Peach latent mosaic viroid in naturally infected sweet cherry trees in southern Italy

Crescenzi, A.[1], Piazzolla, P.[1] and Hadidi, A.[2]

[1]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali – Università degli Studi della Basilicata – C.da Macchia Romana, 85100 Potenza – Italy.

[2]Fruit Laboratory – Agricultural Research Service – United States Department of Agriculture, Beltsville, MD 20705 USA

Corresponding author: A. Crescenzi, Phone: +390971.205700, Fax: + 39.0971.2055703

E-mail:crescenzi@unibas.it

Summary: Peach latent mosaic viroid (PLMVd) was found in naturally infected sweet cherry trees grown in commercial orchards in southern Italy. The viroid was detected in nucleic acid extracts of symptomless leaves by molecular hybridization with a PLMVd cRNA probe. The viroid was transmitted by grafting from sweet cherry to peach seedlings and identified in peach by molecular hybridization.

Key words: graft transmission, dot-blot hybridization, PLMVd, cRNA probe, southern Italy, sweet cherr

For almost two decades *Peach latent mosaic viroid* (PLMVd) was thought to be restricted to peach (*Prunus persica)*, nectarine, or peach hybrid trees. During the last few years, however, it has been shown that other stone fruit trees, such as sweet cherry (*P. avium)*, plum (*P. domestica)* and apricot *(P. armeniaca)*, as well as Japanese plum (*P. salicina)* and mume (*P. mume)* are natural hosts of PLMVd (Faggioli *et al.* 1997; Hadidi *et al.*, 1997; Giunchedi *et al.*, 1998; Osaki *et al.* 1999). In addition, natural infection by PLMVd of cultivated (*Pyrus communi)* and wild pear (*P. amygdaliformis)* has recently been reported (Kyriakopoulou *et al.*, 2001).

PLMVd was detected at a low concentration by reverse transcription-polymerase chain reaction (RT-PCR) in quarantined germplasm at the United States Department of Agriculture plant quarantine facility, Beltsville (MD) and in sweet cherry in Romania and Italy (Hadidi *et al.*, 1997). This viroid was also detected by RT-PCR in sweet cherry trees in Canada and Japan (Hadidi *et al.*, 1997; Osaki *et al.*, 1999) and its identity was confirmed by molecular cloning and nucleotide sequencing of the amplified cDNA products (Hadidi *et al.*, 1997).

We now report that PLMVd infects naturally sweet cherry trees in southern Italy and that it is graft transmissible from sweet cherry to peach GF 305 seedlings.

During a survey for assessing the sanitary status of cherry plants in southern Italy, about 20 samples from different cultivars were collected from several orchards. Budwoods from collected samples were grafted onto peach 'GF 305' to transmit PLMVd, if present, from the naturally infected cherry samples to this PLMVd-susceptible host species. PLMVd is readily transmissible by grafting and budding to this peach cultivar (Desvignes, 1986; Flores *et al.*, 2002). Several months later, leaves of graft-inoculated peach plants remained symptomless.

Total nucleic acids (TNA) were extracted from 250 mg of cherry and peach leaf tissue as described by Hadidi *et al.*, (1990). TNA were denatured by adding an equal volume of formaldehyde denaturation buffer (3 volumes of 20x SSC buffer and 2 volumes of 37% formaldehyde) for 60 minutes at 65 °C. Fifty µl of each sample were applied onto positively charged Nytran^{TM} membranes using a Minifold^{TM} apparatus. After spotting, the TNA were cross-linked to the membranes in an UV Stratalinker oven. Prior to hybridisation, membranes were pre-hybridised for 60 minutes at 55 °C and then hybridised overnight at the same temperature with a digoxigenin (DIG)-labeled SP6 RNA polymerase-generated, full length, PLMVd cRNA probe (Shamloul *et al.*, 1995, Hadidi *et al.*, 1997). After washing, the membranes were incubated in 2x SSC with 1 µg/ml RNase A at room temperature and then subjected to digoxigenin chemiluminescent detection.

Figure 1 shows dot blot hybridisation detection of PLMVd in total nucleic acid extracts from leaf tissues of naturally infected Italian sweet cherry plants *(Fig. 1, d and e)* and grafted peach GF-305 seedlings *(Fig. 1, f)*. PLMVd was detected in naturally infected sweet cherry plants and was successfully graft-transmitted to peach GF-305 seedlings. Molecular hybridisation showed that 5% of the 18 sweet cherry tested plants were naturally infected by PLMVd. Molecular data were confirmed by graft-transmissibility of the viroid to peach 'GF-305' seedlings.

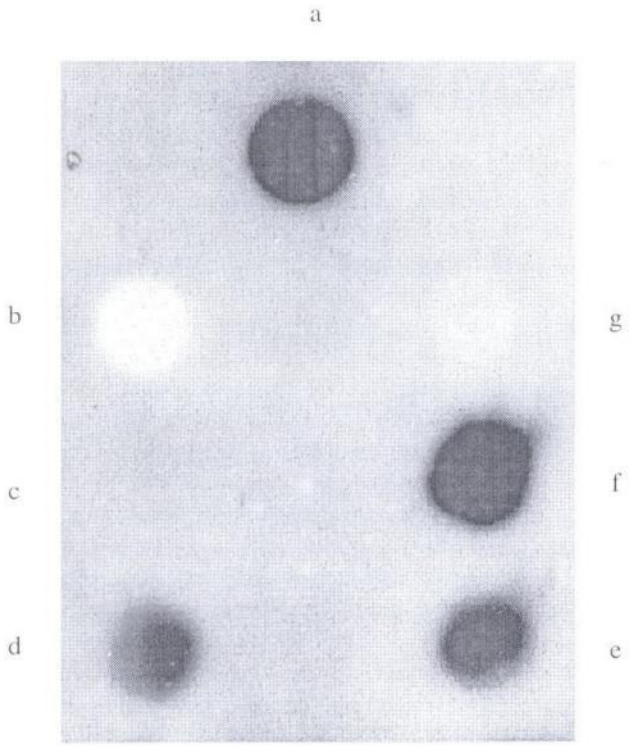

Figure 1 Detection of PLMVd by molecular hybridisation on total nucleic acid extracts from leaf tissue from cherry and peach leaf tissue. DIG-labelled PLMVd cRNA was used as probe. **a)** peach GF-305, PLMVd positive control; **b)** peach GF-305, healthy control; **c)** and **d)** *P. avium* cv Ferrovia plants from orchard; **e)** *P. avium* cv Napoleon plant from orchard; **f)** peach GF-305, experimentally infected by graft inoculation from cherry plant, resulted positive to PLMVd in molecular hybridisation; **g)** *P. avium*, healthy control

Peach latent mosaic viroid was previously reported in Italy by Faggioli *et al.*, (1997). It was showed that it is graft-transmissible from naturally infected symptomless plum trees to peach GF-305 seedlings They based its identification on polyacrylamide gel electrophoretic analysis of RNA extracts of infected plum and grafted peach GF-305 seedlings leaves. On the contrary, we have identified PLMVd by molecular hybridisation with a PLMVd specific cRNA probe.

With the exception of PLMVd in plum, that is associated with plum spotted fruit disease in Italy (Giunchedi *et al.*, 1998), the economic impact of PLMVd in stone fruits other than peach has not yet been determined. Plum fruits with spot symptoms may be of reduced value as they may not be suitable for export (Hadidi *et al.* 2002). The direct economical impact of PLMVd infection on sweet cherry is uncertain. However, because PLMVd is transmitted by aphids (Flores *et al.* 1992), though at a low rate, and by contaminated tools (Hadidi *et al.* 1997), the possibility exists of natural spread to other stone fruit species. Thus, testing for PLMVd in sweet cherry in certification and quarantine programs in Italy and other countries is advisable

References

Desvignes, J. C. (1986): Peach latent mosaic and its relation to peach mosaic and peach yellow mosaic virus diseases. *Acta Horticulturae* **193**: 51–57.

Faggioli, F., Loreti, S. & Barba, M. (1997): Occurrence of peach latent mosaic viroid (PLMVd) on plum in Italy. Plant Disease **81**: 423.

Flores, R., Hernandez, C., Avinent, L., Hermoso, A., Llácer, G., Juarez, J., Arregui, J. M., & Navarro, L., C. (1992): Studies on the detection, transmission and distribution of peach latent mosaic viroidin peach trees. *Acta Horticulturae* **309**: 325–330.

Flores, R., Hernandez, C., Llacer, G., Shamloul, A. M., Giunchedi, L. & Hadidi, A. (2002) Peach latent mosaic viroid in peach. In: Hadidi, A., Flores, R., Randles, J. W., Semancik, J.S. (eds.). *Viroids*. CSIRO Publishing, Collingwood, Victoria, Australia

Giunchedi, L., Gentit, P., Nemchinov, L., Poggi-Pollini, C. & Hadidi, A. (1998): Plum spotted fruit: a disease associated with peach latent mosaic viroid. *Acta Horticulturae* **472**: 571–579.

Hadidi, A., Giunchedi, L., Osaki, H., Shamloul, A.M., Crescenzi, A., Gentit, P., Nemchinov, L., Piazzolla, P. & Kyriakopolou, P. E. (2002): Peach latent mosaic viroid in temperate fruit hosts. In: Hadidi, A., Flores, R., Randles, J.W. & Semancik, J. S. (eds.). *Viroids*. CSIRO Publishing, Collingwood, Victoria, Australia

Hadidi, A., Giunchedi, L., Shamloul, A. M., Poggi-Pollini, C. & Amer, M., A. (1997): Occurrence of peach latent mosaic viroid in stone fruits and its transmission with contaminated blades. *Plant Disease* **81**: 154–158.

Hadidi A., Huang C., Hammond R.W. & Hashimoto J. (1990): Homology of the agent associated with dapple apple disease to apple scar skin viroid and molecular detection of these viroids. *Phytopathology* **80**: 263–268.

Kyriakopoulou, P. E., Giunchedi, L. & Hadidi, A. (2001): Peach latent mosaic viroid and pome fruit viroids in naturally infected cultivated pear *Pyrus communis* and wild pear *P. amygdaliformis:* implications on possible origin of these viroids in the Mediterranean region. *Journal of Plant Pathology* **83**: 51–62.

Osaki, H., Yamaguchi, Y., Sato, Y., Tomita, Y., Kawai, Y., Miyamoto, Y. & Ohtsu, Y. (1999): Peach latent mosaic viroid isolated from stone fruits in Japan. *Annals of Phytopathological Society of Japan* **65:** 3–8.

Shamloul A. M., Minafra A., Hadidi A., Giunchedi L., Waterworth H.E. & Allam E. K. (1995): Peach latent mosaic viroid: nucleic sequencing of an Italian isolate, sensitive detection using RT–PCR and geographic distribution. *Acta Horticulturae* **386**: 522–530.