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SHORT NOTES

Occurrence of dapple fruit of plum in Italy

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Summary. In the course of a field survey of viroids of stone fruit trees in southern Italy, we encountered some particular fruit symptoms in plum cv. Florentia and Sorriso di Primavera, which resembled the symptoms of a disease described in Japan in 1989 and was named dapple fruit disease. Symptoms consisted mainly of discolored spots on the fruit skins, and led to loss of market value. Hop stunt viroid (HSVd) was found in all symptomatic trees, but not in any symptomless samples. No other viroid was found in these trees. All samples were also checked for the more common viruses affecting plum. Apple chlorotic leaf spot trichovirus (ACLSV) was detected in both symptomatic and symptomless trees in similar percentages but no other viruses were found in any of the samples. These results provide evidence for the belief that HSVd is the cause of dapple fruit. This is the first report of dapple fruit in Italy.

Key words: hop stunt viroid.

Introduction

Peach latent mosaic viroid (PLMVd) (Di Serio and Ragozzino, 1996; Giunchedi *et al*., 1997; Hadidi *et al*., 1997; Barba and Faggioli 1998;) and hop stunt viroid (HSVd) (Loreti *et al*., 1998) have both been reported on stone fruits in Italy. PLMVd is associated with various symptoms in peach (Di Serio and Ragozzino, 1996; Desvignes, 1999), whereas HSVd has been reported in many hosts such as hop, cucumber, grapevine, citrus, pear (Shikata, 1990), peach, plum, and more recently apricot and almond (Astruc *et al.,* 1996; Canìzares *et al*.,1999). HSVd is latent in many of these hosts, e.g. grapevine (Shikata, 1990; Polivka *et al*., 1996) and apricot (Astruc *et al*., 1996), but in other cases it has been thought to cause diseases such as dapple fruit, a plum disease found in Japan, which induces chlorotic blotches and spots on peach fruit (Sano *et al*., 1989).

In the course of work on these two viroids in southern Italy, we came across some particular fruit symptoms in plum (*Prunus salicina*) cv. Florentia and Sorriso di Primavera that resembled the disease reported from Japan. Symptoms varied between cultivars: wine-red dappling in cv. Florentia, yellow-orange in cv. Sorriso di Prima-

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vera. In cv. Florentia the discolored spots formed a net-like pattern on the skins (Fig. 1), while the skins of Sorriso di Primavera exhibited irregular reddish lines (Fig. 2). These blemishes, though not extending into the flesh, reduced market value.

In this paper we report on an investigation into the possible causal agent(s) of these symptoms.

Materials and methods

Source of material

Leaves and fruits of the two cultivars, grown on different rootstocks in widely distant parts of Campania and Basilicata, were collected in early summer for laboratory analysis. Thirty-five samples of leaves and fruits per cv. were harvested at random from trees with symptoms. At the same time an equal number of samples was collected in the same orchards from symptomless trees.

Extraction of total nucleic acids

Leaves and fruit skins (0.2–0.4 g) were powdered in liquid nitrogen and homogenised with 900 µl of 0.2 M Tris-HCl pH 8.2, 17.5 µl of 5 M NaCl, 8 µl of 10% Triton X-100 and 2 µl of 2-mercaptoethanol. After centrifugation at 9,000 *g* for 20 min, the pellet was discarded and the supernatant was mixed with 0.5 ml of water-saturated phenol pH 7.0, 100 µl of 5% SDS and 100 µl of 0.1 mM EDTA pH 7.0. The nucleic acids present in the aqueous phase, obtained after a further centrifugation at 9,000 *g* for 20 min, were recovered by ethanol precipitation and then resuspended in 500 µl of sterile distilled water (Faggioli *et al.,* 2001).

RT-PCR

The following one-tube RT-PCR protocol was used: 1.5 µl of diluted TNA was denatured in the presence of 1 ml of reverse primer (75 µM) at 95°C for 5 min, followed by 5 min on ice. Then the RT mix (50 mM Tris-HCl pH 7.4, 75 mM KCl, 5 mM $MgCl_2$, 4 mM dNTPs, 1 mM DTT and 5 U reverse transcriptase M-MLV) was added to the same tube to obtain a final volume of 20 µl. Reverse transcription was performed at 42°C for 45 min, followed by 5 min at 95°C and 5 min at 4°C. Eighty µl of PCR mix (10 mM Tris-HCl pH 9, 50 mM KCl, 0.1% Triton X-100, 2.5 mM $MgCl_2$, 2U Taq polymerase, 0.15 µM forward primer) was added to the same tubes and the PCR was performed as follows: denaturation at 95°C for 45 s, annealing at 60°C for 45 s, extension at 72°C for 1 min, for a total of 35 cycles, followed by final extension for 7 min at 72°C. Products were analysed by electrophoresis in 1% agarose gel followed by staining with ethidium bromide. For HSVd we used the specific primers, VP20-H and VP19-C (Astruc *et al.*, 1996) which amplify the full length of the viroid (about 300 bp); for PLMVd the primers of Loreti *et al*. (1999) were utilised.

Dot-blot hybridization

Five µl of total nucleic acids from each sample was blotted on nylon membranes (N+ Roche Diagnostics GmbH, Mannheim, Germany) previously soaked in 2× SSC. Nucleic acids were then UV cross-linked to membranes. Hybridisation to the specific HSVd and PLMVd digoxigenin-labelled riboprobes followed the protocol of Loreti *et al*. (1999). Full length-HSVd cDNA was kindly provided by T. Candresse.

Serological tests

All samples were also checked for the more common viruses of plums (Nemeth, 1986) with ELISA, using the modifications of Alioto *et al*. (1999) and commercial antisera. To obtain reliable data, shoots from selected trees were taken in November, sealed in plastic bags, and kept at +4°C for 80 days. The twigs were then brought to room temperature with their bases kept in water containing 1% sucrose. Upon flowering, the petals were tested with ELISA.

Results and discussion

All fruits presenting dapple fruit symptoms (Fig. 1 and 2) were found to carry HSVd either by RT-PCR (Fig. 3) or by dot blot-hybridisation analysis, but this viroid was not detected in any symptomless trees. PLMVd was not found in any of the samples, whether with or without symptoms. *Apple chlorotic leaf spot trichovirus* (ACLSV) occurred in 35 of the 70 plum trees with dapple fruit symptoms (50%): 14 (40%) of the cv. Florentia and 21 (60%) of the cv. Sorriso di Primavera. ACLSV was also found in 27 (38.5%) symptomless trees: 11 of the cv. Florentia and 16 of the cv. Sorriso di Primavera (31.4% and 45.7% respectively). No other viruses (*Prunus dwarf* - PDV, *Prunus necrotic ring spot* - PNRSV, *Plum pox*

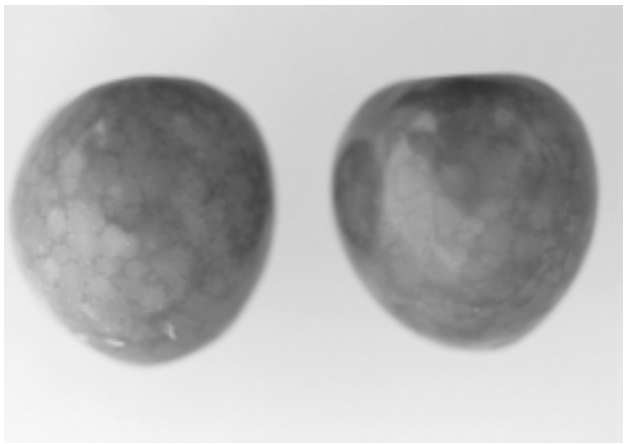

Fig. 1. Fruits of plum cv. Florentia showing the typical discoloured spots forming a net-like pattern on the skin.

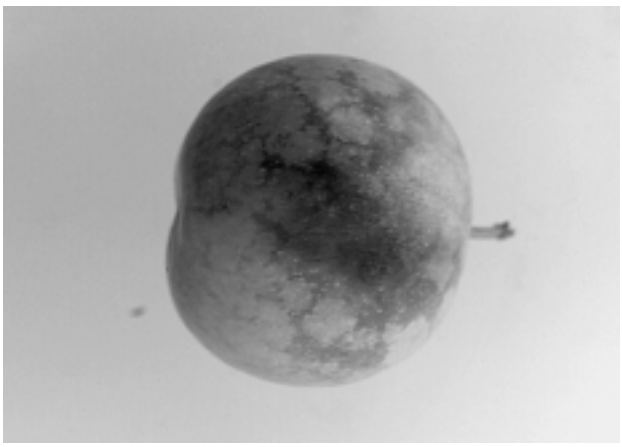

Fig. 2. Fruit of plum cv. Sorriso di Primavera with irregular reddish lines on the skin.

- PPV, *Apple mosaic* - ApMV) were found in the analysed samples (Table 1).

These results suggest that HSVd is the causal agent of dapple fruit disease. This viroid was always found in symptomatic trees, never in symptomless trees, demonstrating a clear association with the disease.

PLMVd was never detected, but ACLSV occurred in both diseased and healthy trees at rates that were not significantly different; however, this virus is known to be widespread in plums, especially those of Sino-Japanese origin (Desvignes, 1999; Amatruda and Ragozzino, unpublished). ACLSV did not seem to induce any particular al-

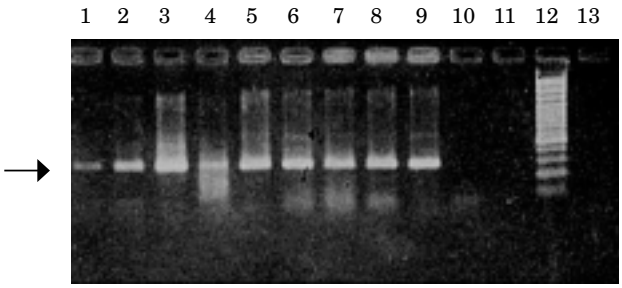

Fig. 3. RT-PCR detection of Hop stunt viroid from symptomatic (lanes 1–8) and symptomless (lane 10, 11) plum trees. Lane 9=infected control; lane 13=healthy control; lane 12=100 bp marker (Genenco).

Table 1. Molecular (RT-PCR and dot-blot hybridization) and serological (ELISA) analysis of plum samples collected in Campania and Basilicata.

	Molecular assays		Serological assay				
	HSVd	PLMVd	ACLSV	PDV	PNRSV	PPV	ApMV
Symptomatic samples							
cv. Florentia	35/35[a]	0/35	14/35	0/35	0/35	0/35	0/35
cv. Sorriso di Primavera	35/35	0/35	21/35	0/35	0/35	0/35	0/35
Total	70/70	0/70	35/70	0/70	0/70	0/70	0/70
Symptomless samples							
cv. Florentia	0/35	0/35	11/35	0/35	0/35	0/35	0/35
cv. Sorriso di Primavera	0/35	0/35	16/35	0/35	0/35	0/35	0/35
Total	0/70	0/70	27/70	0/70	0/70	0/70	0/70

[a] Positive samples / total samples.

terations, although in six trees of cv. Florentia infected with this virus, a few fruits (2–3%) had a deformed style apex.

To our knowledge this is the first report of dapple fruit in Italy and in Europe. Sano *et al.* (1989) reported that the disease was closely related to the occurrence of Hop stunt viroid. This is interesting because HSVd has already been reported on plum in Europe, but in the observed cases it did not cause any symptoms (Astruc *et al.,* 1996; Canizares *et al.*, 1999). An investigation has been started into possible sequence differences between the present isolate and others previously described.

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