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Association of a novel highly divergent monopartite circular ssDNA virus with chlorotic dwarf and dry branches in apple and pear and potential association with symptoms in grapevine

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Symptomatic apple (*Malus domestica* cv. Eva) and pear (*Pyrus* cv. Pera d'água) plants collected in Viçosa, MG, Brazil, showing symptoms of chlorotic dwarf, delay and weakening of budding and dry branches, and one grapevine plant (*Vitis vinifera* cv. Cannonao) collected in Bento Gonçalves, RS, Brazil, showing red, coriaceous leaves and leaf roll, were evaluated for the presence of a circular, ssDNA virus. Using rolling circle amplification, we cloned and sequenced a novel, highly divergent, apparently monopartite circular ssDNA virus. We sequenced a total of 45 complete viral genomes [17 from apple, 26 from pear (18 from one plant and 8 from another) and 2 from grapevine]. Sequence analysis revealed a genome of 3,424-3,442 nts, organized in eight putative functional ORFs, six on the 5' half of the virion-sense strand and two on the 5' half of the complementary-sense strand. A large intergenic region contains a short palindromic sequence capable of forming a hairpin-like structure and the sequence TAGTATTAC, conserved in all nanoviruses. The iteron-like and other conserved sequences are also evidenced in the intergenic region. The virion-sense ORFs encode two putative movement proteins (MP major of 471 nts and MP minor of 234 nts), the coat protein (CP, 714 nts), a putative silencing suppressor protein (237 nts), a protein with putative ribonuclease Z function (405 nts) and a putative transcription factor (186 nts). Those in the complementary-sense strand encode the replication-associated protein (Rep, 945 nts) and a protein with putative RNA-binding function (390 nts). Comparison of the amino acid (aa) sequences showed identities of approximately 35% with nanoviruses and alphasatellites (Rep), and mastreviruses (CP and MPs). A conserved domain found in the CP predicts importin α -dependent nuclear localization and DNA-binding properties. The two MPs (major and minor) have predicted transmembrane domains. PCR-based detection with specific primers was used to evaluate the incidence and the correlation of the new virus with the observed symptoms in apple, pear and grapevine. Additional samples were collected and several asymptomatic apple and pear samples were negative for the presence of the virus. Grapevines with mild symptoms were also negative for the presence of the virus. These results suggest the association of this novel ssDNA virus with the symptoms observed in these plants.

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