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SANITARY IMPROVEMENT OF THE *CITRUS* COLLECTION IN THE BOTANICAL GARDEN OF PALERMO (ITALY)

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SUMMARY - In order to preserve the genetic heritage represented by the historical *Citrus* collection of Palermo Botanical Garden, the *in vitro* germplasm conservation of virus-free plants was carried out. The presence of the main citrus viruses was tested by ELISA and DTBIA and sanitation of some genotypes was carried out by somatic embryogenesis from stigmas and styles.

Key words: Citrus, viruses, ELISA, DTBIA, somatic embryogenesis, Italy

RESUME *Afin de préserver les ressources génétiques de la collection historique des agrumes du Jardin Botanique de Palerme, on a réalisé la conservation in vitro du germoplasme de plantes indemnes de virus. La présence des virus principaux des agrumes a été testée par l'ELISA e le DTBIA; certains genotypes ont été assainis par l'embryogénèse somatique des stygmates et des styles.*

Mots-clés*: Agrumes, virus, ELISA, DTBIA, embryogénèse somatique, Italie.*

INTRODUCTION

The historical *Citrus* collection of Palermo Botanical Garden, which was established in 1789 (Mazzola and Schicchi, 1998), includes cultivars that are rare or absent from the commercial groves, because their characters are not suitable for modern citrus farming. In particular, the cultivated specimens represent a remarkable resource of genotypes, which may risk to be left aside and to be not available or identifiable in the future. Most of them are originated from different Asian sites (tropical and south-eastern Asia) and have been introduced through the exchange of propagating materials between scientific institutions of several countries (i.e. California, Florida, Algeria, Jamaica) for breeding purposes; however, a group of these cultivars are the result of selection practices carried out inside the Botanical Garden, thus showing particular morphological profiles of fruits and leaves.

The collection has never been sanitarily assessed for the virological status, which may be seriously compromised considering that most of the planting material is originated from countries known to be seriously infected by a number of virus and virus-like diseases (Roistacher, 1995). Since most of the infected Citrus *spp*. may often be symptomless, thus representing a potential threat to the local *Citrus* groves, it is of utmost importance to know their sanitary status and to regenerate virus-free material from the infected genotypes for the conservation of the *Citrus* germoplasm.

The main citrus viruses, citrus tristeza virus (CTV), citrus psorosis virus (CPsV) and citrus infectious variegation virus (CVV) can be easily detected by Enzyme Linked Immuno Sorbent Assay-ELISA (Bar Joseph *et al.,* 1979; Davino and Garnsey, 1984; Djelouah *et al.*, 2000) and Direct Tissue Blot Immuno Assay-DTBIA (D'Onghia *et al.*, 2001a), using commercial kits from different producing companies. Moreover, their elimination can be easily obtained through *in vitro* regeneration of healthy plants by

shoot-tip-grafting (Navarro,1992) and, recently, by somatic embryogenesis from styles and stigmas, too (D'Onghia *et al.*, 2000; 2001b).

Object of this study is the enhancement of the Botanical Garden *Citrus* collection by recovering and preserving the genetic heritage of great historical value for its utilization in the framework of breeding programmes and for the biodiversity conservation.

To this aim, the presence of CTV, CPsV and CVV was assessed on all specimens of taxa collected by ELISA and/or by DTBIA.

MATERIAL AND METHODS

A total of 141 plants of 48 *Citrus* cultivars (tab. 1) and specimens belonging to *C. hystrix* DC, *C. nobilis* Lour., *C. myrtifolia* Raf., *C. clementina* Hort. ex Tanaka, *C. bergamia* R. & P., *C. medica* L., *C. meyerii* Y. Tanaka and *C. lumia* Risso, were tested.

Table 1. Main *Citrus* spp. and number of serologically tested cultivars

***Citrus* spp.**	**Cultivars**
C. aurantium L.	10
C. sinensis Osbeck	21
C. limon (L.) Burm. f.	3
C. paradisi Macfad.	5
C. grandis Osbeck	7
C. deliciosa Ten.	2
Total	48

The materials used in the serological assays were ovaries and styles of fresh or frozen flowers, fresh collected leaves. All samples were ELISA and DTBIA tested for the detection of CTV, CPsV and CVV. Particularly, CTV and CPsV were assessed by Double Antibody Sandwich-ELISA (CTV kit from Loewe, Switzerland; CPsV kit from Agritest s.r.l., Italy) and DTBIA (CTV kit from Plant Print, Spain; CPsV kit from Agritest s.r.l., Italy), whereas CVV was detected by Triple Antibody Sandwich-ELISA using polyclonal and monoclonal antibodies (Domaines Agricoles UCP, Morocco).

In ELISA, plant tissues were ground with the extraction buffer and two wells were filled with the sap of each sample. After incubation, monoclonal antibodies (1/1000) were added followed by the use of conjugated anti-mouse IgG (1/1000) for CVV detection (Davino and Garnsey, 1984), whereas for CTV and CPsV, polyclonal antiserum and monoclonal antibodies conjugated with alkaline phosphatase (1/1000) were added, respectively (Bar Joseph *et al.*, 1979; Djelouah *et al.*, 2000). For all viruses, P-nitrophenyl phosphate in substrate buffer was used and readings of the absorbance values were made by using automatic plate reader at 405nm (Titertek Multiskan plus MKII).

In DTBIA the cut surface of the plant material (stem, petiole, peduncles and ovaries sections for CTV, only the ovaries for CPsV) was printed on the nitrocellulose membrane. Each printed membrane was placed in a limp with the addition of 0.1g pf bovine serum albumin (BSA) diluted in 10 ml of distilled water (1% solution). After incubation, monoclonal antibodies conjugated with alkaline phosphatase and specific to the target virus were added. The membrane was let at room temperature for 2 to 3h (Garnsey *et al.*, 1993; D'Onghia *et al.*, 2001a).

Negative and positive controls were used in both assays and each test was repeated twice.

The sanitation of some infected cultivars, important from the scientific and ornamental point of view, was performed by somatic embryogenesis from styles and stigmas tissues following the protocols of (D'Onghia *et al.*, 2000).

RESULTS AND CONCLUSION

Results of serological assays showed the presence of the three viruses in 17 out of 141 plants but only 2 individuals were CTV-infected. They were immediately removed and destroyed. CVV was the most detected virus that infected 11 plants (7.8%) belonging to five *Citrus* spp. CPsV was found in 4 plants (2.8%) each of a different species (Tab. 2).

Few clear-cut CPsV (Fig. 1) and CVV (Fig. 2,3) symptoms were observed. Nevertheless, *C. grandis* 'Shaddock' showed severe CVV yellowing in mature leaves, which is quite unusual to observe, thus indicating the susceptibility of this species to the infection and the severity of the virus strain.

In the case of CTV, it is known that *C. meyerii* is symptomless while *C. sinensis* could have been grafted onto a tolerant rootstock, thus not showing tristeza symptoms, too.

The plantlets regenerated by somatic embryogenesis from styles and stigmas were free from CTV, CPsV and CVV. They were maintained *in vitro* and *in vivo* as virus-tested germplasm collection.

These preliminary results showed the apparently good sanitary status concerning the main citrus viruses of the Botanical Garden *Citrus* collection. It was very interesting to find a high number of CVV-infected trees compared to the other viruses which are usually more spread.

By this activity CTV infected plants were fortunately eradicated, thus eliminating the potential risk of contamination of neighbouring citrus groves.

The sanitized genotypes could replace the eradicated CTV-infected plants thus preserving the original collection. This is also true for other genotypes which were successfully regenerated by somatic embryogenesis as virus-tested material. This procedure can eliminate other undetected agents, which may be present in the tested trees.

Table 2. Results of serological assays: CVV and CPsV-infected *Citrus* species

***Citrus* spp.**	**Cultivars**	**Infected**	
		CVV	**CPsV**
C. aurantium L.		X	X
C. aurantium L	Canaliculata	X	
C. sinensis Osbeck			X
	Dulcis sanguinea	X	
	Sigillata	X	
	Dulcis	X	
C. limon (L.) Burm. f.	'Cajetana'	X	X
C. grandis Osbeck	Conifera	X	
	Shaddock	*X*	
C. deliciosa Ten.	Bombajensis	X	
	Aurantifolius		X

Fig. 1. Psorosis bark scaling in the trunk of *C. limon* 'Cajetana'. Bud-union overgrowth is also shown

Fig. 2. Severe CVV yellowing in *C. grandis* 'Shaddock'.

Fig.3. Symptoms of CVV-crinkly leaves in association or not with variegation in: (a) *C. sinensis* 'Dulcis sanguinea'; (b) *C. grandis* 'Conifera'

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