Indexing of Seeds of Different Citrus Species for Tristeza and Variegation Viruses

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ABSTRACT. Seeds collected from infected citrus trees were indexed for citrus tristeza virus (CTV) and citrus variegation virus (CVV). CTV was detected by Enzyme-Linked Immunosorbent Assay (ELISA), and CVV by ELISA and Serologically Specific Electron Microscopy (SSEM). Seeds of Shamouti sweet orange, alemow and Marsh grapefruit were positive for CTV, whereas seeds of trifoliate oranges were negative. CVV was found at high levels in Volkamer lemon seeds. Virus particles were detected by SSEM in extracts from intact seeds and in seeds with no coats. Seedlings originated from CVV infected seeds were negative when indexed by ELISA.
Index words. ELISA, SSEM.

Seed-transmission of citrus virus, viroids and virus-like diseases has been reported in few cases. Seed transmission of citrus psorosis virus was reported in Carrizo and Troyer citrange (2, 5) and in trifoliate orange (3). Salibe and Moreira (11) reported seed transmission of exocortis viroid (CEV) in different sweet orange cultivars whereas no seed transmission has been shown for citrus cachexia viroid (CCaV) (4). In this paper, we report the detection of CTV and CVV in citrus seeds.

MATERIALS AND METHODS

Viruses. Citrus seeds of Marsh grapefruit, alemow and Shamouti sweet orange, and trifoliate orange (selections English Large, Yamaguchi, Christiansen and New Mexico) imported from Mozambique were used for CTV ELISA tests. Seeds were collected from naturally infected plants with stem pitting symptoms (10) at Umbeluzi, Maputo, Mozambique.

For CVV tests Volkamer lemon seed collected from symptomatic fruits (Fig. 1) of 8-yr-old plants infected with CVV-1 and CVV-2 were used.

ELISA and SSEM. For CTV tests, a conventional double sandwich ELISA (6) was used with a monoclonal antibody from Ingenasa Company in Spain. A polyclonal antibody developed against CVV-2 (7) was used for CVV. Seed collected were rinsed in water, air dried and stored at 4 C for 1-4 months. Intact seed, seed coat and seed without seed coat were tested by ELISA for CTV and CVV.

Each sample of 2-8 seed was ground in liquid N_2 and extracted in PBS-Tween with 2% PVP (extraction buffer) at a ratio of 1:10 (w/v). Young leaves collected from Mexican lime infected with the T4 and T8 strains of CTV and from Volkamer lemon trees infected with CVV-FD (8) were used as positive control.

SSEM was done as previously reported (7). Parlodion-coated 200-mesh copper grids were coated with antibody by floating them on a 1/500 dilution of the antiserum to CVV-2. Antigen was extracted from peeled seed and seed coats as done for ELISA. The grids were positively stained with uranyl acetate in 50% ethanol, and were observed with a Zeiss EM 109 electron microscope at 12,000 to 50,000X.

Chemical treatments. Three different chemical treatments were used to degrade the virus protein of CVV infected seeds: a) seeds were soaked for 12 hr in 10% SDS, and rinsed several times in water and air dried; b) seeds were treated for 15 min with 1% Na_3PO_4, followed by 30 min in 0.5% NaOCl, washed three times in water and air dried; and c) seeds were treated with 20% HCl for 30 min, rinsed three times in water and air dried. After chemical treatment, the seeds were peeled, and seed coats and peeled seeds were tested by ELISA.

Another experiment was done with the seed coats and peeled seeds. After peeling, peeled seeds and seed coats were treated with chemicals as described in (b) and (c).

Transmission test. Two, four and six month-old Volkamer lemon seedlings, originating from CVV-1 and CVV-2 infected seeds were tested by ELISA using young leaves and bark as antigen source.

RESULTS

CTV was detected at different concentrations in seeds of different citrus species. In Marsh grapefruit and Shamouti sweet orange samples the virus was detected at high concentration.

In alemow, CTV was found in the seed coats of few samples at very low titer but CTV was not detected in peeled seeds or in intact seeds. CTV was not detected in any of the trifoliate orange selections tested (Table 1).

Volkamer lemon seeds assayed were infected to different degrees with CVV. In fact, 90% of seeds from trees infected with CVV-1 and 72% from trees infected with CVV-2 were positive. In all cases the highest virus titer was in seed coats (Table 2). CVV virions were detected by SSEM in extracts from seed coats, seeds without coats and seeds. The highest titer of CVV was found in seed coat extracts.

After chemical treatment of intact Volkamer lemon seed with SDS, Na_3PO_4 + NaOCl and HCl, the seed coat was removed and assayed along with the peeled seed and CVV was detected by ELISA only in seed coat. HCl treatment of peeled seed and seed coat degraded the CVV protein (Table 3). None of the 600 Volkamer lemon seedlings tested gave positive results when assayed for CVV by ELISA.

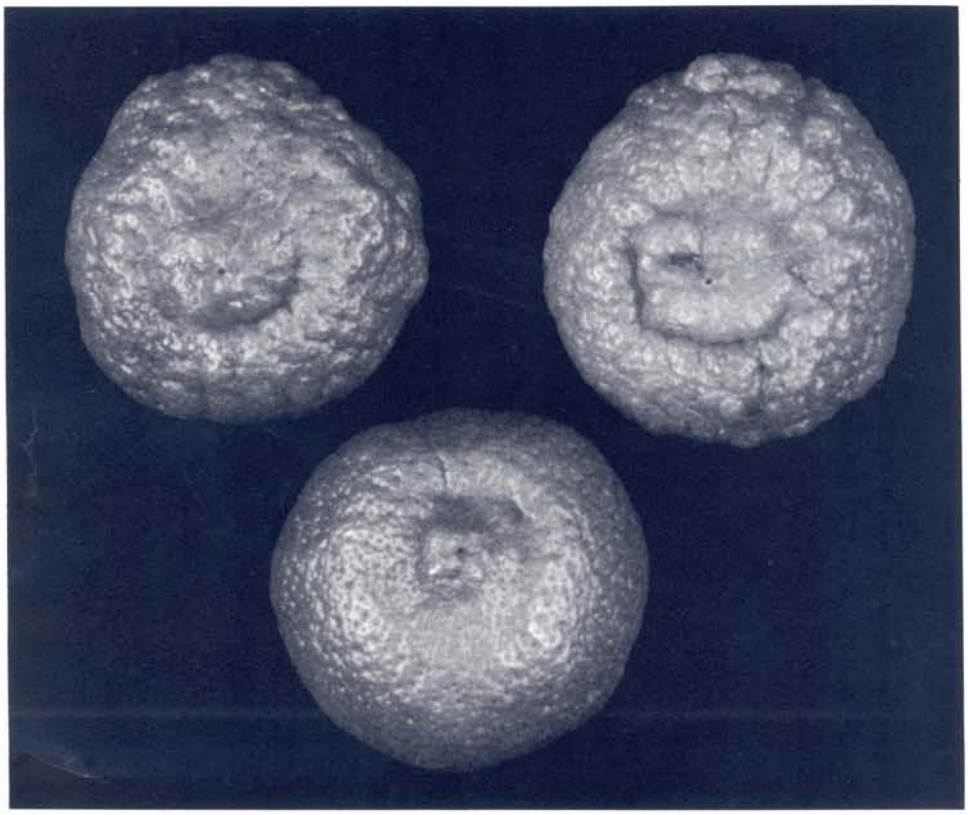

Fig. 1. Volkamer lemon fruits collected from tree inoculated 8 yr previously with CVV-1 (upper). Volkamer lemon fruit collected from tree inoculated 8 yr previously with CVV-2 (lower).

TABLE 1
DETECTION OF CITRUS TRISTEZA VIRUS IN SEEDS OF DIFFERENT CITRUS SPECIES BY ELISA

Species and cultivar	No. positive samples/no. tested[z]	Absorbance value[y] (405nm)
Marsh grapefruit		
Intact seed	15/15	0.828
Peeled seed	15/15	1.160
Seed coat	15/15	1.680
Alemow		
Intact seed	3/15	0.083
Peeled seed	0/15	0.007
Seed coat	15/15	0.140
Shamouti sweet orange		
Intact seeds	12/12	0.785
Trifoliate orange		
Intact seeds		
English Large	0/15	0.002
Yamaguchi	0/15	0.007
Christiansen	0/15	0.006
New Mexico	0/15	0.009
Healthy control		
Intact seed	0/5	0.009
Peeled seed	0/5	0.007
Seed coat	0/5	0.005
Infected control		
leaves	5/5	1.473

[z]Each sample consisted of 5 seeds.
[y]Each value is the mean of all samples.

DISCUSSION

CTV and CVV detection by ELISA (8) and SSEM confirm the sensitivity of such methods in virus diagnosis in seed. In the citrus species tested, CTV has been detected in all the species susceptible to virus infection but no detection in immune species, as trifoliate orange, has been reported.

Wallace (12) reported crinkly leaf virus which is closely related to CVV, was transmitted by seed. Our results confirm the presence of CVV in seeds,

TABLE 2
DETECTION OF CITRUS VARIEGATION VIRUS IN VOLKAMER LEMON SEEDS BY ELISA

Sample	Virus strain	No. positive samples/no. tested[z]	Absorbance value[y] (405 nm)
Peeled seed	CVV-1	44/66	0.197
Seed coat		60/66	0.944
Peeled seed	CVV-2	26/36	0.184
Seed coat		26/36	1.014
Healthy control			
Peeled seed		0/5	0.010
Seed coat		0/5	0.009
Infected control			
Leaves	CVV-FD	5/5	1.101

[z]Each sample consisted of 2 seeds.
[y]Each value is the mean of all samples.

TABLE 3
EFFECTS OF CHEMICAL TREATMENTS OF SEEDS FROM CVV-INFECTED VOLKAMER LEMON

Chemical treatments	Sample	Virus Strain	No. positive samples/no. tested[z]	Absorbance value[y] (405 nm)
Intact seed treatments				
10% SDS	Peeled seed	CVV-1	0/24	0.010
	Seed coat		12/24	0.443
	Peeled seed	CVV-2	0/24	0.007
	Seed coat		21/24	0.380
1% Na_3PO_4 + 0.5% NaOCl	Peeled seed	CVV-1	0/9	0.012
	Seed coat		9/9	1.733
	Peeled seed	CVV-2	0/9	0.010
	Seed coat		9/9	1.510
20% HCl	Peeled seed	CVV-1	0/9	0.009
	Seed coat		9/9	1.263
	Peeled seed	CVV-2	0/9	0.009
	Seed coat		9/9	1.541
Separated seed treatment				
1% Na_3PO_4 + 0.5% NaOCl	Peeled seed	CVV-1	0/6	0.010
	Coated seed		6/6	0.882
	Peeled seed	CVV-2	0/6	0.012
	Coated seed		3/6	1.410
20% HCl	Peeled seed	CVV-1	0/6	0.009
	Coated seed		0/6	0.011
	Peeled seed	CVV-2	0/6	0.012
	Coated seed		0/6	0.011

[z]Each sample consisted of 5 seeds.
[y]Each value is the mean of all samples.

but no seed transmission occurred. Different interpretations have been offered why seed transmission is very low in spite of the systemic nature of most virus diseases (1). The hypothesis that CVV is not able to infect the embryo might explain the lack of seed transmission. Our results confirm that CVV is present in the seed, probably in the superficial layers and not in the embryo. Other additional data is needed to clarify this point.

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