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VIROIDS OF STONE FRUITS: INCIDENCE AND DISEASES IN THE MEDITERRANEAN

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SUMMARY - Two viroids are known to infect stone fruit trees: *Hop stunt viroid* (HSVd) and *Peach latent mosaic viroid* (PLMVd). PLMVd has been found frequently in peach, and occasionally in plum, cherry and apricot germplasm from countries in Europe and Asia. Recent studies have reported it in very significant percentages in several Mediterranean countries. HSVd has been detected also frequently in the Mediterranean in several stone fruit species: i.e. apricot, peach, plum and almond. The application of recombinant DNA technology has permitted the use of molecular hybridisation and Polymerase Chain Reaction (PCR) for viroid detection. These techniques, combined with the application of an easy procedure for the extraction of total nucleic acids, the existence of non-radioactive PLMVd and HSVd - specific probes and primers, are now used for routine diagnosis of stone fruit viroids. More recently, tissue-imprinting hybridisation, a technique that avoids sample extraction and only requires the direct transfer of the plant material (fruits, stem, cuttings, leaves) has been applied successfully to the detection of these viroids in stone fruits.

Key words: Mediterranean, stone fruits, viroids, PLMVd, HSVd, viroid detection

RESUME *- Deux viroïdes sont censés être capables d'infecter les espèces fruitières à noyau: le Hop stunt viroid (HSVd) et le Peach latent mosaic viroid (PLMVd). Le PLMVd a souvent été détecté chez le pêcher et occasionnellement, dans du matériel végétal de prunier, cerisier et abricotier provenant de pays européens et asiatiques. Des études, menées récemment, ont signalé des pourcentages très significatifs dans de nombreux pays méditerranéens. Le HSVd a été rapporté chez différentes espèces fruitières à noyau: abricotier, pêcher, prunier et amandier. Ce viroïde a aussi été mis en évidence fréquemment en Méditerranée. L'application de la technologie du DNA recombinant a permis d'utiliser l'hybridation moléculaire et la réaction en chaîne de la polymérase (PCR) pour la détection des viroïdes. Ces techniques, associées à une procédure facile d'extraction des acides nucléiques totaux et à l'emploi de sondes et amorces non radioactives et spécifiques pour le PLMVd et le HSVd, sont actuellement utilisées dans le diagnostic de routine des viroïdes des espèces fruitières à noyau. Tout récemment, l'hybridation tissue-printing, une technique qui évite l'extraction des échantillons et exige uniquement le transfert direct du matériel végétal (fruits, tige, boutures, feuilles), a été adoptée pour la détection de ces viroïdes chez les espèces fruitières à noyau.*

Mots-clés: *Méditerranée, espèces fruitières à noyau, viroïdes, PLMVd, HSVd, détection des viroïdes*

INTRODUCTION

Viroids are the smallest known plant pathogens and consist of a single-stranded, circular RNA of 246 to 399 nucleotides (Semancik, 1987; Diener, 1991; Symons, 1997; Flores *et al*., 1997; 1998). They cause serious diseases in economically important crops (potato, tomato, cucumber and hop), fruit trees (citrus, apple, peach, grape, apricot, avocado and coconut) and ornamental plants (*Chrysanthemum*, *Coleus* and *Iresine*).

Viroids are not known to code for any protein and therefore they are host-dependent in their life-cycle. According to the present taxonomy, viroids are classified in 7 genera embraced into 2 families: *Pospiviroidae* (characterized by the presence of a central conserved region) and *Avsunviroidae* (characterised by self-cleavage activity).

Two different viroids are known to infect stone fruit trees: *Hop stunt viroid* (HSVd) and *Peach latent mosaic viroid* (PLMVd).

PLMVd

Peach latent mosaic disease was described first in France (Desvignes, 1976; 1980), induced by PLMVd. The viroid occurs frequently in peach, and occasionally in plum, cherry and apricot germplasm from countries in Europe and Asia (Faggioli *et al*., 1997; Hadidi *et al*., 1997).

The principal characteristics of the disease on peach trees includes: delayed flowering 4 to 6 days with similar effects in vegetation and fruit maturity. On infected peach trees, fruits are deformed, dull in colour, discoloured or pigmented. Vegetative buds turn necrotic, drops off and/or produce weak growth. Yield declines in quantity and quality. Occasionally leaves exhibit discoloration in the form of either diffuse mosaic or yellowish mottles: blurred chlorotic blotches, or large areas of bright-yellow or cream (calico mosaic). The leaves are generally not deformed; only a few severe strains induce entirely yellowish foliage, curved inwards and folded, with necrotic notches (Desvignes, 1999). It has been recently observed that variants of PLMVd with specific molecular properties are the causal agents of the peach disease known as 'Calico' (Malfitano *et al*., 2002) PLMVd was found in a high incidence in peach of Japan and U.S. origin (Badenes and Llácer, 1998; Boyé and Gentit, 1998).

Incidence of PLMVd in France is relatively low (about 15%; Desvignes, 1999), it is higher in Italy, (incidence of 20-50%) (Barba and Faggioli, 1999) and Spain (82%) (Badenes and Llácer, 1998). Recent studies carried out in some Middle East countries have shown the presence of this viroid in significant percentages: 40% in Syria (Ismaeil *et al*., 2001), 34% in Lebanon (Choueiri *et al*., 2001) and 29% in Jordan (Al Rwahnih *et al*., 2001). In the Mediterranean and Europe, PLMVd has been reported also in Algeria, Morocco, Greece, Austria, Romania and former Yugoslavia (Diekman and Putter, 1996).

HSVd

HSVd, as its name indicates, was first described as the causal agent of the stunt disease of hops in Japan, but since then it has been found in several plant species, in such fruit crop plants like grapevine, citrus, pear, peach and plum (Shikata, 1990). These plants either showed specific disorders or symptomless. The diseases known as cachexia of citrus (Diener *et al*., 1988; Semancik *et al*., 1988; Levy and Hadidi, 1993) and dapple fruit of plums and peaches (Sano *et al*., 1989) have been associated with sequence variants of HSVd. More recently, HSVd was detected and characterized in apricot (Astruc *et al*., 1996; Kofalvi *et al*., 1997) and almond (Cañizares *et al*., 1999)

Overall sequence homologies (Shikata, 1990) and phylogenetic analysis (Hsu *et al*., 1994) first indicated that HSVd isolates can be separated into three groups (plum-type, hop-type and citrus-type). The fact that these groups often contained isolates coming from a limited number of hosts prompted the suggestion that the group-discriminating sequence variations could in fact represent host-specific sequence determinants which may facilitate or be required for replication in a given host. Later, a detailed phylogenetic analysis of the existing HSVd sequences, together with the new HSVd sequences of *Prunus* isolates determined by Kofalvi *et al*. (1997) resulted in a redefinition of the grouping of variants of this viroid. A bias for the presence of certain sequences and/or structures in some hosts was observed, although no host-determinants were conclusively found.

Surprisingly, this analysis also revealed that a number of HSVd isolates probably arose from recombination events and that the hop-type group itself is likely the result of a recombination event between members of the plum-type and citrus-type groups (Kofalvi *et al*., 1997). Most of the apricot sequence variants are grouped in the plum-type group or in one of the minor recombinant groups (P-C group; Amari *et al*., 2001b). In fact, the high number of new sequence variants grouped in this recombinant P-C group indicates that these recombinant variants are more frequent than previously thought.

Interestingly, no apricot sequence variants were found to belong to the citrus-type group. Analysis of the geographical distribution of the apricot sequence variants revealed that most of the Moroccan variants are phylogenetically related to the Spanish apricot variants whereas the Greek variants were more closely related to the grapevine German variants indicating a different geographical origin for these two groups of apricot HSVd isolates (Amari *et al*., 2001b).

Finally, it is worthy noting that most of the apricot sequence variants from Cyprus are grouped into the recombinant P-C group which could indicate that all of them derived from an intra-specific event or, alternatively, that these events have occurred more often in this country than in others.

This viroid was found quite common in apricots with an average incidence in the Mediterranean of 34%. Cañizares *et al*. (1998; 2001) revealed HSVd in 81% of the apricot trees tested in South-eastern Spain. A lower but still substantial HSVd incidence was recently reported for other Mediterranean countries: 10% in Cyprus, 10% in Morocco, 5% in Greece and 2% in Turkey (Amari *et al*., 2000). More recently, Al Rwahnih *et al*. (2001) reported 19% incidence of HSVd in Jordan, whereas in Syria and Lebanon the incidence was 62% (Ismaeil *et al*., 2001) and 28% (Choueiri *et al*., 2002), respectively (Table 1). Thus, although HSVd is latent in apricot, this host could represent a natural reservoir from which the viroid can potentially be transmitted to other susceptible host crops, including other stone fruits, such as plum and peach.

No means of natural spread has been observed so far for HSVd and experiments are under way to check its presence in pollen and seeds of apricot (K. Amari, V. Pallás and M.A. Sánchez-Pina, unpublished information).

Table 1. HSVd incidence in apricot in several Mediterranean countries**

Countries	Nr of trees (infected/tested)	Incidence rate (%)
Spain[M]	123/152	80.9
Syria	15/24	62.5
Italy	42/113	37.2
Lebanon	36/130	27.6
Jordan	5/26	19.2
Cyprus	9/86	10.4
Morocco	3/29	10.3
Greece	3/59	5.0
Turkey	1/49	2.0
Albania	0/20	0.0
Malta	0/2	0.0
Total	237/690	34.3

[M]Murcia region; **Information collected in the following papers: Cañizares *et al*. (1998); Amari *et al*. (2000); Al Rwahnih *et al*. (2001); Ismaeil *et al.* (2001), Ferreti *et al*. (2001) and Choueiri *et al*. (2002).

VIROID DETECTION

At the moment, unlike for bacterial and fungal diseases no chemical treatments can be applied as a field control of viroid diseases, and therefore early detection by means of sensitive diagnostic methods is the main way to control them (Mathews, 1991; Hull,1993; Pallás *et al*., 1998a, b). As serological techniques can not be applied to viroid diagnosis because of the lack of coat protein, their detection must rely on bioassays or by direct detection of genomic viroid RNA.

Biological detection can be carried out by indexing all year round in the greenhouse on specific woody indicators, but the process is time - consuming, expensive and still unreliable, specially for HSVd, and not appropriate for screening large populations, mostly due to the requirements of greenhouse facilities and plant care. Likewise, gel electrophoresis techniques, used on the basis of the distinct mobility of small circular viroid RNAs, are not suitable for numerous samples.

During the last years, the application of recombinant DNA technology has permitted the use of molecular diagnostic methods e.g. molecular hybridisation and Polymerase Chain Reaction (PCR) (Pallás *et al*., 1998b). These techniques, combined with the application of rapid procedure for the extraction of total nucleic acids, the existence of non-radioactive PLMVd and HSVd specific probes and primers (Shamloul *et al*., 1995; Astruc *et al*., 1996) are now used for routine diagnosis of stone fruit viroids. More recently, tissue-imprinting hybridisation, a technique that avoids sample extraction and only requires the direct transfer of the plant material (fruits, stem, cuttings, leaves) has been applied to the detection of HSVd in apricot trees (Astruc *et al.,* 1996; Cañizares *et al.,* 2001; Amari *et al.,* 2001a). The use of petioles has allowed the monitoring of HSVd in apricot trees over an entire growing season (Amari

et al., 2001a) and the diagnostic tool of choice for large scale in the frame of the Mediterranean Network of Fruit Tree Viruses (MNFTV) (this volume).

CONCLUSIONS

The recently recorded high viroid incidence, even in local varieties, is surprising and their real impact on production remains to be established. One plausible explanation for this observation could be that viroids and the local varieties of the corresponding stone fruit trees have co-existed for long time without any important deleterious effect for the hosts. Only under determined environmental conditions or after the introduction of foreign varieties, less adapted to the local conditions, some kind of disorders could appear. If that were the case, more preventive actions should be addressed specially for those viroid-host combinations for which the incidence level has been shown to be extraordinary high such as in the case of HSVd-apricot and PLMVd-peach.

REFERENCES

Al-Rwahnih, M., Myrta, A., Abou-Ghanem N., Di Terlizzi, B. and Savino, V. (2001). Viruses and viroids of stone fruits in Jordan. *EPPO Bull*. 31: 95-98.

Amari, K., Cañizares, M.C., Myrta, A., Sabanadzovic, S., Srhiri, M. Gavriel, I. Çaglayan, K. Varveri, C. Gatt, M., Di Terlizzi, B. and Pallás, V. (2000). First report of hop stunt viroid (HSVd) from some Mediterranean countries. *Phytopath. medit*. 39: 271-276.

Amari, K., Cañizares, M.C., Myrta, A., Sabanadzovic, Di Terlizzi, B. and Pallás, V. (2001a). Tracking Hop stunt viroid infection in apricot trees during a whole year by non-isotopic tissue printing hybridisation. *Acta Hort*. 550: 315-320.

Amari, K., Gomez, G., Myrta, A., Di Terlizzi, B. and Pallás V. (2001b). The molecular characterization of 16 new sequence variants of Hop stunt viroid reveals the existence of invariable regions and a conserved hammerhead-like structure on the viroid molecule. *J. Gen. Virol.* 82: 953-962.

Astruc, N., Marcos, J.P., Macquaire, G., Candresse, T. and V. Pallás (1996). Studies on the diagnosis of hop stunt viroid in fruit trees: Identification of new hosts and application of a nucleic acid extraction procedure based on non-organic solvents. *Eur. J. Plant Pathol*. 102: 837- 846.

Badenes, M.L. and Llácer, G. (1998). Occurrence of peach latent mosaic viroid in American peach and nectarine cultivars in Valencia, Spain. *Acta Hort*. 472 (2): 565-570.

Barba, M. and Faggioli, F. (1999). I viroidi agenti causali di malattie degenerative dei fruttiferi. *Frutticoltura* 2: 69-73.

Boyé, R. and Gentit P. (1998). Sanitary status of stone fruit industry in the Mediterranean countries: France. In *Stone fruit viruses and certification in the Mediterranean countries: problems and prospects*. (B. Di Terlizzi, A. Myrta, V. Savino, eds), Bari, Italy. Options Méditerranéennes Série B. nº 19. pp. 47-49.

Cañizares, M.C., Aparicio, F., Amari, K. and Pallás, V. (2001). Studies on the aetiology of Apricot 'Viruela' Disease. *Acta Hort*. 550: 249-258.

Cañizares, M.C., Marcos, J.F. and Pallás, V. (1998). Studies on the incidence of hop stunt viroid in apricot trees (Prunus armeniaca) by using an easy and short extraction method to analyze a large number of samples. *Acta Hort*. 472 (2): 581-585.

Cañizares, M., Marcos, J. and Pallás, V. (1999). Molecular characterization of an almond isolate of hop stunt viroid (HSVd) and conditions for eliminating spurious hybridization in its diagnostics in almond samples. *Eur. J. Plant Pathol*. 105: 553-558.

Choueiri, E., Abou Ghanem-Sabanadzovic, N., Khazzaka, K., Sabanadzovic, S., Di Terlizzi, B., Jreijiri, F. and Martelli, G.P. (2001). First record of Peach latent mosaic viroid in Lebanon. *Journal of Plant Path*. 83 (2): 225-227.

Choueiri, E., Abou Ghanem-Sabanadzovic, N., Khazzaka, K., Sabanadzovic, S., Di Terlizzi, B., Myrta, A., El-Zammar, S., Jreijiri, F., Savino, V. and Martelli, G.P. 2002. First record of *Hop stunt viroid* on apricot in Lebanon. *Journal of Plant Path*. 84 (1): p. 69.

Desvignes, J.C. (1976). The virus diseases detected in greenhouse and in field by the peach seedling GF 305 indicator. *Acta Hort*. 67: 315-323.

Desvignes, J.C. (1980). Different symptoms of the peach latent mosaic. *Acta Phytopathol. Acad. Sci. Hung*. 15: 183-190.

Desvignes, J.C. (1999). *Virus diseases of fruit trees*. Centre techniques interprofessionnel des fruits et légumes. CTIFL, Paris.

Diener, T. (1991). Subviral pathogens of plants: Viroids and viroidlike satellite RNAs. *FASEBJ* 5: 2808-2813.

Diekman, M. and Putter C.A.J. (1996). *FAO/IPGRI Technical Guidlines for Safe Movement of Germplasm. N. 16. Stone Fruits*. Food and Agriculture Organization of United Nations, Rome/International Plant Genetic Resources Institute, Rome.

Diener, T., Smith, D., Hammond, R., Albanese, G., La Rosa, R. and Davino, M. (1988). Citrus B viroid identified as a strain of hop stunt viroid. *Plant Dis*. 72: 691-693.

Faggioli, F., Loreti, S. and Barba, M. (1997). Occurrence of peach latent mosaic viroid (PLMVd) on plum in Italy. *Plant Dis*. 81: p. 423.

Ferretti, L., Faggioli, F., Ragazzino, E., Pasquini, G. and Barba, M., (2001). Diffusione di virus e viroidi in germoplasma di drupacee nell'Italia centro-meridionale. In *Atti progetto POM A32-Volume II* Locorotondo, 4-7 dicembre 2001. pp 667-673.

Flores, R., Di Serio, F. and Hernandez, C. (1997). Viroids: The non-coding genomes. *Seminars in Virology*, 8: 65-73.

Flores, R., Randles, J., Bar-Joseph, M. and Diener T., (1998). A proposed scheme for viroid classification and nomenclature. *Arch. Virol.* 143: 623-629.

Hadidi, A., Giunchedi, L., Shamloul, A.M., Poggi Pollini, C. and Amer, A.M. (1997). Occurrence of peach latent mosaic viroid in stone fruits and its transmission with contaminated blades. *Plant Dis*. 81: 154-158.

Hsu, Y-H., Chen, W. and Owens, R.A. (1994). Nucleotide sequence of a hop stunt viroid variant isolated from citrus growing in Taiwan. *Virus Gen*. 9: 193-195.

Hull, R. (1993). Nucleic acid hybridization procedures. In *Diagnosis of plant virus diseases*. Matthews R.E.F. (ed). CRC Press, Inc. Boca Raton.

Ismaeil, F., Abou Ghanem-Sabanadzovic, N., Myrta, A. Di Terlizzi, B. and Savino, V. (2001). First record of Peach latent mosaic viroid and Hop stunt viroid in Syria. *Journal of Plant Path*. 82 (2): p. 227 (disease note).

Kofalvi, S., Marcos, J.F., Cañizares, M.C., Pallás, V. and Candresse, T. (1997). Hop stunt viroid (HSVd) sequence variants from Prunus species: evidence for recombination between HSVd isolates. *J. Gen. Virol.* 78: 3177-3186.

Levy, L. and Hadidi, A. (1993). Direct nucleotide sequencing of PCR-amplified DNAs of the closely related citrus viroids IIa and IIb (Cachexia). In *Proceedings of the 12th Conference of the International Organization of Citrus Virologists*, Riverside, pp. 180-186.

Malfitano, M., Di Serio, F., Covelli, L., Ragazzino, A., Hernández, C. and Flores, R. (2002). Peach latent mosaic viroid variants inducing peach calico contain a characteristic insertion. In *Abstracts of XII International Congress of Virology*, Paris, July 27th - August 1st 2002.

Mathews, R.E.F. (1991). *Plant Virology*, 3rd ed. Academic, San Diego.

Pallás, V., Más, P. and Sánchez-Navarro, J.A. (1998a). Detection of plant RNA viruses by non-isotopic dot-blot hybridization. In *Plant Virus Protocols: from Virus Isolation to Transgenic Resistance* Foster, G., Taylor, S. (eds). Humana Press, Totowa, NY. pp 461-468.

Pallás, V., Sánchez-Navarro, J.A., Más, P., Cañizares, M.C., Aparicio, F. and Marcos, J.F. (1998b). Molecular diagnostic techniques and their potential role in stone fruit certification schemes. In *Stone fruit viruses and certification in the Mediterranean countries: problems and prospects.* Di Terlizzi, B., Myrta, A., Savino, V. (eds) Bari. Options Méditerranéennes Série B. nº 19. pp 191-207.

Sano, T., Hataya, T., Terai, Y. and Shikata, E. (1989). Hop stunt viroid strains from dapple fruit disease of plum and peach in Japan. *J. Gen. Virol.* 70: 1311-1319.

Semancik, J., Roistacher, C., Rivera-Bustamante, R. and Duràn-Vila, N. (1988) Citrus Cachexia viroid, a new viroid of citrus: relationship to viroids of the exocortis disease complex. *J. Gen. Virol.* 69: 3059-3068.

Semancik, J.S. (1987). *Viroids and viroid-like pathogens*. Boca Raton, CRC Press.

Shamloul, A.M., Minafra, A., Hadidi, A., Waterworth, H.E., Giunchedi, L. and Allam, E.K. (1995). Peach latent mosaic viroid: nucleotide sequence of an Italian isolate, sensitive detection using RT-PCR and geographic distribution. *Acta Hort*. 386: 522-530.

Shikata, E. (1990). New viroids from Japan. *Sem. Virol*. 1: 107-115.

Symons, R.H. (1997). Plant pathogenic RNAs and RNA catalysis. *Nucleic Acids Res.* 25: 2683-2689.