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Detection of CTV by immunoprinting in the Eastern Mediterranean Region of Turkey

M. A. Yilmaz, S. Baloglu
University of Cukurova, Faculty of Agriculture, Department of Plant Protection, Adana – Turkey

SUMMARY –.Direct immunoprinting was used to detect CTV in samples taken from different Turkish provinces, including those which had already been found positive by ELISA.
This technique is as reliable as ELISA if fresh tissues are used in the printing. It may prove very useful during certification mainly in the nursery.

Key words: citrus, tristeza, closterovirus, ELISA, immunoprinting, diagnosis, Turkey

RESUME *–.La méthode d'immunoprinting a été utilisée pour la détection du CTV sur des échantillons prélevés dans différentes provinces turques, y-compris celles qui avaient donné une réaction positive au test ELISA. Cette technique est aussi fiable que le test ELISA, pourvu que des tissus frais soient utilisés lors du printing. Elle peut s'avérer utile dans le cadre de la certification, notamment en pépinière.*

Mots-clés: *agrumes, tristeza closterovirus, ELISA, immunoprinting, diagnostic, Turquie*

Introduction

Given the presence of citrus tristeza virus (CTV) in Turkey where sour orange rootstocks are extstensively used, this pathogen poses serious threats to the Turkish citrus industry.

In any certification programme, the choice of the best diagnosis method is of the utmost importance in terms of reliability, user friendliness, cost and enviromental impact. In previous comparative trials by ELISA, several Mediterranean CTV strains have been detected using monoclonal and polyclonal antibodies of different manufacturing companies. The use of direct-immunoprinting ELISA method for the detection of CTV is a new attempt. In this study it was applied to determine the presence of CTV in the Eastern Mediterranean region of Turkey.

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Materials and methods

A kit of direct-immunoprinting ELISA by Plant Print, Spain, was used. Several samples were tested from: (i) CTV-positive trees previously detected by ELISA (Boubker *et al.*, 1998); (ii) CTV-suspected trees in the groves; (iii) healthy-looking plants close to CTV positives; (iv) CTV isolates on indicator plants kept under greenhouse conditions; (v) one CTV isolate from Ethrog citron which was partially or extensively purified. Citrus orchards were inspected in different districts of Icel (Igdir), Adana (Kozan) and Hatay (Dortyoò, Erzin) provinces (Table I).

Table I - Samples tested in the present survey

Location	Species	Cultivars	Codes	N. Samples
Dörtyol (Hatav)	*Citrus sinensis* L. Osbeck	Washington Navel	DTR	5
	Fortunella spp.	Kumquat	KUM	3
	C. aurantifolia x *Fortunella sp.*	Limequat	LiQT	2
Igdir (ICEL)	*C. sinensis*	Shamouti	T	13
	C. limon (L.) Burm	Kütdiken	TIL	1
Kozan (Adana)	*C. sinensis*	W. Navel	KTR	9
Yenice (ICEL) commercial nursery	*C. reticulata* (L.) Blanco	Cleopatra	KLT	1
	C. myrtifolia Raf.	Chinotto	CHT	2
	C. aurantifolia x Fortunella *sp.*	Limequat	LKT	2
		Variegated limequat	LVT	3
Greenhouse	*C. medica (L.).*	Ethrog citron	DT	2
		Ethrog citron	PPT*	1
		Ethrog citron	EPT**	1
	C. aurantifolia (Christ.) Swingle	Mexican lime	GTR	24
			Total	**69**

* PPT: partially purified CTV
** EPT: extensively purified CTV

Prints were made, as described by Garnsey *et al.* (1993), from freshly cut young shoots and occasionally from petioles and fruit pedicels, and examined under the following conditions:

- Samples were printed on the nitrocellulose membrane directly in the field (Test I);
- Samples were kept a week at 4°C and then printed (Test II) ;
- Some of the ELISA CTV-positive samples were kept for two weeks at -20°C before printing (Test III).

After drying, blotted membranes were incubated in a 1% solution of bovine serum albumine and than washed. Immunoblotting was done by exposing the blotted membranes to monoclonal antibodies specific to CTV linked with alkaline phosphatase in 10 ml of buffered physiological water.

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The development of the membrane was made by solving one tablet of BCIP-NBT Sigma Fast in 10 ml of distilled water and incubating until the appearance of purple violet colour in the positive control, that was already blotted in the membrane, as well as the negative.

The reaction was stopped by washing the membrane with water and, after drying, the reading was made by using a magnification lens.

Results and discussion

With test I, 33 out of 69 samples were CTV-positive while with test II the number of positives dropped slightly (31 out of 69). All positive plants were already known to be CTV-infected according to previous tests. Freezing of samples proved highly detrimental as shown by the negative detection observed with Shamouti oranges (Test III).

Partially and extensively purified materials from the same CTV isolate were also positive. The negative results were from plants which were suspected to be CTV-infected or were growing in the proximity of CTV positive trees in the field. In addition, when some of the CTV positive samples were tested from their petioles and pedicels, they gave positive reactions as well, regardless the sampled parts.

Table II - Results of immunoprinting under different conditions for the detection of CTV.

Code	Test I		Test II		Test III	
	N. samples	positive	N. samples	positive	N. samples	positive
DTR	5	0	5	0	-	-
KUM	3	3	3	3	-	-
LiQT	2	0	2	0	-	-
T	13	10	13	10	5	0
TIL	1	0	1	0	-	-
KTR	9	0	9	0	-	-
KLT	1	0	1	0	-	-
CHT	2	0	2	0	-	-
LKT	2	0	2	0	-	-
LVT	3	0	3	0	-	-
DT	2	2	2	2	-	-
PPT	1	1	1	1	-	-
EPT	1	1	1	1	-	-
GTR	24	16	24	14	-	-
Total	**69**	**33**	**69**	**31**	**5**	**0**

As shown in Table 3, immunoprint ELISA method was also compared with previous ELISA test using Kits from different sources (Spain, Switzerland, France and Morocco). There was a

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fairly good agreement between the results of classical and immunoprinting ELISA except for the source IZMH4 that was negative with samples taken directly from the field, and for sources KTR1 and KTR4 that were consistently negative. It should be noted however, that these samples had given very low ELISA readings with the antiserum used for immunoprinting. It can therefore be concluded that immunoprinting can be reliably used for the diagnosis of CTV. Samples should be freshly printed on to the membrane in spring when young shoots are available. The use of this method as well as classical ELISA can be very useful for the preliminary screening in the production of primary sources and be easily applied for mass diagnosis in the nursery phase of certification as further control in the areas where CTV is present.

Table III - Results obtained with different ELISA methods and kits for CTV detection

Samples	ELISA kits				Immunoprint	
	Mabs Spain	Pabs Switzerland	Pabs France	Mabs Morocco	Laboratory	Field
T 12	1.495	1.732	1.520	0.323	++++	+++
T 13	0.947	1.376	1.156	0.392	NT	NT
T 21	0.660	0.869	0.314	0.209	++++	++++
GTR 1	0.936	1.250	0.741	0.360	-	++
GTR 3	2.841	2.739	2.647	2.654	+++	++++
GTR 4	1.949	2.095	0.886	1.258	+++	-
KTR 1	0.277	1.091	0.934	0.233	-	-
KTR 4	0.266	0.660	1.020	0.608	-	-
KUM 3	2.715	2.700	2.743	2.713	++++	+++
KUM 4	2.877	2.948	2.903	2.922	++++	+++
KUM 5	2.719	1.852	2.826	0.130	+++	+++
(-)Control	0. 097	0.148	0.090	0.080	-	-
(+)Control	1.826	1.239	1.253	1.251	++	+

Mabs = Monoclonal antibodies
Pabs = Polyclonal antibodies

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