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in

Myrta A. (ed.), Di Terlizzi B. (ed.), Savino V. (ed.).
Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 45

2003
pages 89-93

Article available on line / Article disponible en ligne à l'adresse :

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To cite this article / Pour citer cet article

Shalaby A.A., Sahar A.Y., Mazyad H. **Occurrence and molecular detection of Plum Pox Virus strains in Egypt.** In : Myrta A. (ed.), Di Terlizzi B. (ed.), Savino V. (ed.). *Virus and virus-like diseases of stone fruits, with particular reference to the Mediterranean region.* Bari : CIHEAM, 2003. p. 89-93 (Options Méditerranéennes : Série B. Etudes et Recherches; n. 45)

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OCCURENCE AND MOLECULAR DETECTION OF *PLUM POX VIRUS* STRAINS IN EGYPT

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SUMMARY - Plum pox potyvirus EL Amar strain was detected in naturally infected apricot, peach and plum trees showing chlorotic rings, pale green lines and spots on the leaves, pale yellow rings and deformation on the fruits and typical spots on the stones collected from different locations of Egypt. Symptom observations followed by DAS-ELISA were used to identify the presence of PPV. Strain identification was done by RT-PCR, IC-RT-PCR, RFLP and sequence analysis using primers specific for PPV-C (cherry), PPV-D (Dideron), PPV-M (Marcus) and El-Amar strains. Results indicated that PPV-EA (El-Amar) is the only strain of the virus found in Egypt.

Key words: Egypt, stone fruits, PPV, virus strain, PCR, ELISA

RESUME *- Des isolats de PPV ont été collectés des vergers d'abricotier, pêcher et prunier infectés naturellement dans différentes localités en Egypte. L'observation des symptômes et l'application de la DAS-ELISA ont permis d'identifier des isolats de PPV. L'identification des souches a été effectuée par RT-PCR/RFLP et PCR avec des amorces spécifiques pour le PPV-C (cerisier), le PPV-D (Dideron) et PPV-M (Marcus). Les résultats ont indiqué que le PPV-EA (El-Amar) est la seule souche du virus repérée en Egypte.*

Mots-clés: *Egypte, espèces fruitières à noyau, PPV, souche du virus, PCR, ELISA.*

INTRODUCTION

Stone fruit trees are affected by a large number of viruses that exhibit very different biological properties as well as structural characteristics and genome expression strategies. *Plum pox virus* (PPV) is the most devastating viral disease worldwide of stone fruit including peaches, apricots, plums, nectarines, almonds and cherries. The disease significantly limits stone fruit production in areas where it is established. More than 100 million stone fruit trees in Europe are infected. Plum Pox was first discovered in Bulgaria in 1915 (Atanasoff, 1932), and gradually spread throughout most of Europe by the 1970s (Roy and Smith 1994), and later in the 1980s to the Middle East (Egypt) (Mazyad *et al*., 1992), India (Thakur *et al*., 1992) and Chile (Acuna, 1994). In 1999, PPV was detected in Pennsylvania (USA) (Levy *et al*., 2000), immediately after in Canada (Thomson *et al*., 2001). There are four PPV strains that can infect stone fruits. Most of the strains can infect several *Prunus* and herbaceous hosts. Many strains are spreading throughout different regions particularly in Europe and the Mediterranean countries. PPV-D was first isolated from apricots in France. It can also infect plums, peaches and nectarines naturally but does not infect cherry. It is well established in many European countries and the only strain found in the Western hemisphere. Aphids are not very efficient in transmitting D strain from infected to non-infected trees, being sometimes referred to as the "non-epidemic" form of PPV. An aggressive PPV-D isolate on peach has been observed and characterized in France but its distribution is unknown. The strain of Sharka identified in Ontario, Nova Scotia and Pennsylvania is the D strain, while PPV-M was first identified in the peach originated from Greece, but now it has been found in many European and Mediterranean countries. It is usually more aggressive in peach, but it has been isolated from naturally infected plum and apricot also. This strain is transmitted from infected to non-infected trees more efficiently by aphids than the D strain and is considered the "epidemic" form of PPV. Once established in a region, M strain can spread quickly and is very difficult to eliminate. On the other hand, PPV-Cherry has been the only strain isolated from sweet and sour cherry in nature. Both strains are considered as 'non-epidemic" forms of the virus. PPV-C strain is reported from several European countries (Kerlan and Dunez, 1979; Wetzel *et al*., 1991; Nemchinov and Hadidi, 1996; Crescenzi *et al*., 1996), and PPV-El Amar was isolated from apricots in Egypt, but can also infect plum and apricot in nature.

This study reports the molecular characterization of PPV isolates in Egypt and the differentiation between EL Amar and other strains (C, D and M) using PCR technique (RT-PCR, IC-RT-PCR and RFLP).

MATERIALS AND METHODS

PPV isolates used in this study were collected from naturally infected apricot, peach and plum orchards in different locations in Egypt exhibiting chlorotic ring spot symptoms.

DAS-ELISA (Clark and Adams 1977), RNA extraction using silica-capture method (Boom *et al*., (1990), and Immuno-capture PCR (Wetzel *et al*., 1992) were used in laboratory tests. 20-mer primer 5`-ACCGAGACCACTACACTCCC-`3 (homologous to PPV-RNA nucleotides 9036-9056) and a 20-mer primer 5`-CAGACTACAGCCTCGCCAGA-`3 (complementary to PPV-RNA nucleotides 9330-9310) encoding the carboxyl terminus of the coat protein gene of PPV (P1 and P2) were used as described by Wetzel *et al*. (1991). In addition, two complementary primers (PD and PM) (Cambra *et al*., 1998) were used with P1 primer to differentiate between D and M strain. For PPV-C, specific oligonucleotide primers (Nemchinov and Hadidi, 1998) from the 5` terminus of the coat protein gene of the sour cherry strain of PPV (PPV-SoC) (Nemchinov *et al*., 1996) were used. PCR-amplified products using P1 and P2 primers were digested using *RsaI* enzyme (Promega). PCR products were sequenced and computer analyses were done for all PPV strains. The phylogenetic tree observed the identity between the various strains.

RESULTS

Plum pox potyvirus EL Amar strain was detected in naturally infected apricot, peach and plum trees showing PPV symptoms, collected from different locations of Egypt (Fig. 1).

Fig. 1. Distribution of PPV in different locations in Egypt i.e. Fayum, Sinai, Alexandria and Al Arish.

In Reverse transcription polymerase chain reaction (RT-PCR) analysis, a DNA fragment of the expected size (243 bp) was amplified from leaf extracts of infected apricot, peach and plum trees (Fig. 2).

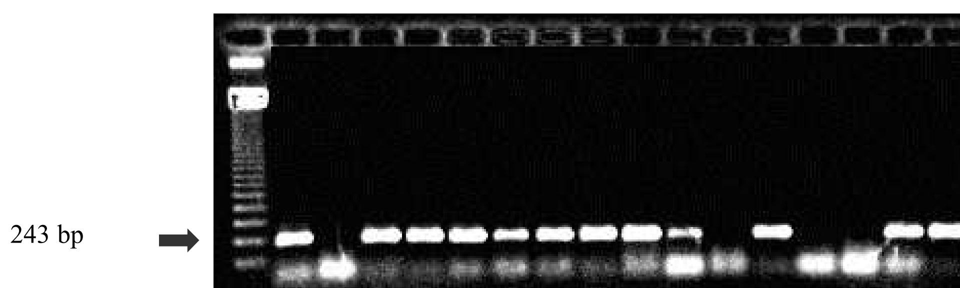

Fig. 2. Agarose gel electrophoresis analysis of amplified plum pox virus (PPV) from PPV-infected apricot, peach and plum trees. Lane M, 123 bp DNA ladder marker-GIBCOBRL; lane 1, 2, 3, 4, 5, 6, 7, 8, 9 and lane 10, PPV from infected stone fruit tissues (apricot, peach and plum); lane 11, 13 and lane 14, uninfected tissues; lane 12, 15 and lane 16, positive control of infected tissue with PPV-M, PPV-D and PPV-C respectively.

On the other hand, tests confirmed that immunocapture RT-PCR can be more sensitive than RT-PCR (Wetzel *et al*., 1992; Candresse *et al*., 1994), and the use of an antibody in IC/RT-PCR enhances specificity of the test (Brandt and Himmler, 1995) (data not shown). Furthermore, RT-PCR reaction with two other complementary primers (PD and PM) indicated that no amplification signals were obtained from samples tested in RT-PCR except for the positive control. Another set of specific primers was used in RT-PCR to detect the presence of PPV-C, but except for the positive control, no amplified products were observed from extracts of infected tissue. The PCR products were then subjected to *RsaI* digestion. Only the positive control of PPV- D was digested with this enzyme giving a restriction profile characteristic of D-type isolates. During our screening for PPV strains in Egypt, the results demonstrate that the only strain present in Egypt is El-Amar.

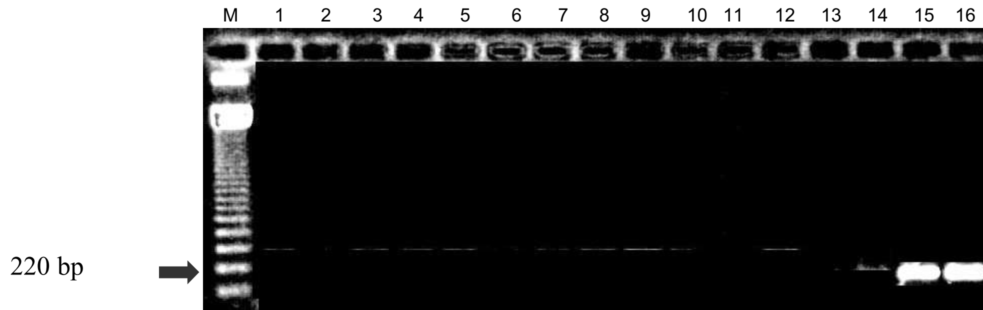

Fig. 3. Agarose gel electrophoretic analysis of RT-PCR amplified products using specific primers designed to amplify the presence of PPV-C, but except for the positive control, no amplified products were observed from extracts of infected tissue. Lane M, 123 bp DNA ladder marker-GIBCOBRL; lane 15 and lane 16 , positive control infected with PPV-C.

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PPV-el amar AAAGACTAACACATGTGGAGGTATAACCTCACTGAATGTGCCGCAATTAAAGCGGAGAAAAGGATGCTGACAGGAATCTA   80
PPV-C       ---ct---cttgggtga---tctag...---tcc--ctgttttt-ga-tcctgtt--c-tcctt-t--ttgcttt-atag   77
PPV-D       ---ct---c-tgggtga---tcta-...---tcc-g-tgttttt-ga-tcctgtt--c-tcctt-t--ccgcttt-atag   77
PPV-M       ---ct---ctcgggtga---tctag...---tccg-ctgt.ttt-ga-tcctgtt--c-tcctt-t--ccgcttt-a-ag   76

PPV-el amar AAAACAATTGGGTGACTAGACTCTCACCCAAGTAGAGTTTATG.....................................  123
PPV-C       c-gtac--cca----ggtttta-ctc-at-t--cctag-ctgttattgtcgaacacaggcccttgtatctgatgtagaga  157
PPV-D       c-gtac---ca----ggtttta-ctc-at-t--tctag-ctgttattgtcgaacacaggcccttgtatctgatgtagcga  157
PPV-M       cggtac---ca----ggttcaa-ctc-at-t--gctag-ctgttattgtcaaacacaggcccttgtatctgatgtagcga  156

PPV-el amar .....................ATAGATACCGAGACCACTACAAGGGCGAATTCGCGGCCGCTAAATTCAATTCGCCCTAT  182
PPV-C       gtgtttcactccattcgggtt----t-ctt-t-caagag-----------------------------------------  237
PPV-D       gtgcttcactccattcgggtt----t-ctt-t-caagag-----------------------------------------  237
PPV-M       gtgcttcactccattcgggtt----t-ctt-t-caagag-----------------------------------------  236

PPV-el amar AGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCG  262
PPV-C       --------------------------------------------------------------------------------  317
PPV-D       --------------------------------------------------------------------------------  317
PPV-M       --------------------------------------------------------------------------------  316

PPV-el amar CCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCA  342
PPV-C       --------------------------------------------------------------------------------  397
PPV-D       --------------------------------------------------------------------------------  397
PPV-M       --------------------------------------------------------------------------------  396

PPV-el amar GCCTATACGTACGGCAGTTTAAGGTTTACACCTATAAAAGAGAGAGCCGTTATCGTCTGTTTGTGGATGTACAGAGTGAT  422
PPV-C       --------------------------------------------------------------------------------  477
PPV-D       --------------------------------------------------------------------------------  477
PPV-M       --------------------------------------------------------------------------------  476

PPV-el amar ATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGGCCAGTGCACGTCTGCTGTCAGATAAAGTCTCCCCGTGAAC  502
PPV-C       ------------------------------------------------------------------agtct---------  557
PPV-D       ---------------------------------------------------------------.--agtct---------  556
PPV-M       ---------------------------------------------------------------.--agtct.--------  554

PPV-el amar TTTA.CCCGGTGGTGCATATCGG.GGATGAAAGCTGG.CGCATGATGANCCCCGATATGGCCAAGTGTGCC         570
PPV-C       ----c------------------n-------------c----------c-a-----------.--------         627
PPV-D       ----.------------------.-------------.----------c-a-----------.--------         623
PPV-M       ----.------------------.-------------.----------c-a-----------.--------         621
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Fig. 4. Multiple sequence alignment by DNAMAN method (DNAMAN, sequence analysis software program, Lynnon Biosoft, Canada) between partial nucleotide sequences of El-Amar strain of PPV (Upper line) were compared with sequences of PPV-C, PPV-D and PPV-M

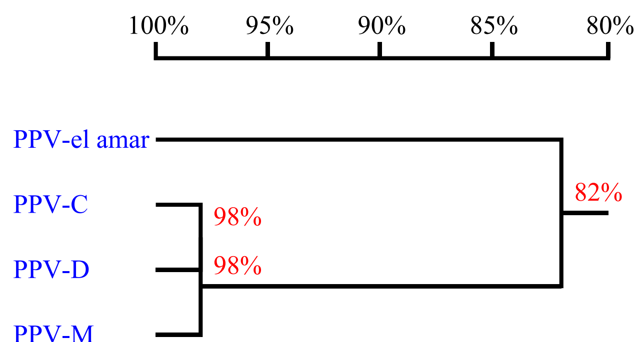

Fig. 5. Homology tree and comparison of the 3` terminal region sequence of the four strains of PPV.

CONCLUSIONS

Symptomatic leaf samples from apricot, peach and plum were positive for PPV in DAS-ELISA confirming the presence of the virus in different locations of Egypt. RT-PCR method and RFLP analysis indicated that all samples by PPV-El-Amar strain, which was the only PPV strain identified in Egypt.

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