Information pest: *Plum pox virus*

Plum pox virus is a regulated plant pathogen included in European legislation as a quarantine pest.
Sharka, caused by *Plum pox virus* is the most devastating viral disease worldwide in terms of agronomic impact and economic importance of stone fruits including peaches, apricots, plums, nectarines, almonds, sweet and sour cherries. The disease was first described in 1917 in Bulgaria; since then, it has progressively spread to a large part of Europe, India and America. The introduction of infected plant propagation material is considered the most important means of long-distance spread of *PPV* which is transmitted by many aphids species.

PPV consists of several strains; the most common are PPV-M and PPV-D.

Infected plants may not show symptoms for several months and symptoms are often transient in appearance. *PPV* symptoms on stone fruits depend on host species and cultivar and the strain of the virus. Symptoms may appear on leaves, petals, fruits and stones. They are particularly conspicuous on leaves in spring: chlorotic spots, bands or rings, vein clearing, or even leaf deformation in peaches. Some peach cultivars may also show flower breaking symptoms. Infected fruits show chlorotic spots or rings.

Introduction

The PCR *Plum pox virus* set has been developed by Qualiplante based on Wetzel et al. (1991b).

This method is referred in the Appendix 3 of the PM7/032 (1) Diagnostic protocols for regulated pests: *Plum pox potyvirus*, European and Mediterranean Plant Protection Organization Bulletin (2004) 34, 155-157.

A verification was performed by Qualiplante (data not published). Moreover, the performance of the method was evaluated during an Italian project and obtains the following validation data on symptomatic plants collected in spring:

- sensibility: 100%
- specificity: 100%
- relative accuracy: 100%
- analytical sensitivity: 10^{-6}
- analytical specificity: 100%
- repeatability: 100%
- reproducibility: 92,03%.

The sensitivity and the relative accuracy decrease when plants collected in spring are not symptomatic and (66%;83%) and when plants are collected in winter (65%, 82%).

The primer pair was designed on homologous regions of different PPV strains (PPV-D, PPV-M and PPV El Amar) and amplifies a 243 bp product.

Intended use

The PCR set is validated for the detection of *Plum pox virus* (PPV) in One-Step End-Point RT-PCR. Suitable tissues are plant leaves, flowers and buds. The best period for analysis is spring (temperature less than 28°C) on symptomatic plants.

For leaves: the test portion is constituted by the basal part of each leaves of the sample. The sample preparation can be carried out as follows:

- overlap the leaves of the sample in the same direction from the basal part,
- cut and delete all the petioles,
- cut the leaf blade in order to obtain the same surface for each of the leaves constituting the sample,
- cut the basal part of the leaves, perpendicular to the central vein, to obtain a total weight of sample equal to 1 gram.

For flowers: does not require particular preparation:

- cut the flowers constituting the sample to obtain a total weight of the sample equal to 1 gram.

If the sample weighs more than 1 gram of flowers, collect a fragment of each flower as representative as possible until the total weight required for the sample is obtained.

For buds: does not require particular preparation. If the sample consists of several branches, it is necessary to take buds of each branch, as representative as possible, until the total weight required of 1 gram is reached. Buds can be removed from twigs with a scalpel.

Set format and content

Two sets are available for 24 and 96 tests.

Article N°	Product name
7PPV-P2	PCR *Plum pox virus* set 24
7PPV-P9	PCR *Plum pox virus* set 96

Content	set 24	set 96
Direct Master Mix	24 tests 7PPV--P2-DM-	2x48 tests 7PPV--P9-DM-
RT-Enzyme	24 tests 7PPV--P2-RT-	96 tests 7PPV--P9-RT-
Positive Control	3 tests 7PPV--P2-PC-	8 tests 7PPV--P9-PC-
Negative Control	3 tests 7PPV--P2-NC-	8 tests 7PPV--P9-NC-

This product should be used only for research purposes.

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Storage conditions

This set can be shipped at room temperature but upon receipt it should be stored immediately at the recommended storage temperature: **from -30 ° C to -10 ° C**.
Avoid prolonged exposure to light and repeated freeze and thaw cycles.

Shelf life

If the set is correctly stored, at constant-temperature freezer, its performance is guaranteed until the expiration date indicated on the tubes label.

Materials and equipment (not provided)

- RNA extraction tools and reagents
- Nuclease-free filter tips and micropipettes
- Optical grade nuclease-free tubes/plate
- Disposable latex or vinyl gloves
- DNA ladder and loading-dye buffer
- PCR thermal cycling
- Agarose gel reagents and apparatus

Nucleic acids extraction

Extract RNA from samples according to your usual protocol. Upon request, Qualiplante can recommend you an extraction method.

Preparation of the PPV 1-Step master mix

a) Slowly thaw Direct Master Mix and RT-Enzyme by placing it on ice or at 4°C.
b) Shake briefly Direct Master Mix and RT-Enzyme and spin down the liquid.
c) In a new tube called **PPV 1-Step master mix**, mix 17,5 µl of Direct Master Mix and 0,5 µl of RT-Enzyme per reaction. Do not forget to count the Positive Control and the Negative Control in the number of reactions to prepare.

Example:	**1 rxn**	**10 rxns**
Direct Master Mix	17,5 µl	175,0 µl
RT-enzyme	0,5 µl	5,0 µl

d) Store the **PPV 1-Step master mix** by placing it on ice or at 4°C.

Reaction set-up

a) Shake briefly **PPV 1-Step master mix** and spin down the liquid.
b) Add 18 µl of **PPV 1-Step master mix** (without RNA template) to each PCR tubes or wells of an optical-grade PCR plate.
c) Add 2 µl of RNA template to the **PPV 1-Step master mix**. Do not forget to prepare a PCR tube or well of an optical-grade PCR plate for the Positive Control and the Negative Control.

Components	**Volume/PCR tube or well**
RNA template or Positive control or Negative control	2 µl
PPV 1-Step master mix	18 µl
Total Volume / PCR tube or well	20 µl

In order to confirm the absence of any reagent's contamination, we strongly recommend including a no-template control (e.g. DEPC water) in the assay.

Run and thermal cycling

a) Seal carefully the PCR tubes or PCR plate. Centrifuge briefly to collect components at the bottom of the PCR tubes or wells of the plate. Protect from light before thermocycling.
b) Load the PCR tubes or plate into the thermal-cycler and follow the thermal cycling below:

Steps	Temp (°C)	Time	Cycle(s)
Reverse transcription	50°C	15 min	1
Enzyme activation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing and elongation	60°C	60 sec	
Storage	4°C	∞	-

Agarose gel electrophoresis

Prepare an agarose gel **at 2% w/v in 0,5X-TBE buffer**.

Gel loading:
- load the DNA ladder (for example 100-1'000 bp DNA step ladder)
- load 10 µl of PCR products from the previous step adding the loading-dye buffer (not provided in the set).

Run: run the gel electrophoresis for 50-60 minutes at 80V.

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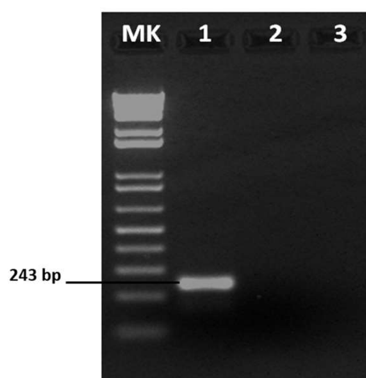
Results analysis

ANALYSIS VALIDATION

Plum pox virus is detected when a 243 bp DNA fragment is observed. The analysis is validated when:

- ✓ 1 DNA fragment of 243 bp is visible in the positive control lane.
- ✓ No DNA fragment is visible in the negative control lane.

The picture below represents a 0,5X-TBE 2% agarose gel showing the cDNA amplification in a sample infected by PPV:



MK: DNA ladder – **1: PPV positive sample** or Positive Control **- 2:** healthy sample or Negative Control - 3: No template control.

RESULTS INTERPRETATION

The specific product of PPV is a 243 bp DNA fragment.

- ✓ A sample is **positive** when a 243 bp specific DNA fragment is present in the PCR reaction.
- ✓ A sample is **negative** when no fragment is present in the PCR reaction.

The table below summarizes the results interpretation:

Fragment size **243 bp**	Interpretation
-	Negative
✓	**POSITIVE** *Plum pox virus*

Special handling instructions

This set was designed to be used by laboratory staff trained to follow the usual molecular biology precautions. Always perform the tests in a nuclease-free work environment. Always wear gloves when handling samples containing DNA/RNA and the components of the set. Do not touch any set components with an ungloved hand. Use appropriate laboratory disposable parts. Use nuclease-free tubes and filter tips to avoid degradation and cross-contamination. Do not use components from sets with different batch numbers in the same test procedure. Do not interchange reagents with other sets. To avoid cross-contamination, use separate rooms for (a) nucleic acids extraction, (b) preparation of the Master Mix and (c) amplification. To avoid cross-contamination and obtain reliable results, it is essential to strictly follow the protocol in this manual. Avoid unnecessary freeze-thaw cycles of the set components. Do not use reagents after their expiration date.

Troubleshooting

Post-PCR data analysis shows no amplification, or amplification plots look grossly abnormal:

Possible causes	Corrective actions
Evaporation of the sample due to inadequate sealing of the plate	Repeat the test using the appropriate tools to seal correctly the plate
Consumables are not appropriate for the method	Repeat the test using consumables recommended by the thermal cycler supplier
The quality of nucleic acid extracted is low	Repeat the extraction step. Ensure that the method of extraction has been performed correctly. In case of doubt, contact us
Abnormal amplification	Centrifuge the plate briefly to spin down the contents and eliminate any air bubbles

No amplification reaction is observed in the positive control well, while other samples are positive:

Possible causes	Corrective actions
The positive control provided with the set was not added into the reaction well	Repeat the test. If the problem persists, contact us

An amplification plot is observed in the negative control well:

Possible causes	Corrective actions
Contamination of the negative control or the Master Mix with target-positive nucleic acid	Repeat the test by applying appropriate quality procedures to prevent contamination. Seal the plate correctly

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