Ilarviruses of *Prunus* spp.: Diagnosis, genetic diversity and movement within and among plants

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Prunus spp. are affected by a large number of viruses, causing significant economic losses through either direct or indirect damage, which results in reduced yield and fruit quality. Among these viruses, members of the genus *Ilarvirus* (isometric labile ringspot viruses) occupy a significant position due to their distribution worldwide. Although symptoms caused by these types of viruses were reported early in the last century, their molecular characterization was not achieved until the 1990s, much later than for other agronomically relevant viruses. This was mainly due to the characteristic liability of virus particles in tissue extracts. In addition, ilarviruses, together with *Alfalfa mosaic virus*, are unique among plant viruses in that they require a few molecules of the coat protein in the inoculum in order to be infectious, a phenomenon known as genome activation. Another factor that has made the study of this group of viruses difficult is that infectious clones have been obtained only for the type member of the genus, *Tobacco streak virus*. Four ilarviruses, *Prunus necrotic ringspot virus*, *Prune dwarf virus*, *Apple mosaic virus*, and *American plum line pattern virus*, are pathogens of the main cultivated fruit trees. As stated in the 9th Report of the International Committee on Taxonomy of Viruses, virions of this genus are "unpromising subjects for the raising of good antisera." With the advent of molecular approaches for their detection and characterization, it has been possible to get a more precise view of their prevalence and genome organization. This review updates our knowledge on the incidence, genome organization and expression, genetic diversity, modes of transmission, and diagnosis, as well as control of this peculiar group of viruses affecting fruit trees.

Ilarviruses share common biological properties and infect a wide range of *Prunus* species and plant families other than the family *Rosaceae*. PNRSV was initially described in 1941 on peach. The name "prune dwarf" was derived from the stunting and leaf malformation symptoms observed on Fellenberg prune (*P. domestica*). The diseases induced by PNRSV and PDV in stone fruits were known commonly as "ringspot diseases." ApMV was first described in apple and later on stone fruits in Bulgaria. Plum line pattern disease was first reported in Kentucky (United States) by Valleau and subsequently APLPV was identified as the causal agent. Ilarviruses have been considered as latent pathogens when affecting fruit trees and, consequently, their economical incidence has been underestimated. PNRSV causes significant crop losses depending on the host. Previous reports have shown that PNRSV is responsible for yield losses of up to 15 % in sweet cherry and of up to 100 % in peach. PNRSV can reduce bud-take in nurseries, decrease growth of fruit (10 to 30 %) and fruit yield (20 to 60 %), delay fruit maturity, and increase susceptibility to winter injuries in orchards. Yield losses caused by PDV may reach up to 50 % in sour cherry, whereas it causes low bud-take in nurseries (40 to 50 % compared with healthy stocks) and slower growth of young trees. PDV is probably the most damaging almond-infecting ilarvirus and causes chlorotic mottle, line pattern, and occasionally, stunted vegetation in the Mediterranean region. PDV and PNRSV act synergistically in mixed infections, causing peach stunt disease and often leading to a progressive decline, provoking the death of stone fruit trees. Little is known about the real economic impact of ApMV and APLPV on stone fruit production. However, it has been shown that ApMV infection may result in growth reduction and yield losses in other crop species (hazelnut), and that it may cause reduced apple bud-take in nurseries and losses of 46 % in Golden Delicious cultivars. Given the scenario in which ilarviruses are proven to be economically important, it is quite surprising that their molecular characterization lagged behind that of other plant viruses and was delayed until the beginning of the 1990s due essentially to their extreme lability. Determination of the complete sequence of ilarvirus genomes contributed to the emergence of molecular approaches aimed at the study and diagnosis of these viruses.

We now have a more informed picture about their diagnosis, incidence, genomic organization, and expression, and about the potential mechanisms by which they are transmitted. However, we are still far from knowing how these viruses exert their pathogenic effects in susceptible hosts. Studies of host–virus interactions are still lacking for this type of virus and only experimental hosts have been primarily used to date. The use of high throughput technologies would be useful to advance ilarvirus– host interactions to identify key genes involved in pathogenicity functions in their respective hosts and help to design strategies for their control.

Bibliographic References

Amari, K., Burgos, L., Pallás, V. and Sánchez-Pina, M.A. 2007. Prunus necrotic ringspot virus early invasion and its effects on apricot pollen grains performance. Phytopathology 97:892-899.

Barba, M. and Hadidi, A. 2011. DNA microarrays and other future trends in detection and typing of viruses, viroids and and phytoplasmas. Pages 361-372, *in*: Virus and

virus-like diseases of pome and stone fruits. A. Hadidi, M. Barba, T. Candresse and W. Jelkmann, eds. APS Press, St Paul, MN, USA.

Bol, J.F. 2005. Replication of alfamo- and ilarviruses: role of the coat protein. Annu. Rev. Phytopathol. 43:39-62.

Herranz, M.C., Al-Rwahnih, M., Sánchez-Navarro, J.A., Elena, S.F., Choueiri, E., Myrta, A. and Pallás, V. 2008. Low genetic variability in the coat and movement proteins of American plum line pattern virus isolates from different geographic origins. Arch. Virol. 153:367-373.

Herranz, M.C., Sánchez-Navarro, J.A., Aparicio, F. and Pallás, V. 2005. Simultaneous detection of six stone fruit virases by non-isotopic molecular hybridizationusing a unique riboprobe or `polyprobe´. J. Virol. Methods 124:49-55.

Herranz, M.C., Sanchez-Navarro, J.A., Sauri, A., Mingarro, I. and Pallas, V. 2005. Mutational analysis of the RNA-binding domain of the Prunus necrotic ringspot virus (PNRSV) movement protein reveals its requirement for cell-to-cell movement. Virology 339:31-41.

James D, Varga A, Pallas V and Candresse, T. 2006. Strategies for simultaneous detection of multiple plant viruses. Can. J. Plant Pathol. 28: 16-29.

Más, P. and Pallás, V. 1995. Non-isotopic tissue-printing hybridization: a new technique to study long-distance plant virus movement. J. Virol. Methods 52:317-326.

Mink, G.I. 1993. Pollen- and seed-transmitted viruses and viroids. Annu. Rev. Phytopathol. 31:375-402.

Pallas, V., Aparicio, F., Herranz, M. C., Amari, K., Sanchez-Pina, M. A., Myrta, A., and Sanchez-Navarro, J. A. 2012. Ilarviruses of Prunus spp.: A continued concern for fruit trees. Phytopathology 102:1108- 1120.

Pallás, V. and Garcia, J.A. 2011. How do plant viruses induce disease? Interactions and interference with host components. J. Gen. Virol. 92:2691-2705.

Pallás, V., Faggioli, F., Aparico, F. and Sanchez-Navarro, J.A. 2011. Molecular Hybridization Techniques for Detecting and Studying Fruit Tree Viruses and Viroids. Pages 335-342 *In*: Virus and virus-like diseases of pome and stone fruits. A. Hadidi, M. Barba, T. Candresse and W. Jelkmann, eds. APS Press, St Paul, MN, USA.

Pallás, V., Más, P. and Sánchez-Navarro, J.A. 1998. Detection of plant RNA viruses by nonisotopic dot-blot hybridization. Methods Mol. Biol. 81:461-468.

Peiró, A., Pallás, V. and Sánchez-Navarro, J.A. 2011. Simultaneous detection of eight viruses and two viroids affecting stone fruit trees by using a unique polyprobe. Eur. J. Plant Pathol. DOI 10.1007/s10658-011-9893-0

Sánchez-Navarro, J.A., Aparicio, F., Herranz, M.C. and Pallás, V. 2005. Simultaneous detection and identification of eight stone fruit viruses by one step RT-PCR. Eur. J. Plant Pathol. 111:77-84.

Sánchez-Navarro, J.A., Herranz, M.C. and Pallás, V. 2006. Cell-tocell movement of Alfalfa mosaic virus can be mediated by the movement proteins of Ilar-, bromo-, cucumo-, tobamo- and comoviruses, and does not require virion formation. Virology 346:66-73.

Scott, S.W., Bowman-Vance, V. and Bachman, E.J. 1992. The use of nucleic acid probes for the detection of Prunus necrotic ringspot virus and prune dwarf virus. Acta Hortic. 309:79-83.

Scott, S.W., Zimmerman, M.T., Ge, X. and MacKenzie, D.J. 1998. The coat proteins and putative movement proteins of isolates of Prunus necrotic ringspot virus from different host species and geographic origins are extensively conserved. Eur. J. Plant Pathol. 104:155-161.

Untiveros, M., Perez-Egusquiza, Z. and Clover, G. 2010. PCR assays for the detection of members of the genus ilarvirus and family Bromoviridae. J. Virol. Methods 165:97-104.

Uyemoto, J. K., and Scott, S. W.1992. Important diseases of Prunus caused by viruses and other graft-transmissible pathogens in California and South Carolina. Plant Dis. 76:5-11.