

Comparative Viroid Detection of Cuban Viroid Isolates Using Biological Indexing and Sequential PAGE

J. M. Pérez, I. Peña, and R. Pérez

ABSTRACT. Detection of nine citrus viroid isolates in a collection from different parts of Cuba, was evaluated by biological indexing and sequential PAGE (sPAGE). All isolates induced symptoms in citron, while three also caused cachexia symptoms in Parson's Special mandarin and Clemeline 11-20. Sequential PAGE showed that two of the isolates contained only citrus exocortis viroid (CEVd), while all the others were mixtures of two or three different viroids. In addition to CEVd, citrus viroids II-a, II-b and III were identified. Detection by sPAGE was possible any time from 2.5 to 14 mo after inoculation, irrespective of whether samples were collected in spring (mean temperature 23.8°C) or summer (mean temperature 30.4°C).

Different procedures can be used for detecting citrus viroids including bioassay, sequential PAGE and molecular hybridization (1, 4, 5). Citron Arizona 861-S-1 under high temperature is a good biological indicator and source of high viroid titer (2). We compared bioassay with sPAGE on a collection of Cuban viroid isolates, which differ in their reactions on indicators and electrophoretic mobility of infecting viroids (3), at different times of the year and at different bioassay incubation times.

Nine viroid isolates, believed to be infected with exocortis and/or cachexia, from different regions in

Cuba were graft inoculated onto citron, Parson's Special mandarin and Clemeline 11-20. One exocortis isolate was also mechanically inoculated onto *Gynura aurantiaca*. Samples were collected for viroid extraction at various times after inoculation, from 2.5 mo to 14 mo.

Symptom development was noted on the indicators. Healthy and infected young citron and *Gynura* shoots (4 to 6 g) were subjected to RNA extraction and analyzed by sPAGE as previously described (1, 3). The following viroid isolates were used as standards: E-1 (CEVd), E-183 (CVD IIa and CVD-III), CVD-

TABLE 1
BIOASSAY AND SPAGE OF CUBAN VIROID ISOLATES TESTED AFTER DIFFERENT INCUBATION TIMES

| Isolate | Incubation (mo) | Bioassay ^a | | | | sPAGE ^b | | | |
|--------------|-----------------|-----------------------|---------------|-----|------|--------------------|---------|---------|---------|
| | | Citron | <i>Gynura</i> | PSM | Clem | CEVd | CVd-IIa | CVd-IIb | CVd-III |
| E-183 | 2.5/5 | + | | | | | * | | * |
| E-15 | 2.5 | +++ | | | | * | | | * |
| E-C-881 | 5 | +++ | | ++ | ++ | * | | * | * |
| E-77 | 2.5 | +++ | +++ | | | * | | | |
| E-183-II-III | 14 | ++ | | | | | * | | * |
| E-890 | 10 | +++ | | | | * | * | | * |
| E-C-IV | 14/10 | ++ | | ++ | ++ | | | * | * |
| E-66 | 12 | + | | | | | | | * |
| E-L-1-37 | 12 | + | | | | | * | | * |
| Healthy | | | | | | | | | |

^a= symptom severity severe (+++), moderate (++) , mild (+); PSM = Parson's Special Mandarin; Clem = Clemeline 11-20.

^b* = Band present at expected size of the viroid.

IIIId, CVd-IIb (cachexia), the last two kindly provided by N. Duran-Vila (IVIA, Moncada, Spain).

Table 1 displays the results of the bioassays and sPAGE. All isolates developed symptoms on citron ranging from severe to mild. CEVd was detected by sPAGE in only those with severe symptoms. Only one isolate (E-77) was infected only by CEVd; the others all had two or three viroids present. Both bioassay and sPAGE detected cachexia in two isolates. Moderate or mild symptoms in the citron were associated

with mixed infections of two of the following, CVd-IIa, CVd-IIb and CVd-III.

Positive results with sPAGE were obtained with four isolates 2.5 mo after inoculation, even though mean temperatures at this time (spring) were only 23.8°C. Other isolates analyzed in summer (30.4°C, 5 mo after inoculation), and at 10, 12 or 14 mo all gave positive results. Severe symptoms in citron indicate the presence of CEVd, but can mask the presence of other viroids which can be detected by sPAGE.

LITERATURE CITED

1. Duran-Vila, N., J. A. Pina, J. F. Ballester, J. Juárez, C. N. Roistacher, R. Rivera-Bustamante, and J. S. Semancik
1988. The exocortis disease: A complex of viroid RNAs. In: *Proc. 10th Conf. IOCV*, 152-164. IOCV, Riverside, CA.
2. Duran-Vila, N., J. A. Pina, and L. Navarro
1993. Improved indexing of citrus viroids. In: *Proc. 12th Conf. IOCV*, 202-211. IOCV, Riverside, CA.
3. Pérez, J. M.
1993. Los viroides en el cultivo de los cítricos. Evento Internacional de tristeza de los cítricos, Tamaulipas, Mexico.
4. Pérez, J. M., I. Peña, J. C. Rodríguez, and R. Candelaria
1986. Transmisión de aislados de exocortis a hospedantes herbáceos. Verificación de aislados severos por electroforesis en geles de poliacrilamida. *Mem. Simp. Intern. Citricult. Trop. Habana*, 2: 160-167.
5. Roistacher, C. N.
1991. *Graft-Transmissible Diseases of Citrus. Handbook for Detection and Diagnosis*. FAO, Rome. 286 pp.