Detection of Citrus Vein Enation Virus Using Cereal Yellow Dwarf Virus ELISA Kits

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ABSTRACT. Citrus vein enation virus (CVEV) has been proposed as a possible member of the Luteoviridae family on the basis of its persistent transmission by aphids, the presence of isometric virus-like particle in extracts of infected leaves and in cells of enations and viruliferous aphids. Five commercially available Luteoviridae ELISA kits (cereal yellow dwarf (CYDV)-RPV, CYDV-RMV, barley yellow dwarf-MAV+PAV, potato leaf roll, and beet western yellows) were tested against extracts of CVEV-infected rough lemon tissues. CYDV-RMV and CYDV-RPV gave positive reactions with extracts of infected young bark. The other ELISA kits failed to detect CVEV. Young bark tissue was found to give the best results. Isolates from California, Spain and Australia also reacted positively with the CYDV-RMV and RPV antisera. This finding supports the proposal that CVEV is a member of the Luteoviridae family.

Citrus vein enation virus (CVEV) is persistently transmitted by the aphid species *Toxoptera citricida* (5), *Aphis gossypii* and *Myzus persicae* (2). Isometric virus-like particles have been observed by electron microscopy in the phloem of vein enations and in viruliferous aphids (4), and small numbers of isometric particles have been isolated from enations (1). These findings led to the suggestion that CVEV is probably a member of the Luteoviridae (1).

It was our intention to purify enough virus to produce antibodies, and to develop an ELISA kit. However, before embarking on such an exercise we decided to test several commercially available ELISA kits for well characterized members of the Luteoviridae, since this family has been described as having a serological continuum (7). DAS-ELISA kits for the following viruses were purchased from Sanofi Diagnostics Pasteur (Marnes La Coquette, France): potato leaf roll (PLRV), beet western yellows (BWYV), barley yellow dwarf (BYDV)-PAV+MAV, cereal yellow dwarf (CYDV)-RPV and RMV. Extracts of CVEV-infected rough lemon leaves, mid veins and young bark were made and used for each of the kits as per manufacturer's instructions. The leaf and mid vein extracts gave unsatisfactory results, and only bark extracts were subsequently used.

The PLRV, BWYV and BYDV-PAV+MAV kits all gave negative results with the CVEV-infected tissue (Table 1). Both CYDV-RMV and CYDV-RPV kits gave positive results, but the former gave higher OD readings; it also gave a higher reading with its own positive control

TABLE 1
REACTION OF A SOUTH AFRICAN ISOLATE OF CITRUS VEIN ENATION VIRUS IN INFECTED ROUGH LEMON BARK EXTRACTS WITH VARIOUS LUTEOVIRIDAE ELISA KITS

	Absorbance (405nm)		
ELISA kit	Negative control	Positive control[z]	CVEV
PLRV	0.066	1.788	0.075
BWYV	0.020	0.872	0.047
BYDV-PAV+MAV	0.040	0.141	0.050
CYDV-RPV	0.057	0.182	0.199
CYDV-RMV	0.172	1.749	0.907

[z]Positive virus controls supplied with the kit.

TABLE 2
REACTION OF VARIOUS CITRUS VEIN ENATION VIRUS (CVEV) ISOLATES IN INFECTED BARK EXTRACTS WITH TWO CEREAL YELLOW DWARF VIRUS (CYDV) ELISA KITS

Virus isolate[y]	Absorbance (405 nm)[z]	
	CYDV-RMV kit	CYDV-RPV kit
CYDV-RMV positive control	1.749	nd
CYDV-RMV negative control	0.103	nd
CYDV-RPV positive control	nd	0.182
CYDV-RPV negative control	nd	0.037
CVEV (South Africa)	0.907	0.199
CVEV (California 701)	0.928	0.207
CVEV (California 702)	1.074	0.222
CVEV (California 703)	1.421	0.122
CVEV (Spain)	1.089	0.175
CVEV (Australia AW 15)	0.234	0.072
CVEV (Australia field)	0.317	0.076

[z]nd = not done.
[y]CYDV-RMV and CYDV-RPV positive and negative controls supplied with the kit.

(1.749) than CVYD-RPV gave with its own control (0.182), possibly indicating a higher antigenicity of the former. Jacomino (3) has also reported a negative result using PLRV antiserum with CVEV-infected tissue, the only Luteoviridae antiserum he tested.

To test whether other isolates of CVEV would react with CYDV antisera, we obtained peeled, dried young green bark of CVEV-infected lemons or Mexican limes from California, Spain and Australia. Extracts of these isolates were then tested against CYDV-RPV and CYDV-RMV. Again, positive reactions with the CYDV-RMV and CYDV-RPV kits were obtained, the former giving higher absorbance readings (Table 2).

We conclude that CVEV is a member of the Luteoviridae family, most closely related serologically to CYDV-RMV and RPV, now classified as poleroviruses (6). Further characterization of CVEV by PCR using Luteroviridae specific primers and sequencing is now being conducted to determine how closely related CVEV may be to the two CYDV serotypes.

Note added in proof CVEV-infected bark samples obtained from New Zealand and China also gave positive results with CYDV-RMV antiserum.

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