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VALIDATION OF THE 'TISSUE-PRINTING' TECHNIQUE FOR DETECTING STONE FRUIT VIROIDS

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SUMMARY - A survey was carried out to validate the tissue-printing technique for detecting *Peach latent mosaic* (PLMVd) and *Hop stunt* (HSVd) viroids in stone fruit samples. The membranes were pressed in different countries and hybridised in a central laboratory. Clear-cut positive results were obtained in many samples for both viroids. The technique is fast, easy and reliable. These advantages made tissue-printing technique an appropriate method for a first screening to evaluate the sanitary status of stone fruit trees.

Key words: tissue-printing, molecular hybridisation, PLMVd, HSVd, stone fruits

RESUME *- Une enquête a été réalisée afin de valider la technique tissue-printing pour la détection du Peach latent mosaic viroid (PLMVd) et du Hop stunt viroid (HSVd) dans des échantillons d'espèces fruitières à noyau. Les membranes ont été préparées dans divers pays et hybridées dans un laboratoire central. Des résultats sûrement positifs ont été obtenus pour les deux viroïdes dans plusieurs échantillons. Cette technique est rapide, facile et fiable et par conséquent, elle est tenue pour une méthode convenable à utiliser dans une première phase de criblage, lorsqu'on évalue l'état sanitaire des espèces fruitières à noyau.*

Mots-clés: *tissue-printing,hybridation moléculaire, PLMVd, HSVd, espèces fruitières à noyau*

INTRODUCTION

Viroids are entities composed exclusively of RNA, and therefore, serological techniques can not be applied to viroid diagnosis because of the lack of viroid-encoded proteins. Detection procedures for viroids must relied either on bioassays or on the direct detection of the RNA. Bioassays have been largely used as a detection tool even before the etiology of viroid-induced diseases was demonstrated. In spite of their unquestionable sensitivity, the utilization of bioassays for screening large numbers of samples is time consuming and expensive. Likewise, gel electrophoresis techniques, used on the basis of the distinct mobility of small circular viroid RNAs, has also been used for detection purposes but it is not suitable for large sample numbers. Molecular hybridisation is the preferred method of choice for detecting stone fruit viroids. This method has been extremely facilitated by the use of non-isotopic riboprobes (Pallás *et al*., 1998a,b). One of the key steps in this technique is the sample preparation. Until very recently most of the methods generally used for viroid extraction require phenol or other toxic organic solvents, making them undesirable for diagnostic laboratories that manage large number of samples. An extraction method that avoids the use of phenolics, previously described for obtaining plant genomic DNA (Dellaporta *et al*., 1983), has been used to enrich partially purified extracts in viroid-like RNAs (Pallás *et al*., 1987) and viroids (Astruc *et al*., 1996; Cañizares *et al*., 1999).

TISSUE-PRINTING

In spite of these improvements, any extraction procedure is a tedious step and inconvenient for routine analysis. Sample manipulation is reduced to a minimum using tissue-printing technique. It avoids sample extraction by directly imprinting the samples (stem, cutting, leaf) onto a nylon or nitrocellulose membrane. This technique was first described to detect proteins by immunocytolocalization (Cassab and Varner, 1987) and later applied to RNA detection (McClure and Guilfoyle, 1989). The technique was then adapted for detection and localisation of plant viruses (Mansky *et al*., 1990; Chia *et al*., 1992). Regarding the viruses and viroids of stone fruit trees, the nonisotopic tissue-printing hybridisation has been applied to *Cherry leaf roll virus* (CLRV) (Más and Pallás, 1995), *Prunus necrotic ring spot virus (*PNRSV) (Sánchez-

Navarro and Pallás, unpublished data), *Apple chlorotic leaf spot virus* (ACLSV) (Cañizares *et al*., 2001) *Peach latent mosaic viroid (*PLMVd) (Faggioli and Barba, 2001) and *Hop stunt viroid* (HSVd) (Romero-Durbán *et al*., 1995; Astruc *et al*., 1996). Immuno-tissue printing has been used for the detection of ACLSV and *Plum pox virus (*PPV) (Knapp *et al*., 1995).

The imprint hybridisation technique can be applied not only for diagnostic purposes with the obvious advantage of reducing the test times (see previous references) but also to study viroid distribution within the infected plants (Stark-Lorenzen *et al*., 1997; Amari *et al*., 2001).

In order to validate the imprint hybridisation technique for large-scale detection of stone fruit viroids (PLMVd) and (HSVd), a study was carried out in the frame of MNFTV. The sample imprinting of nylon membranes were done in different Mediterranean countries for a total of about 200 samples of peach, plum and apricot. After the pressing of the transversal section of petioles by duplicates, the membranes had no particular treatment. Successively, the membranes were sent to and developed at the Instituto de Biologia Molecular y Celular de Plantas-CSIC in Valencia (Spain). Clear signals (positive reactions) were found for PLMVd (Fig. 1) and HSVd. The detailed results of this work will be reported in a separated paper.

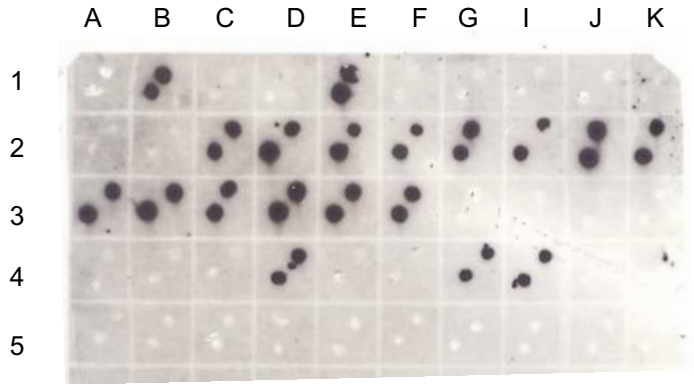

Fig. 1. Application of the tissue-printing technique for the detection of PLMVd in peach trees. Transversal sections of petioles were pressed onto nylon membranes (by duplicates) and hybridised against a PLMVd-specific riboprobe.

CONCLUSIONS

In addition to minimum manipulations of samples, other evident advantages of the tissue-printing technique are the following:

samples can be processed in the field in very distant geographical areas from the place they are going to be analysed, avoiding the problems of plant quarantine;
once 'processed', samples do not need any special conservation before final analysis;
'processing' is fast and easy and it does not need any special training to the operator and thus, it can be done in places where no laboratory equipment exist;
the technique can be applied even in the months of the year that no foliar tissue is present. We were able recently to detect PLMVd in peach cuttings from Canada during December when field temperatures were around 0º C (Pallás, unpublished).

All the above advantages make tissue-printing technique an appropriate method for a first screening to evaluate the sanitary status of stone fruit trees.

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