Virus and Virus-Like Diseases of Citrus in Tunisia

A. Najar[1], N. Duran-Vila[2], A. Khlij[1], and J. M. Bové[3]

[1]Institut de la Recherche Agronomique de Tunisie, Tunis, Tunisia; [2]Instituto Valenciano de Investigaciones Agrarias, 46113 Moncada, Spain; [3]INRA / Université Victor Segalen Bordeaux 2, Villenave d'Ornon, France

ABSTRACT. The citrus area in Tunisia amounts to 16,200 ha on which local cultivars of sweet orange (*Citrus sinensis*), common mandarin, Clementine and lemon are grown. Trees are relatively old and symptoms of scaly bark psorosis, concave gum (blind pocket), impietratura, and cachexia, are frequently seen in the orchards. As sour orange is the exclusive rootstock, symptoms of exocortis have never been observed, except in southern Tunisia where all trees of a sweet orange orchard on Rangpur lime showed severe scaling symptoms and bud union constriction. The incidences of *Citrus tristeza virus* (CTV) and *Citrus psorosis virus* (CPsV) were determined by direct tissue blot immuno-assay (DTBIA). All samples tested negative for CTV, whereas 20.3% were positive for CPsV. *Spiroplasma citri*, the causal agent of citrus stubborn disease, has been detected by PCR in sweet orange trees, as well as in *Circulifer haematoceps*, the vector of *S. citri*. The incidence of citrus viroids was determined by graft inoculation on Etrog citron, sPAGE analysis and molecular hybridisation. All samples were infected with at least two viroids, with *Citrus exocortis viroid* (CEVd), *Citrus bent leaf viroid* (CBLVd), *Hop stunt viroid* (HSVd), *Citrus viroid III* (CVd-III) and *Citrus viroid IV* (CVd-IV), occurring in respectively 68.4%, 32.7%, 67.8%, 81.0% and 2.3% of the sources tested.

The citrus area in Tunisia amounts to 16,200 ha, 77% of which is located in the Cap Bon region, 50 km South of Tunis (Fig. 1), an area characterized by a mild climate and high rainfall. The main varieties grown in this area are: Maltaise orange, acidless orange, Navel orange, Valencia Late orange, Clementine, lemon, Wilking mandarin and sour orange (Fig. 2).

Trees less than 10 yr of age, trees between 10 and 39 yr of age, and trees older than 40 yr constitute 32%, 38% and 30% of the trees observed. The relatively large number of old trees explains why symptoms of scaly bark psorosis, concave gum (blind pocket), impietratura, and cachexia, can frequently be seen in the orchards. As sour orange is the exclusive rootstock in the old orchards, symptoms of exocortis have never been observed. However, in Southern Tunisia, near Mides (Fig. 1), where some citrus were grown previously, all trees of a sweet orange orchard on Rangpur lime showed severe exocortis symptoms and bud union constriction.

The citrus industry in Tunisia is based mainly on the production of local cultivars of sweet orange, common mandarin, Clementine and lemon. With sour orange as the only rootstock in the major growing areas, the presence of tristeza disease in the Mediterranean basin is a threat to the Tunisian citrus industry, and new rootstocks giving tristeza tolerant rootstock/scion combinations are urgently needed as an alternative to sour orange. Since some promising rootstocks are known to be sensitive to viroids, a survey was conducted to determine if the cultivars being grown presently in Tunisia were infected with viroids. The different cultivars [and numbers of source trees of each cultivar tested during this survey] were Maltaise demi-sanguine [26], Maltaise sanguine [9], Maltaise blonde [20], orange double-Fine [4], Washington navel [12], Valencia late [12], common mandarin [29], Cassar Clementine [42], Lunari lemon [5], and Eureka lemon [11], for a total of 170 sources.

Fig. 1. Map of Tunisia showing the citrus growing areas.

Etrog citron plants graft-inoculated with tissue from these cultivars developed symptoms characteristic of viroid infection. Further, sPAGE analysis (4) and molecular hybridisation using viroid specific probes (3) revealed that all cultivars were infected with at least two viroids. *Citrus exocortis viroid* (CEVd), *Citrus bent leaf viroid* (CBLVd), *Hop stunt viroid* (HSVd), *Citrus viroid III* (CVd-III) and *Citrus viroid IV* (CVd-IV) were found to be widespread, occurring in respectively 68.4%, 32.7% 67.8% 81.0% and 2.3% of the 174 sources tested.

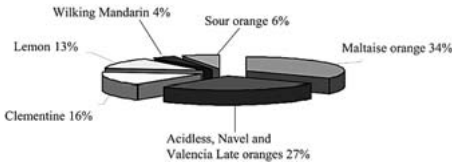

Fig. 2. A schematic showing percentages of main citrus cultivars grown in Tunisia.

The incidence of *Citrus tristeza virus* (CTV) and *Citrus psorosis virus* (CPsV) was determined by direct tissue blot immunino-assay (DTBIA), using ovaries collected during flowering time from 1,296 sources. All samples tested negative for CTV. For CPsV, 20.3% (263 of 1,296) of sources gave positive assays. Clementine was the most infected cultivar with 31.8% (49 of 154) of the sources infected, followed by Valencia late at 23.2% (19 of 82) and Maltaise at 19.4% (108 of 558) of the sources infected. Lemon sources were least infected at 3.2% (1 of 31). A Tunisian CPsV isolate (Tun 4) from a Maltaise sweet orange was characterized by biological indexing and serology using TAS-ELISA with 24 monoclonal antibodies. Tun 4 showed similarities with a strain from Italy (IAM 191 X), but differences from Algerian, Moroccan, and Egyptian strains (1). Leaf symptoms of Petri's variegation were conspicuous in the spring.

Spiroplasma citri, the causal agent of citrus stubborn disease, has been detected by PCR in citrus trees from Bou Salem and El Fahs, as well as in *Circulifer haematoceps*, the leafhopper vector of *S. citri*. The leafhopper *Circulifer opacipennis*, which is found exclusively on *Salsola kali*, also carried the spiroplasma (2). Conversely, in the regions of Hammamet and Gobba, tests of trees showing some symptoms were negative. This can be explained by the fact that in these regions the temperatures are not very high and therefore not favourable for the multiplication of *S. citri*.

In summary, citrus trees in Tunisia are relatively old and carry several graft transmissible diseases. The renewal of old orchards and the establishment of new plantings should be made taking into consideration that the presence of the tristeza disease in the Mediterranean basin is a threat to the Tunisian citrus industry and that rootstocks other than sour orange should be used. The following steps were taken in order to implement this process:

a) A sanitation program for the production of plants free of virus and virus-like agents was initiated in 1994 and a national reservoir of disease-free plants is now available. This reservoir includes standardized local cultivars as well as varieties imported from the INRA station at Corsica in order to increase the range of cultivars available and to expand the production up to a 8-9 mo period. This reservoir contains mother trees to supply budwood to the nurseries.
b) Legislation has been updated and a certification program will be implemented in 2005.
c) A rootstock assay has been initiated recently (September, 2004) to generate information regarding which rootstocks are more suitable for the Tunisian growing conditions and also which are most compatible with the Maltaise sweet orange, the most important Tunisian cultivar.

ACKNOWLEDGMENTS

This work was partially supported by project AECI 41/03/P financed by the Spanish Agency of International Cooperation (AECI). The authors would like to thank the Gi-fruits (Groupement inter-professionel des fruits) for its cooperation in collecting samples during the surveys.

LITERATURE CITED

1. Khlij, A.
2003. Prospection des principaux champignons (*Phytophthora* spp. et *Phoma tracheiphila*) et du virus de la psorose des agrumes dans la région du Cap-bon. Master of Science thesis. Instituto Agronomico Mediterraneo di Bari.
2. Najar, A., S. Bouhachem., J. L. Danet., C. Saillard., M. Garnier, and J. Bové
1998. Présence en Tunisie de *Spiroplasma citri*, l'agent causal du stubborn des agrumes et de son vecteur, la cicadelle *Circulifer haematoceps*. Contamination de *C. haematoceps* et de *C. opacipennis* par *S.citri*. Fruits 53: 391-396
3. Palacio, A., X. Foissac, and N. Duran-Vila
2000. Indexing of citrus viroids by imprint hybridisation. Eur. J. Plant Pathol. 105: 897-903.
4. Rivera-Bustamante, R., R. Gin, and J. S. Semancik
1986. Enhanced resolution of circular and linear forms of viroid and viroid like RNA by electrophoresis in a discontinuous-pH system. Analyt. Biochem. 156: 91-95.