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A survey for citrus psorosis-associated virus in a citrus collection of Southern Italy

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SUMMARY - The presence of citrus psorosis-associated virus (CPSaV) in a international citrus collection held at the Mediterranean Agronomic Institute of Valenzano (BA) and at the Dipartimento di Protezione delle Piante dalle Malattie, University of Bari - Italy (DPPM-UBA) was assessed using a DAS-ELISA kit produced at the Istituto di Fitovirologia Applicata (IFA)-CNR Torino, Italy (Garcia *et al.*, 1997). CPSaV was widespread in most of the citrus varieties. ELISA confirmed the results obtained by previous indexing, with one exception of a local clementine cv. 'Fedele'. In two 'Navelina' samples ELISA was more sensitive than indexing in detecting the virus. The reliability and sensitivity of ELISA test can represent an important step toward routine serological indexing of this virus and eventual certification.

Key words: citrus, psorosis, ELISA, diagnosis, Italy

RESUME *- La présence du virus associé à la psorose des agrumes (CPSaV) a été évaluée dans une collection internationale d'agrumes, soumis au préalable à des essais d'indexage pour la psorose, à l'Institut Agronomique Méditerranéen de Valenzano (Bari) et au Dipartimento di Protezione delle Piante dalle Malattie de l'Université de Bari, Italie (DPPM-UBA). A cette fin, un kit DAS-ELISA a été utilisé, produit par l'Istituto di Fitovirologia Applicata (IFA)-CNR Turin, Italie (Garcia et al., 1997). CPSaV s'est avérée être largement répandue chez la plupart des variétés d'agrumes. L'ELISA a confirmé les résultats obtenus par indexage, à l'exception d'un cultivar local de clémentinier, "Fedele". Pour deux échantillons de "Navelina", l'ELISA a été plus sensible que l'indexage dans la détection du virus. La preuve de la fiabilité et de la sensibilité du test ELISA représente un résultat important en vue d'un indexage sérologique de routine de ce virus et pour l'éventuelle certification.*

Mots-clés*: agrumes, psorose, ELISA, diagnostic, Italie.*

Introduction

The Italian citrus industry is concentrated in the southern regions (Sicily, Calabria, Apulia, Basilicata and Campania). In Apulia most citrus trees are cultivated along the Ionian coast and to a lesser extent in Gargano and Salento, stretching over 10,000 ha.

The most important citrus species are the Navel oranges ('Naveline', 'Washington Navel', 'Newhall'), mandarins ('Avana common' mandarin and 'Tardivo di Ciaculli'), clementines ('Common' clementine and the local ecotypes 'Fedele', 'Precoce di Massafra', 'Spinoso' and 'Gentile') and, recently introduced, Satsuma.

Most of the virus and virus-like diseases have been reported in this region and psorosis is surely the most severe (D'Onghia, 1998) as in many Mediterranean countries (Roistacher, 1993).

Psorosis is a complex of diseases (Psorosis A, psorosis B and ringspot), which are related by cross protection (Derrick *et al.*, 1991, Roistacher and Calavan, 1965; Roistacher 1993), being often symptomless in most citrus varieties. The putative causal agent is a filamentous virus belonging to a new taxon called 'spirovirus' (Derrick *et al.*,1993), or 'ophiovirus' (Garcia *et al.*, 1994; Milne *et al.*, 1996).

Besides the classical biological indexing, ELISA has been successfully used for CPSaV detection by a kit produced at the IFA-CNR Torino, Italy (Garcia *et al.*, 1997).

The use of this antiserum in DAS-ELISA is here reported for the analysis of citrus plants of different varieties and origins, held in the citrus collection of the IAM-B and of the DPPM-UBA, already biologically indexed for psorosis (A.M. D'Onghia and V. Savino, unpublished information). Ten American psorosis isolates served as positive controls. A number of concave gum isolates for a possible relationship to psorosis were also included in the test.

Materials and methods

A total of 177 samples were taken in May and September 1996 from all plants in a variety collection held at IAM-B and the DPPM-UBA, representing different citrus varieties mainly coming from Apulia (Southern Italy) and from Lebanon, Spain and Turkey (Table 1). All of these had previously been indexed to Madame Vinous sweet orange and Tangor dweet seedlings and scored as positive or negative for psorosis (Roistacher, 1993). These samples were tested by DAS-ELISA using an antiserum produced against CtRSV-4 (Garcia *et al.*, 1997). Wells of flexible plates (Falcon 3911) were coated with IgG at 1.4 µg/ml in 0.1M sodium carbonate buffer pH 9.6 plus 0.01% sodium azide, then incubated for about 2 h at 37°C. Extracts were prepared from freshly collected young citrus leaf samples and ten times diluted in phosphate-buffered saline with 0,05% tween 20 (PBS-T) containing 2% polyvinylpyrrolidone, and incubated overnight at 4° C. Conjugated alkaline phosphatase, diluted 1/1000 in PBS-T, was added and incubated for 2h at 37°C. The substrate (1mg/ml of paranitrophenyl phosphate) was then added, and the colour developed at room temperatures. The results were based on

mean absorbance values for the 2 wells loaded with each sample, considering positive the values 3 times higher than healthy controls. As representative example, isolate CtRSV 4 was giving an absorbance value 15 times higher than healthy control. Ten American psorosis isolates (P200, P203, P203M, P205, P208, P209, P213, P214, P215M, P216, P216M; courtesy of C.N. Roistacher, Riverside, California) free of other known viruses or viroids were used as positive controls. Concave gum (CG) isolates CG301, CG302 and CG306 (courtesy of C.N. Roistacher) were also tested to evaluate a possible relationship between CG and psorosis, often apparently occurring in mixed infection. The second series of tests, in September 1996, was run to check the results obtained in May from the same plants.

Results and Discussion

The ELISA results (Table 1) indicate that CPSaV is widespread in most of the citrus varieties tested. All the plants that indexed positive for psorosis were also positive in ELISA, except for one psorosis sample of a local clementine cv. Fedele. These results suggest that the ELISA test was highly reliable as a detector of the psorosis disease as recognized biologically.

Two samples of cv. Navelina, which indexed as negative in the biological test, were found to be positive by ELISA in both the May and September test, suggesting that in these cases the ELISA was more sensitive than indexing in detecting the psorosis virus.

All the plants of cv. Navelina, infected by concave gum (D'Onghia *et al.*, 1992) and showing strong oak leaf pattern symptoms in the field and index tests, proved to be psorosis-positive in ELISA. No local concave gum source that was psorosis negative was found. However, the three CG sources from the USA were clearly psorosis-negative, so it is assumed that the level of mixed infection with the two agents is very high in the local material tested.

The similar results obtained by indexing and ELISA demonstrated the high sensitivity and reliability of the serological assay in detecting psorosis and confirmed the strong correlation between psorosis symptoms and the presence of a virus related to CtRSV-4. These results represent an important step towards routine CPSaV diagnosis in the laboratory, and an additional aid for certification with respect to this virus.

The positive ELISA results with two samples that had indexed as healthy could indicate that these were mild strains that might nevertheless be dangerous in the context of other germplasm or in synergism with other viruses.

Table I - DAS-ELISA results for detecting CPSaV in a citrus variety collection of IAM-B and DPPM-UBA already indexed for psorosis.

Citrus varieties	Tested	Positive by indexing	Positive by DAS-ELISA	Origin
Sweet Orange *(Citrus sinensis)*				
- Navelina	29	11	**13**	Italy-Spain
- Tarocco	4	2	2	Italy
- Shamouti	15	1	1	Lebanon
- Washington Navel	6	3	3	Italy-Lebanon
- Valencia late	4	0	0	Italy
Mandarin and Hybrids *(C.reticulata)*				
- Common	11	1	1	Italy-Spain
- Freemont	5	2	2	Turkey
- Orlando	11	0	0	Italy
Clementine *(C. reticulata)*				
- Common	20	2	2	Italy
- Fedele	5	**4**	3	Italy
- Gentile	6	2	2	Italy
- Precoce di Massafra	4	2	2	Italy
- Spinoso	5	0	0	Italy
Satsuma *(C. unshiu)*				
- Myiagawa	14	8	8	Italy-Lebanon
Lemon *(C. limon)*				
- Sfusato	4	1	1	Italy
- Monachello	3	0	0	Lebanon
- Femminello	3	0	0	Italy
- Interdonato	2	0	0	Italy
- Saasly	3	2	2	Lebanon
Kumquat *(Fortunella margarita)*				
- Miagawa	1	1	1	Italy
Lime *(Citrus limettioides)*				
- Palestine sweet	2	0	0	Italy
- Mexican	2	0	0	Italy
Citron *(Citrus medica)*	15	2	2	Italy
Chinotto *(Citrus myrtifolia)*	1	0	0	Italy
Grapefruit *(Citrus paradisi)*	2	0	0	Italy
TOTAL	**177**	**44**	**45**	

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