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388

Detection of three viruses in apples by taqman real time RT-PCR.

(Detecção de três vírus em macieiras por meio de TaqMan RT-PCR em tempo real.)

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Apple (*Malus* spp.) viruses heavily affect the development and longevity of plantas and cause significant economic damages to apple production and fruit quality. These pathogens are the target of virus removal procedures of propagative material followed by laborious diagnostic assays. Real time PCR using specific fluorophore-marked probes (TaqMan) are increasing their acceptance in virus diagnosis as a consequence of its advantages over conventional PCR. The objetive of this work was to study the efficiency of Real time RT-PCR for detection of three virus species of apples: *Apple stem pitting virus* (ASPV), *Apple chlorotic leaf spot virus* (ACLSV) and *Apple mosaic virus* (ApMV). Oligonucleotides and fluorophore 6-FAM labeled-probes and the quencher TAMRA were designed based on regions of consensus in the coat protein genes of these viruses. Real time RT-PCR reactions were set up folowing Osman & Rowhani (J. Virol. Methods 154:69-75. 2008). Total RNA of healthy apples and of plants proved infected by these viruses were used in presence/absence-type assays using the reaction kit *TaqMan Master Mix One-Step RT-PCR* (Applied Biosystems). For indexing, total RNA of 20 apple accesses regenerated after heat therapy and/or tissue culture were used. It was possible to detect the three viruses with high sensitivity in infected samples (C_T = 22-27, threshold cycles of positive controls). Real time RT-PCR allows a significant increase in the speed, reliability and specificity of assays for viral pathogen diagnosis in apple plants.

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