# Distribution of *Citrus psorosis virus* in Morocco

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ABSTRACT. Using direct tissue blot immunoassay (DTBIA) for detection, a survey of *Citrus psorosis virus* (CPsV) was made in the main citrus-growing areas of Morocco at flowering time. The major citrus varieties from 16 commercial groves, one varietal collection, and the premultiplication plot of the *Domaines Agricoles* certification program conducted by the *Unité de Contrôle des Plants*, UCP-Rabat, were investigated. Virus was detected from flowers by DTBIA or from leaves by ELISA. The highest CPsV incidence (50%) was observed in commercial groves of the Gharb region. The varietal collection carried 19% infection, whereas plants of basic material category from the premultiplication plot were CPsV-free. Among citrus species, sweet orange was the most infected, with Valencia late showing 57.6% infection, followed by Washington navel and Salustiana (40% and 35.5%). Four Moroccan citrus accessions used as CPsV-positive controls in the certification program were investigated biologically and serologically. Three of these sources (M2, M3 and M4) induced leaf mottling and shock symptoms in indicator plants and reacted with different CPsV monoclonal antibodies. M1 elicited only oak leaf patterns in the indicators and gave no serological reactions, thus proving not to be a psorosis source. Serological comparison with other psorosis isolates of different geographical origin using a panel of monoclonal antibodies showed that M2, M3 and M4 share some similarities with Lebanese strains of the virus.

*Index words. Citrus psorosis virus*, detection, DTBIA, Morocco.

The most important citrus types grown in Morocco are Clementines and sweet oranges (Navelina, Valencia late, Salustiana, Vernia late and local common blood oranges). Lemon is grown mainly for local consumption, and sour orange is virtually the only rootstock used (8). Several graft-transmissible pathogens are known to affect the Moroccan citrus industry among which psorosis is considered one of the most widespread (2, 9, 10, 11).

Now that reliable protocols for the serological detection of *Citrus psorosis virus* (CPsV) by ELISA and direct tissue-blot immunoassay (DTBIA) have been developed (4, 5, 6, 7, 12, 14), the incidence and distribution of this virus was evaluated in the main citrus species grown in Morocco. Some local isolates were also characterized biologically and serologically.

The investigations concerned the major citrus species and cultivars (Valencia late and Salustiana sweet oranges, mandarin and Clementine) from 16 commercial orchards, a varietal collection, and the premultiplication plot of the *Domaines Agricoles* certification program conducted by the "*Unité de Contrôle des Plants*" (UCP), Rabat.

The survey was carried out at flowering time in 2000, testing a total of 746 trees. Two groves (Valencia late and Salustiana), were located in the Gharb area; seven additional groves were in Souss Massa, and seven more in Haouz. Five flowers, or six mature leaves from trees that were not in bloom, were collected at random in the orchards and from plants showing bark-scaling and psorosis-like symptoms on the leaves (mottling, flecking, ringspots).

DTBIA was done as described by D'Onghia et al. (6), dissecting ovaries from closed flowers with a sharp razor blade. Two prints per ovary were made on a BioRad 0.45 µm transblot nitrocellulose membrane using fresh tissue or after storage at -20°C. Blotted membranes were allowed to dry for 20-30 min at room temperature before soaking in a 1% solution of BSA for 2 h at room temperature or overnight at 4°C on a

shaker. After washing, blotted membranes were exposed for 3 h to an alkaline phosphatase-conjugated monoclonal antibody (Mab Ps29) at 1:250 dilution in conjugate buffer (12). Serological reactions were developed by dissolving one tablet of BCIP-NBT Sigma fast in 10 ml distilled water and the membranes were incubated until a purple-violet colour appeared in the positive control. The reaction was stopped by washing with tap water and, after drying at room temperature, membranes were observed with a 10-20× lens.

Mature leaves from non-flowering trees were assayed by ELISA (12) using a commercial kit (Agritest, Italy). Ovary tissue and leaves from infected and healthy trees were included as positive and negative controls.

Four 20-yr-old Moroccan accessions of sweet orange (1 Valencia late, 2 Washington navel and 1 Shambar grapefruit), used as CPsV-positive controls at UCP and previously checked for the absence of other pathogens (*Citrus tristeza virus* and *Citrus variegation virus*, *Spiroplasma citri*, Citrus cachexia viroid and *Citrus exocortis viroid*), were characterized biologically and serologically; these are referred to below as M1-M4 respectively.

Each source was indexed on Madame Vinous sweet orange, Dweet tangor and Carte noire mandarin, in a climatized greenhouse at 22-24°C (13), and inspected weekly for symptoms. The Moroccan sources were also mechanically inoculated to several herbaceous hosts grown at 25°C (*Chenopodium quinoa*, *C. amaranticolor*, *C. bushmanium*, *C. capitatum*, *C. berlandeii*, *C. foetidum*, *Nicotiana occidentalis*, *N. benthamiana*, *Phaseolus vulgaris*, *Vigna unguiculata* and *Gomphrena globosa)* using different buffers (3, 5, 14).

Twenty-four monoclonal antibodies (Mabs) against an Italian CPsV isolate (4, 12) and two others (2A3, 13C5) raised against isolate CPsV-4 from Florida (1), kindly supplied by Dr. D. Alioto, University of Naples, were used for testing CPsV sources from Morocco and elsewhere (Egypt, Lebanon, Italy, USA) by TAS ELISA (4).

About 30% of the trees tested (228 of 746) were CPsV-infected, 209 from commercial orchards and 19 from the varietal collection. No infected trees were found in the premultiplication plot. Infection rate was almost 50% in commercial groves (172 trees infected out of 346 tested) in the Gharb area, while in the south (Souss Massa) and south-west it was 9% (9 out of 100) and 28% (28 out of 100), respectively (Fig. 1).

Overall, sweet orange proved to be the most infected, showing 57.6% infection in Valencia late, 40% in Washington navel and 35.5% in Salustiana. Infection rates were highest in the Gharb area, where most of the tested Valencia late and Salustiana oranges came from.

Among the remaining citrus species, Ortanique mandarin was the most infected (40 out of 70), whereas the 10 tested trees of Clementine Nour were CPsV-free (Fig. 1).

The average virus incidence in the varietal collection was 19%, Washington navel being the most infected (50%), whereas Clementine Nour and Eureka lemon were negative (Fig. 2).

With the Moroccan accessions M1-M4, all mechanical inoculations to test plants failed, whereas positive responses were obtained by graft transmission. Flecking and mottling were induced in young leaves of Madam Vinous, Dweet tangor and Carte noire mandarin by M2, M3 and M4, whereas shock symptoms developed in Madam Vinous and Dweet tangor with M3 and M4. Oak leaf patterns in Dweet tangor were induced by M1 and M2.

As shown in Table 1, 13 of the Mabs tested (Ps 1, 5, 7, 11, 13, 15, 19, 21, 23, 26, 27, 28, 31) did not react, whereas the others reacted except with source M1. Mabs Ps 25 and 30 reacted with only two

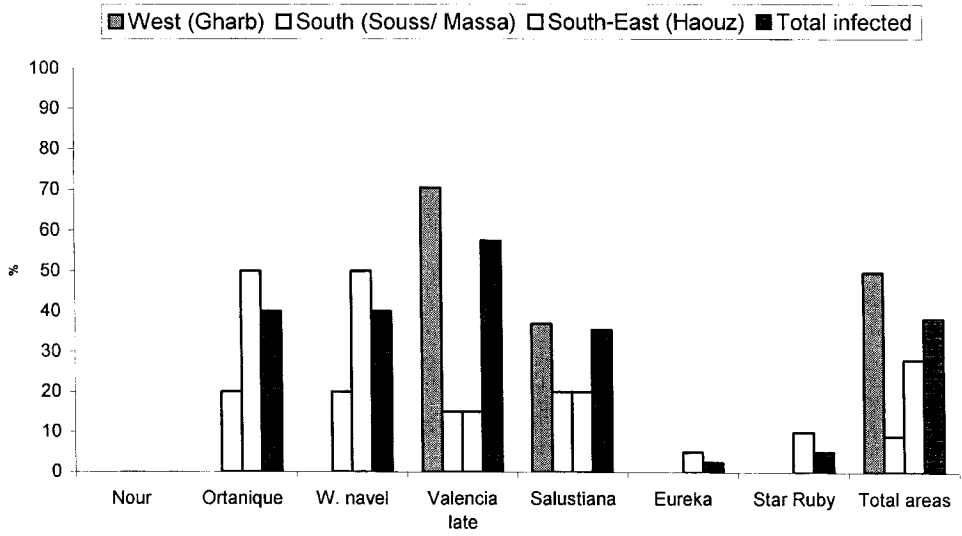


**Fig. 1. Incidence of *Citrus psorosis virus* infection in the areas indicated.**

sources (M2 and M4), whereas Ps 29 reacted with M3 and M4. The latter Mab was used for production of the commercial kit utilized for the survey and did not react with source M2. Source M1 did not react with any Mab and presumably did not carry CPsV. The oak-leaf patterns induced by M1 and M2 indicate the presence of a different agent.

Serological comparison among psorosis sources of different origins using the whole panel of monoclonal antibodies showed some similarities between Moroccan and Lebanese isolates (Table 1).

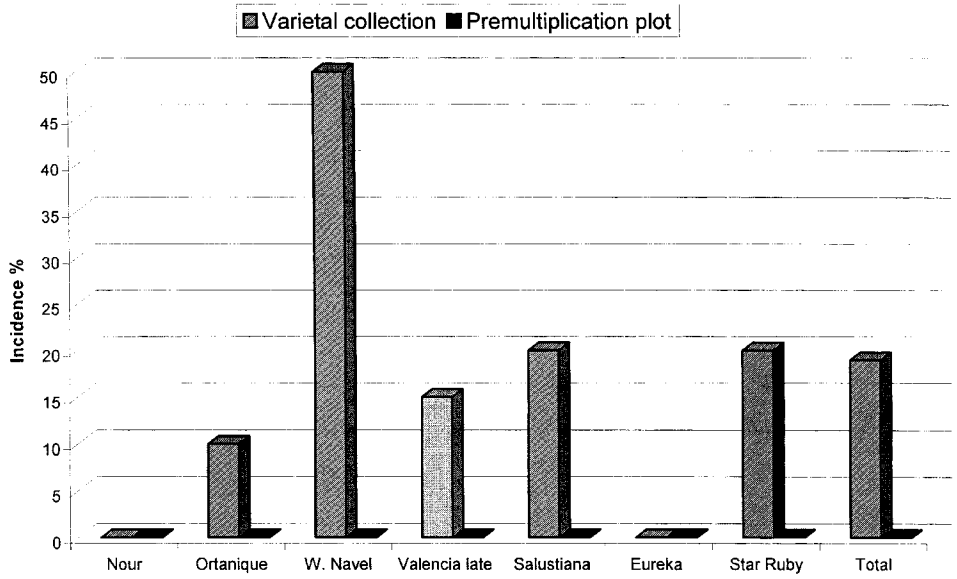


**Fig. 2. Incidence of *Citrus psorosis virus* infection in the varietal collection and premultiplication plot.**

TABLE 1
USE OF MONOCLONAL ANTIBODIES (MABS) TO DIFFERENTIATE *CITRUS PSOROSIS VIRUS* (CPSV) ISOLATES FROM DIFFERENT REGIONS[z]

| Mabs | Ps 01 | Ps 04 | Ps 05 | Ps 06 | Ps 07 | Ps 08 | Ps 11 | Ps 12 | Ps 13 | Ps 14 | Ps 15 | Ps 17 | Ps 18 | Ps 19 | Ps 21 | Ps 23 | Ps 25 | Ps 26 | Ps 27 | Ps 28 | Ps 29 | Ps 30 | Ps 31 | Ps 33 | 2A3 | 13C5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | Egypt | | | | | | | | | | | | | |
| CPsV Sources | | | | | | | | | | | | | | | | | | | | | | | | | | |
| CPsV E1 | — | + | — | + | — | — | — | + | — | — | — | + | + | — | — | — | + | + | — | + | + | — | — | — | + | + |
| CPsV E4 | — | + | — | + | — | — | + | + | — | — | + | + | + | — | — | — | — | — | + | + | + | — | — | — | + | + |
| | | | | | | | | | | | | | Lebanon | | | | | | | | | | | | | |
| 160X | — | + | — | + | — | + | — | + | — | — | + | — | + | — | — | — | + | — | — | + | + | + | — | + | + | + |
| 165X | — | + | — | + | — | + | — | + | — | — | + | — | + | — | — | — | + | — | — | + | + | + | — | + | + | + |
| | | | | | | | | | | | | | Italy | | | | | | | | | | | | | |
| 191X | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 195X | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| USA | | | | | | | | | | | | | | | | | | | | | | | | | | |
| P200 | — | + | — | + | — | + | — | + | + | — | + | + | — | + | + | — | + | — | — | — | — | — | + | + | + | + |
| P203 | — | — | — | + | — | + | — | + | — | — | + | — | — | — | — | — | + | — | — | — | — | + | + | + | + | + |
| | | | | | | | | | | | | | Morocco | | | | | | | | | | | | | |
| M1 | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| M2 | — | + | — | + | — | + | — | + | — | + | — | + | + | — | — | — | + | — | — | — | — | + | — | + | + | + |
| M3 | — | + | — | + | — | + | — | + | — | + | — | + | + | — | — | — | — | — | — | — | + | — | — | + | + | + |
| M4 | — | + | — | + | — | + | — | + | — | + | — | + | + | — | — | — | + | — | — | — | + | + | — | + | + | + |

[z]Incorporating data from (4).

The fact that many trees were tested by DTBIA at the UCP laboratory in Morocco using ovary tissue confirms that this procedure can be used for large scale diagnosis of CPsV. Whereas the incidence of CPsV in Morocco was found to be high (38.3%) in commercial groves, especially in the Gharb area (50%), the selection carried out by the UCP was apparently effective, as no CPsV was detected in the premultiplication plot.

## LITERATURE CITED

1. Alioto, D., M. Gangemi, S. Deaglio, P. Sposato, E. Noris, E. Luisoni, and R. G. Milne
1999. Improved detection of citrus psorosis virus using polyclonal and monoclonal antibodies. Plant. Pathol. 48: 735-741.
2. Bové, J. M.
1995. *Virus and Virus-like Diseases of Citrus in the Near East Region*. FAO Rome, 518 p.
3. Derrick, K. S., R. F. Lee, B. G. Hewitt, G. A. Barthe, and J. V. da Graça
1991. Characterization of citrus ringspot virus. In: *Proc. 11th Conf. IOCV*, 386-390. IOCV, Riverside, CA.
4. Djelouah, K., O. Potere, A. M. D'Onghia, and V. Savino
2000. Production of monoclonal antibodies to citrus psorosis virus. In: *Proc. 14th Conf*. IOCV, 152-158. IOCV Riverside, CA.
5. D'Onghia, A. M., K. Djelouah, D. Alioto, M. A. Castellano, and V. Savino
1998. ELISA correlates with biological indexing for the detection of citrus psorosis-associated virus. J. Plant Pathol. 80: 157-163.
6. D'Onghia, A. M., K. Djelouah, D. Frasheri, and O. Potere
2001. Citrus psorosis virus detection by direct tissue blot immunoassay. J. Plant Pathol. 83: 139-142.
7. Martin, S., D. Alioto, R. G. Milne, J. Guerri, and P. Moreno
2002. Detection of *Citrus psorosis virus* in field trees by direct tissue blot immunoassay in comparison with ELISA, symptomatology, biological indexing and cross-protection tests. Plant Pathol. 51: 134-141.
8. MEDAGRI
2001. *Yearbook of Agricultural and Food Economics in the Mediterranean and Arab Countries*. CIHEAM-IAM Montpelier, France, 426 p.
9. Nhami, A. and A. Kissi
1978. Inventaires des viroses et des maladies similaires affectant le verger agrumicole marocain. Maroc. Fruits 529: 5-12.
10. Nhami, A. and J. J. Bourge
1979. Selection sanitaire en agrumiculture au Maroc. Al-Awamia 57: 29-39.
11. Nhami, A., and A. Zidane
1984. Une contribution de la SO.DE.A.; l'amélioration sanitaire des agrumes. Maroc Fruits 616: 2-3.
12. Potere, O., D. Boscia, K. Djelouah, V. Elicio, and V. Savino
1999. Use of monoclonal antibodies to citrus psorosis virus for diagnosis. J. Plant Pathol. 81: 209-212.
13. Roistacher, C. N.
1991. *Graft-Transmissible Diseases of Citrus. Handbook for Detection and Diagnosis*. FAO, Rome, 286 p.
14. Roistacher, C. N., A. M. D'Onghia, and K. Djelouah
2000. Defining psorosis by biological indexing and ELISA. In: *Proc. 14th Conf. IOCV*, 144-151, IOCV, Riverside, CA.