus), induces decrease of production, incomplete ripening of grapes and progressive decline. Cultivars and rootstocks differ in their susceptibility to the corky bark disease. Some are symptomless carriers or exhibit mild symptoms, while others suffer rapid decline. The objective of this work was to characterize partially three isolates of GVB collected from different grapevine species and Brazilian geographical regions: GVB, the isolate named CS, was collected from cv. Cabernet Sauvignon (Vitis vinifera) exhibiting dark red spotted leaves and mild curling down of leaf edges, maintained in Bento Goncalves, Rio Grande do Sul State; the isolate IS-SVF was collected from cv. Isabel (V. labrusca) showing bark swelling and longitudinal cracking of mature canes in Sao Vicente Ferrer, Pernambuco State, and the isolate CO was collected from symptomless cv. BRS Cora (hybrid grapevine) in Jales, Sao Paulo State. The symptoms could not be associated with a single virus, since these plants could be infected by two or more virus. Total RNA was extracted from infected grapevines by capture on silica and the complete coat protein (CP) gene of GVB was RT-PCR-amplified, cloned into pGEM-T Easy vector and sequenced (two clones/isolate). An expected fragment of 594 nucleotides (bp) (coding for 197 deduced amino acids) was amplified by RT-PCR, using specific primers for GVB (6445v and 7038r), from the three different infected grapevine sources. The obtained sequences showed a low variability of coat protein genes among the three GVB isolates. Nucleotide and amino acid identities were higher than 99% among themselves. GVB GenBank accession codes are KF040331 (CO), KF040332 (IS-SVF) and KF040333 (CS). Different grapevine symptoms vary according to combinations of cultivar or host species with viral isolates, strains or species. In this work, high homologous coat protein sequences of three GVB isolates from symptomatic and symptomless grapevines and from distant geographical regions are involved in a