Development of polyvalent RT-PCR detection tests for the identification of new viruses from the family Closteroviridae

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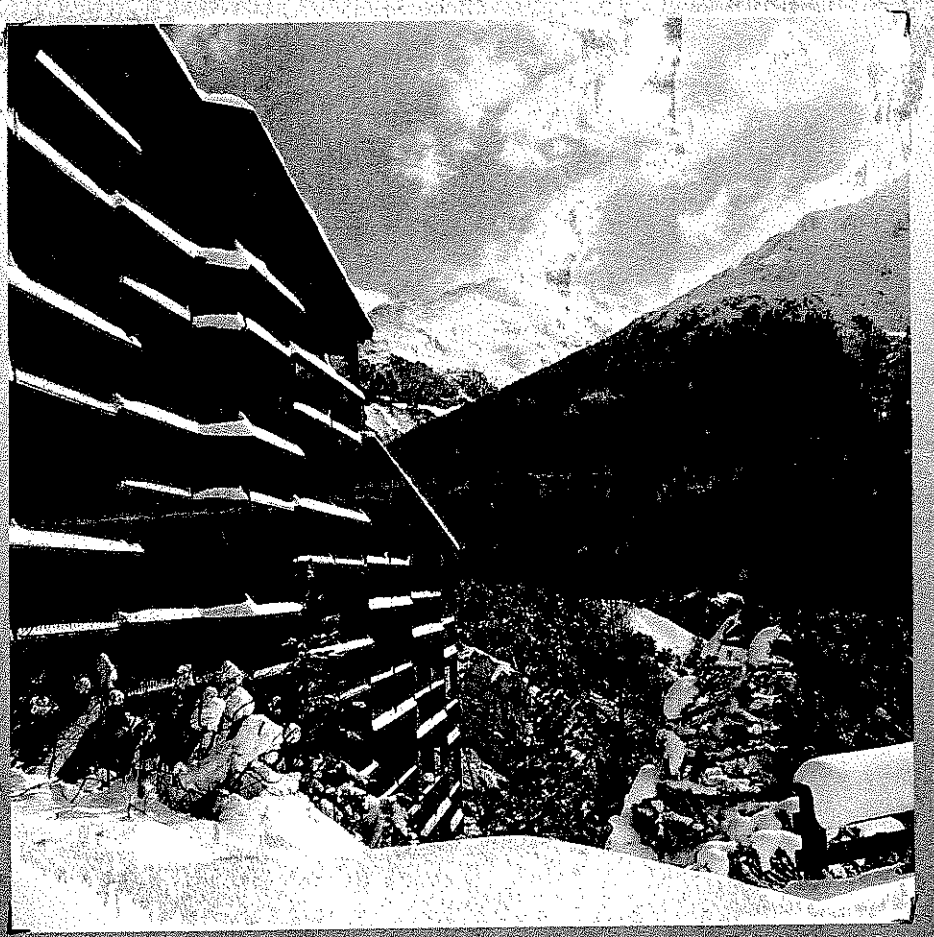

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16- Development of polyvalent RT-PCR detection tests for the identification of new viruses from the family *Closteroviridae*.

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The *Closteroviridae* family contains the *Closterovirus, Ampelovirus* and *Crinivirus* genera and, in addition, a few as yet unclassified viruses. Some important closteroviruses [*Citrus tristeza virus* (CTV)] and some criniviruses [*Lettuce infectious yellows virus* (LIYV), *Potato yellow vein virus* (PoYVV) *Tomato chlorosis virus* (ToCV), *Tomato infectious chlorosis virus* (TiCV) and *Cucurbit yellow stunting disorder virus* (CYSDV)] are classified as quarantine agents while some Ampeloviruses [Grapevine leafroll associated viruses, GLRAVs] are important in certification programs. Rapid and reliable polyvalent methods allowing the detection of multiple viruses, including novel, previously uncharacterized agents, are important for routine diagnosis as well as for etiology studies as they allow to reduce labor and other costs. Although some polyvalent assays have been reported for the *Closteroviridae* (Karasev et al., 1994; Tian et al., 1996; Dovas & Katis, 2003), their use is frequently difficult and yield sometimes inconsistent results.

We have therefore tried to develop a polyvalent test based on nested RT-PCR for the detection of members of the three genera of the *Closteroviridae* family. Based on the work published by Foissac et al. (2005), replicase protein gene sequences from all *Closteroviridae* sequences in databanks sequences were aligned and used to design four degenerated primers containing inosines covering the viral genomic variability for each of the three genera. These primers were then used in polyvalent nested RT-PCRs. The validation of this test was performed on a range of materials infected with known agents belonging to the *Closteroviridae* family. In the three tests, sequencing of the 128-bp amplified fragment and comparison with sequences available in databanks allowed efficient detection and identification of all evaluated the members of the three genera. We are currently evaluating the tests using a range of field sample and, particularly, of *Prunus* materials. A positive amplification signal was obtained in Apricot (*Prunus armeniaca*) material from the Czech Republic and sequence analysis of the amplified fragment revealed the presence of *Little cherry virus-1* (LChV1). Although this cherry-infecting virus was recently reported in peach, plum and almond (Matic et al., 2010), this detection seems to represent the first report of LChV-1 in apricot. We are also currently trying to develop a multiplex assay for the complete *Closteroviridae* family by joining together is a single reaction these 3 polyvalent assays.

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Foissac et al. 2005. Phytopathology, 95, 617-625.
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