Assessment of the main stone fruit viruses and viroids in Algeria

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Abstract

In order to improve the sanitary status of the propagating material of stone fruits, a field survey was conducted to assess the main viruses and viroids affecting stone fruits in selected growing areas and their distribution on the collected material by using serological and molecular detection methods.

Serological assays were carried out to detect *Plum pox virus* (PPV), *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV), Apple mosaic virus (ApMV) and *Apple chlorotic leaf spot virus* (ACLSV). Moreover, tissue-print hybridization was performed to detect *Peach latent mosaic viroid* (PLMVd) and *Hop stunt viroid* (HSVd).

Among nearly 2000 trees tested, no PPV infection was detected, while 14% of them positively reacted to at least one virus. The highest infection rate (18%) was reported in both nurseries and commercial orchards. PNRSV was the most detected virus (9%), followed by ApMV (3%) and PDV (1.5%). Cherry was the most infected species (20%). As for viroids, a high infection rate was recorded for PLMVd (9%) and HSVd (5%); the highest infection rate was reported in mother blocks and varietal collections.

Keywords: Algeria, *Prunus*, virus, viroids, ELISA, tissue-print hybridization, sanitary status.

Introduction

The Algerian stone fruit industry accounts for a production of about 257,848 tons covering a total surface area of 165,490 ha (MADR, 2006). The creation of new stone fruit orchards in Algeria is always based on the use of standard propagation material that is often not certified, thus causing a major risk of establishment of virus and viroid diseases according to their mode of transmission by grafting.

Different surveys were conducted on stone fruit species in Algeria for sanitary assessment, mainly based on visual observations. *Prune dwarf virus* (PDV), *Prunus necrotic ring spot virus* (PNRSV) *Apple chlorotic leaf spot virus* (ACLSV) (Aouane, 2003) and *Peach latent mosaic viroid* (PLMVd) (Torres et al., 2004) were hence reported. Another recent study, performed in Eastern Algeria, allowed the detection of the major viruses cited above and, for the first time, *Hop stunt viroid* (HSVd) (Rouag et al., 2008). This study was undertaken to assess the presence of the main viruses (PPV, PNRSV, PDV, ApMV and ACLSV) and viroids (PLMVd and HSVd) affecting stone fruits in selected growing areas and to verify their distribution by using serological and molecular detection methods.

Materials and methods

Survey and collection of samples: A survey was carried out over the last three years (2006-2008) in the main Algerian stone fruit growing areas: Algiers, Blida, Medea, Skikda, Constantine, Ain Temouchent, Sétif, Bordj Bou Arreidj, Mila, M'sila and Batna (Fig. 1). One thousand seven hundred fifteen samples were collected in spring and autumn and tested by ELISA for virus detection, while direct tissue print hybridisation was performed for viroid detection. Besides the 574 samples collected from commercial orchards, 553 were taken from mother blocks, 354 from variety collections and 227 from nurseries (Tab. 1). Sampling was performed on all the surveyed trees grown in mother blocks and under the screenhouse, while, for variety collections, sampling was hierarchically carried out on selected trees from each variety. In commercial orchards, samples were randomly collected on the basis of field observations; on each tree four shoots were taken from the tree quadrant.

Fig. 1 Geographical distribution of monitored areas.

Tab. 1 Samples collected from tree sources.

	N° of collected samples				
Species	**Nurseries**	**Mother blocks**	**Commercial orchards**	**Varietal collections**	**Total**
Apricot	57	55	180	65	357
Peach	34	153	137	94	418
Plum	24	171	125	21	341
Almond	37	59	57	163	316
Cherry	49	105	75	18	247
Other Prunus spp	26	10	0	0	36
Total	227	553	574	354	1715

In general, sample collection was performed on European plum (*Prunus domestica*), peach (*Prunus persica*), apricot (*Prunus armeniaca*), almond (*Prunus amygdalis*), sweet cherry (*Prunus avium*), sour cherry (*Prunus cerasus*) and Myrobalan (*Prunus cerasifera*) trees.

Virus detection: 1715 samples were tested by Double antibody sandwich-ELISA (DAS-ELISA) as reported by Clark and Adams (1977) for PPV, PNRSV, PDV and ApMV detection and by DAS-simultaneous ELISA (Flegg and Clark, 1979) for ACLSV. The ELISA test was performed by using extracts from young leaves of the collected samples and the serological commercial kits used were purchased from Loewe (Germany). The sample was considered positive if its optical density was three times higher than the negative control.

Viroids detection: Tissue-printing hybridization was carried out for PLMVd and HSVd detection on the 1715 collected samples. From each sample, the petioles of three leaves were printed onto the nylon membrane (Hybond N+, AP Biotech) (Pallás et al., 2003). The membranes imprinted in autumn were stored at 4°C and two weeks later covered with plastic envelope and exposed to UV rays for 2-3 min to fix the nucleic acid.

Hybridization was run using PLMVd and HSVd specific riboprobes labelled with dig-11 dUTP, according to the protocol provided by the Roche Company. For the detection of PLMVd, membranes were hybridized with a specific riboprobes RF43 5'd (CTG GAT CAC ACC CCC CTC GGA ACC AAC CGC T) 3'antisense and RF44 5'd (TGT GAT CCA GGT ACC GCC GTA GAA ACT) 3' sense, amplifying a 337 bp fragment as described by Ambrós *et al*. (1998) and VP19 5'd (GCC CCG GGG CTC CTT TCT CAG GTA AG) 3' antisense and VP20 5'd (CGC CCG GGG CAA CTC TTC TCA GAA TCC) 3' sense, amplifying a 297 bp fragment as described by Astruc *et al*. (1996) for the detection of HSVd.

Results and Discussions

Field observations: During the field survey, more than 3000 trees were visually inspected. Generally speaking, the age of inspected trees varied from 5 to 15 years, except for the varietal collections where the trees were older (more than 25 years old). During the survey, field symptoms were observed in the different species, such as the weak development of the trees, the death of peach and cherry trees, chlorosis, tatter leaves, riddled leaves and fruit cracking (Fig. 2). In some cases, it was impossible to observe field symptoms associated to viruses, taking into account the high infestations of aphids and other pests and pathogens.

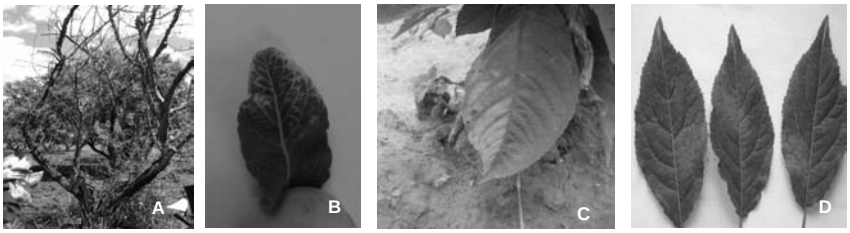

Fig. 2 Main field symptoms observed in the monitored stone fruit growing areas **A**: declining trees; **B**: vein clearing on almond; **C**: chlorosis on cherry; **D**: yellowing on plum.

Virus infection: 248 out of 1715 tested samples reacted positively to at least one virus, showing an infection rate of 14.46%. The highest infection rate was reported on cherry (20.65%) followed by plum (13.78%), peach (12.92%), apricot (11.48%) and almond (11.39%). Interestingly, species used as rootstocks were the most infected ones, where 19 samples out of 36 reacted positively to at least one virus. Ilarviruses (PNRSV, PDV and ApMV) were the most widespread, in particular PNRSV (9%), while ApMV (3%) was detected only in the Eastern area of Algeria. No PPV infected tree was detected from any tested samples (Tab. 2). Nurseries showed the highest level of infection (18.06%), whereas the variety collections (7.06%) displayed the lowest infection rate. The infection rate in mother blocks (12.84%) was relatively high, considering the destination of the propagating material. These results generally match the infection levels observed in other Mediterranean countries (Myrta et al., 2003).

Tab. 2 Distribution of virus infection.

	N° of samples			N° of infections				
Species	**Tested**	**Infected**	**Infection rate (%)**	**PNRSV**	**ApMV**	**PDV**	**ACLSV**	**PPV**
Apricot	357	41	11,48	18	9	8	3	0
Peach	418	54	12,92	40	8	6	0	0
Plum	341	47	13,78	19	16	5	1	0
Almond	316	36	11,39	26	2	3	3	0
Cherry	247	51	20,65	35	10	5	1	0
Other *Prunus*	36	19	52,78	10	7	2	0	0
Total	1715	248	14,46	148	52	29	8	0
Infection rate %	14 %			9 %	3 %	1.5 %	0.5 %	0

Viroid infection: 219 out of 1715 tested samples showed a clear positive reaction, indicating a 12.77% general infection rate. The most infected species were peach (40.19%) followed by apricot (7.28%), cherry (5.26%) and plum (3.52%). Almond and other species used as rootstocks were found free from viroids. The individual viroid incidence reflected a high infection rate for HSVd (5 %) and PLMVd (9%) (Tab. 3). The highest infection rate was reported on mother blocks (18.63%), followed by variety collections (15.82%), commercial orchards (12.02%) and nurseries (6.61%). Similar to virus infection, Algerian viroid infection results match the infection levels observed in European and Mediterranean countries (Torres et al., 2004).

Tab. 3 Results of tissue-printing hybridization on stone fruit viroids in Algeria (2004).

	N° of samples			N° of infection	
Species	**Tested**	**Infected**	**Infection rate (%)**	**HSVd**	**PLMVd**
Apricot	357	26	7,28	11	26
Peach	418	168	40,19	68	108
Plum	341	12	3,52	4	8
Almond	316	0	0,00	0	0
Cherry	247	13	5,26	1	13
Other *Prunus*	36	0	0,00	0	0
Total	1715	219	12,77	84	155
Mean of infection (%)	12,77 %			5 %	9 %

Conclusion

The present study represents the first large-scale survey conducted in the different stone fruit growing areas of Algeria. The survey confirmed the results provided by previous studies (Aouane, 2003; Torres *et al.*, 2004; Rouag et al., 2008) on the presence of fruit tree infectious agents (ACLSV, PNRSV, PDV, HSVd, PLMVd) in Algeria and their wide distribution, while the most important and devastating virus (PPV) was not detected in the collected samples. Therefore, the prompt establishment of effective PPV monitoring is necessary to control the introduction and spread of this pathogen. The large-scale survey allowed us to detect heavy virus and viroid infections, especially in mother blocks that are considered the main source for the distribution of propagating material and can therefore contribute to the rapid and wide dissemination of these agents. It is imperative to continue investigations and analyses to know the real sanitary status with regards to transmissible infections through the propagating material, which may contribute to the success of any certification program for rosaceous stones fruit species.

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