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DETECTION AND CHARACTERIZATION OF *PLUM POX VIRUS* ISOLATES

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SUMMARY - Sharka is a devastating disease affecting many European and Mediterranean areas where stone fruit species are cultivated. The need for a rapid and sensitive detection of the disease, and for the identification of the virus strains as well, stimulated the development of several methods of laboratory diagnosis. In this paper the latest diagnostic methods are listed and briefly discussed. The activity of a European Project for the selection and validation of diagnostic protocols is also presented.

Key words: PPV, diagnosis, ELISA, PCR, MAbs, strain characterization

RESUME *- La Sharka est une maladie destructrice qui affecte bon nombre de régions européennes et méditerranéennes où sont cultivées les espèces fruitières à noyau. L'exigence d'une détection rapide et efficace de la maladie et de l'identification des souches du virus a favorisé la mise au point de diverses méthodes de diagnostic au laboratoire. Dans ce travail, on présente et discute brièvement les méthodes de diagnostic les plus récentes. En même temps, on illustre l'activité d'un Projet Européen pour la sélection et la validation des protocoles de diagnostic.*

Mots-clés: *PPV, diagnostic, ELISA, PCR, Mabs, caractérisation des souches*

INTRODUCTION

Sharka is one of the most devastating diseases of stone fruits. The disease severely affects apricot, peach and plum trees because it reduces quality and premature dropping of the fruits (Dunez and Sutic, 1988; Németh, 1994). *Plum pox virus* (PPV) has occasionally been reported in cherry trees (Crescenzi *et al*., 1997). PPV is a member of the *Potyvirus* genus in the *Potyviridae* family (Berger *et al*., 2000). PPV particles are flexuous rods about 700 x 11 nm composed of a single stranded RNA molecule close to 10,000 nucleotides which is encapsulated with 2,000 subunits of a single coat protein (CP).

The disease was described for the first time in 1917 on plums and in 1933 on apricot in eastern Europe (Bulgaria). Since then, the virus has progressively spread to a large part of the European continent, around the Mediterranean basin and in the Near and Middle East. It has been found also in India and in the Americas (Chile, USA and Canada).

The introduction of infected plant propagating material is the most important means of long distance spread of PPV. In addition, the virus is non-persistently transmitted by a number of aphid species existing in each region.

PPV STRAINS

Marcus and Dideron

The numerous PPV isolates differ in biological and epidemiological properties such as aggressiveness, aphid transmissibility and symptomatology. Two main groups Dideron (D) and Marcus (M) were serologically established (Kerlan and Dunez, 1976). The PPV isolates belonging to D or M groups show different epidemiological behaviour. The M isolates spread more readily by aphids than D isolates and cause more severe symptoms in peach trees. The D isolates are able to naturally infect apricot and plum trees and rarely spread from these hosts to peach trees. The existence of these two groups is also based on: (i) different serologic pattern or reaction with D or M specific monoclonal antibodies, (ii) different electrophoretic mobility of the viral CP, (iii) nucleotide sequence information,

either complete or partial including the 3' terminal region of the genome of several PPV isolates, (iv) sequence analysis of polymerase chain reaction (PCR) fragments corresponding to the C-terminal region of the PPV CP gene and RsaI restriction fragment length polymorphism (RFLP), and (v) different variants of PCR, heminested-PCR, nested-PCR and Co-operational PCR (Co-PCR) using specific primers including colorimetric detection of the amplicons with D or M specific probes.

El Amar and Cherry

Two additional groups of PPV isolates are: El Amar (EA) and Cherry (C). PPV-EA isolates are different in nucleotide sequence (Wetzel *et al*., 1991a) and contain specific epitopes (Myrta *et al*., 1998). Group C was more recently described, after the discovery that some PPV isolates were able to infect cherry trees, and reported molecular and serological differences with the other groups (Nemchinov and Hadidi, 1996; Nemchinov *et al*., 1996) and specific reactions with monoclonal antibodies (Myrta *et al*., 2000).

Tools for strain identification

The specific identification of the strains is of utmost importance for the optimal management of the disease (i.e. eradication of newly introduced strains where the virus is already endemic; choice of propagating material not sensitive to the epidemic spread of specific strains). So, it is important to have access to simple diagnostic tools suitable for both the timely discovery of PPV foci and reliable strain identification.

When serological techniques are adopted, detection of any PPV isolate (universal) can be achieved using monoclonal antibody 5B-IVIA (Cambra *et al*., 1994) or polyclonal antibodies. Selective and specific detection of PPV-D (Cambra *et al*., 1994), PPV-M (Boscia *et al*., 1997), PPV-EA (Myrta *et al*., 1998), and PPV-C (Myrta *et al*., 2000) isolates is possible using commercially available ELISA kits. This method correlates very well with molecular methods (Candresse *et al*., 1998) but, in addition, gives the advantage to type the four strains in a single test. Furthermore, it is less laborious and require facilities and expertises available in the majority of the phytosanitary laboratories of the Mediterranean countries. The few discrepancies reported between serological and molecular methods are probably due to the existence of natural recombinants between Marcus and Dideron, as recently discovered in Slovakia (Glasa *et al.*, 2002).

Molecular hybridisation techniques (Varveri *et al*., 1988), the different electrophoretic mobility of dissociated coat protein (CP) (Bousalem *et al*., 1994; Pasquini and Barba, 1994), and different PCR-based assays have been developed for PPV detection (Korschineck *et al*., 1991; Wetzel *et al*., 1991b, 1992; Candresse *et al*., 1994, 1995; Levy *et al*., 1994; Olmos *et al*., 1996) and for the simultaneous detection and typing of virus isolates (Olmos *et al*., 1997). Different systems of viral target preparation prior to PCR have been developed based on immunocapture (Wetzel *et al*., 1992) or, without the need of extract preparation, on print and squash capture-PCR (Olmos *et al*., 1996). Nested-PCR in a single closed tube (Olmos *et al*., 1999) has been applied for sensitive detection of PPV targets in plant material and in single aphids. A co-PCR system using a universal probe for hybridisation (Olmos *et al*., 2002), has been described providing similar sensitivity to nested-PCR.

International cooperation

The huge number of technical possibilities for detecting and typing PPV isolates has allowed the rapid collection of data on the presence and evolution of the geographical distribution of the different strains of the virus, as demonstrated by the activity of the MNFT reported in this publication. However, in order to further validate the data acquired in this way, projects have been implemented and carried out with the aim to approving officially the related reagents and protocols, after adequate analysis and comparison in internationally known laboratories. In this frame, a Project, financed by the European Union (SMT 4-CT98-2252) and named DIAGPRO (Diagnostic protocols for organisms harmful to plants), has been directed to the production of high quality standardised diagnostic protocols for a sub-set of harmful organisms, including PPV, for approval by the Plant Health Standing Committee of the EU.

The whole project was co-ordinated by the CSL (Central Science Laboratory, York, UK - Dr. Christine Henry) (http://www.csl.gov.uk/prodserv/know/diagpro), but the responsibility for the development, validation and preparation of diagnostic protocols for *Plum pox virus* was held by the IVIA (Instituto Valenciano del Investigaciones Agrarias, Valencia, Spain - Dr. Mariano Cambra).

The main objectives of the project are: (i) review all the diagnostic methods; (ii) select and improve

those with greatest potential in pilot studies; (iii) compare selected schemes in EU-wide ring tests by IVIA and selected intercomparison study laboratories representing statutory testing laboratories in most Member States; and (iv) produce finalised diagnostic protocols for submission to the Plant Health Standing Committee for approval.

In the case of PPV the project considered not only the universal diagnosis of the virus, but also, for the obvious reasons discussed above, the specific identification of the two main strains of the virus, Marcus and Dideron.

Three protocols were selected and submitted to ring testing: DASI-ELISA with the universal monoclonal antibody 5B, and the strain specific MAbs 4D (Dideron) and AL (Marcus); IC-RT-PCR with general (P1-P2) and specific (P1-PD and P1-PM) primers; Co-operational amplification (Co-PCR) and hybridisation with general and specific (Marcus and Dideron) probes.

Sixteen laboratories from 9 different EU countries were involved and the results discussed in a specific meeting in York in October 2002. Detailed results will be published soon, but, in general, even limited to the specific detection of two strains only, all three methods have shown high levels of accuracy in both general detection and strain characterization.

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