Detection of *Peach Latent Mosaic Viroid* by PCR

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Abstract

Peach latent mosaic viroid (PLMVd) is widespread in peach all over the world. It has never been reported from the Czech Republic. That is why we adapted specific and sensitive method for its detection, PCR, to be able to prove its possible occurrence and for certification purposes. Primers PLMVdR, PLMVdF1 and PLMVdF2 were designed on the basis of published RNA sequences. Products of amplification are 208 and 114 bp long for PLMVdF1 and PLMVdF2, respectively. Four PLMVd isolates from Dr Di Serio (CNR Bari) were used as standards. *Potato spindle tuber viroid* and *Hop latent viroid* infected plant material and also healthy material were used to check detection specifity. Both RNA extraction from plant material and PCR were optimalized so that this method of PLMVd detection can also be used for certification purposes.

Keywords: PLMVd; PCR; peach; certification

INTRODUCTION

Peach latent mosaic viroid (PLMVd) is one of the most important viroids in stone fruit trees. For the first time it was isolated in 1988 (FLORES & LLACER 1988) even when the disease it causes has been known since thirties of the last century (DESVIGNES 1986). The main host of PLMVd is peach with symptoms like leaf mosaic, flower breaking, deformation and cracking of fruits. Presence of PLMVd in other stone fruit species (apricots, cherries, plums) was also reported (FAGGIOLI *et al*. 1997; HADIDI *et al*. 1997) but experimentally only peaches could be infected (DESVIGNES 1986). PLMVd is probably widespread all over the world as it could be detected in 25% of trees from Europe, USA, China and Japan (DESVIGNES 1986). Currently PLMVd is detected either by biological tests by grafting onto GF 305 rootstock or by revers PAGE. Both these methods are rather time consuming (for biological test several months) or the sensitivity is low (electrophoresis). For certification of seed material there is need to have fast, sensitive and reliable method of PLMVd determination. That is why PCR is being developed in several laboratories (SHAMLOUL & HADIDI 1999).

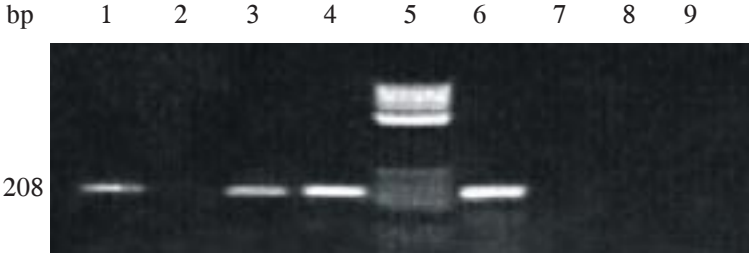
MATERIAL AND METHODS

Four isolates of PLMVd were obtained from Dr Di Serio (CNR Bari) and multiplied by budding onto GF 305 rootstocks (3 buds per rootstock). One part of plants was transferred to glasshouse at the beginning of December to initiate the growth. Leaves were taken during vegetation both from glasshouse grown and later also from outside grown plants and RNA was extracted. Four methods of RNA extraction were used: method of ROBERTSON *et al*. (1991), method of ROBINSON (1992) and extraction by kits from Qiagen and Adgen. Healthy leaves and also plant material infested by *Potato spindle tuber viroid* and *Hop latent viroid* were used as controls. On the basis of published sequences of PLMVd three primers were designed using Williamstone Enterprises program, one reverse (PLMVdR) and two forward (PLMVdF1 and F2). One of them (F1) gives with reverse primer product 208 bp long and second (F2) gives product 114 bp long. Reverse transcription was done in 25 μl reactions containing 5 μl reaction buffer, 0.5 μl (5 U) AMV reverse transcriptase (Promega), 0.5 μl (20 U) Rnasin (Promega), 10 pmol of reverse primer, 0.1mM of each dNTP, 1 μl RNA and DEPC

treated water to 25 μl. This mixture was incubated at 42°C for one hour and boiled for 5 min. PCR was done in 25 μl reaction containing 2.5 μl buffer for Dynazyme DNA polymerase, 1 μl (2 U) Dynazyme (Finnzymes), 10 pmol of each primer, 0.1 mM of each dNTP and 2 μl of cDNA. A PTC 200 thermocycler (MJ Research) was programmed to 5 min at 70°C, 40 cycles of 1 min 94°C, 30 s 58°C and 1 min 72°C, and final 4 min incubation at 72°C. Products were visualized after electrophoresis in 1% agarose gel with ethidium bromide on UV transilluminator.

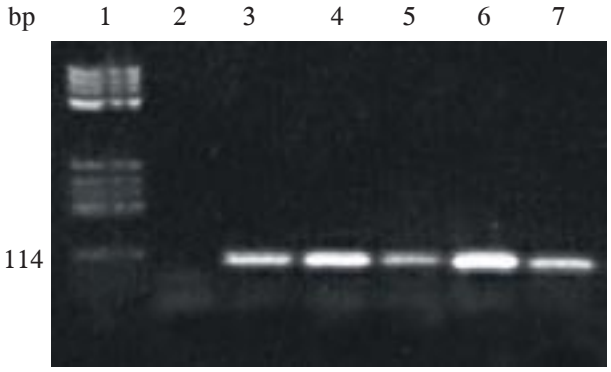
RESULTS AND DISCUSSION

Both pairs of primers R+F1 and R+F2 gave products of predicted lenght with all four PLMVd isolates (Figures 1 and 2). Neither RNA extracted from healthy leaves nor from other viroids gave any product (data not shown). There were no substantial differences among various RNA extraction methods if young leaves were used. The only exception was kit from Adgen, where no products were formed any time. When older leaves from glasshouse grown plants were extracted only kit from Qiagen was successful to provide RNA giving PCR products (Figure 3). The explanation for this may be in high concentration of phenolic compounds in older leaves inhibiting reverse transcription and/or PCR reactions. These compounds can be copurified using phenol/chloroform extraction of RNA but they are eluted from affinity purified RNA when using kit from Qiagen. Similar results were obtained by CAMBRA *et al*. (pers. commun.) in



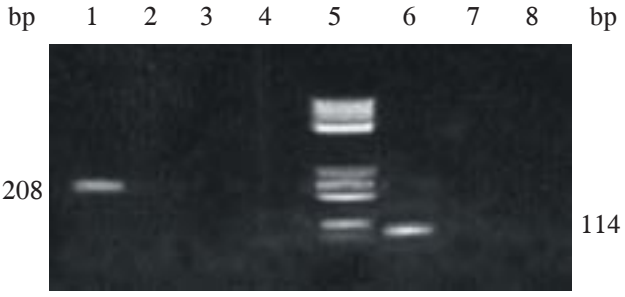
Lane 1: RNA of isolate 1 from glasshouse plant
Lane 2: RNA of isolate 2 from glasshouse plant
Lane 3: RNA of isolate 3 from glasshouse plant
Lane 4: RNA of isolate 4 from glasshouse plant
Lane 5: Molecular marker
Lane 6: RNA of isolate 1 from outside plant
Lane 7: RNA from healthy plant
Lane 8: Healthy plant
Lane 9: Healthy plant

Figure 1. PCR products using primers PLMVdR and PLMVdF1, young leaves, Qiagen RNA extraction



Lane 1: Molecular marker
Lane 2: RNA from healthy plant
Lane 3: RNA of isolate 1 from glasshouse plant
Lane 4: RNA of isolate 2 from glasshouse plant
Lane 5: RNA of isolate 3 from glasshouse plant
Lane 6: RNA of isolate 4 from glasshouse plant
Lane 7: RNA of isolate 1 from outside plant

Figure 2. PCR products using primers PLMVdR and PLMVdF2



Lane 1: Qiagen PLMVdR and PLMVdF1
Lane 2: Robinson PLMVdR and PLMVdF1
Lane 3: Robertson et al. PLMVdR and PLMVdF1
Lane 4: Adgen PLMVdR and PLMVdF1
Lane 5: Molecular marker
Lane 6: Qiagen PLMVdR and PLMVdF2
Lane 7: Robinson PLMVdR and PLMVdF2
Lane 8: Qiagen PLMVdR and PLMVdF2

Figure 3. PCR products using primers PLMVdR and PLMVdF1, PLMVdR and PLMVdF2, isolate 1. old leaves of glasshouse plant. Different RNA extraction

detection of olive viruses. Highly necrotized sugar beet roots after infection by BNYVV contained also high amount of inhibitors preventing product formation in PCR after phenolic RNA extraction (RYŠÁNEK 1999). So, if RNA extraction method is modified according to the leaf age PCR can be successfully used for PLMVd detection.

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