Comparison of Dot Blot, ELISA, and RT-PCR Assays for Detection of Two Cucumber Mosaic Virus Isolates Infecting Banana in Hawaii

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ABSTRACT

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The coat protein genes of two cucumber mosaic virus (CMV) isolates infecting banana plants in Hawaii were cloned and sequenced. Based on nucleotide and amino acid sequence comparisons, both isolates belong to CMV subgroup I. One isolate (CMV-Hawaii), which is common in banana plants in the state of Hawaii and induces mild mosaic symptoms, shares 99% sequence identity (both nucleotide and amino acid sequences) with CMV-C strain. Another isolate (CMV-Oahu), which was found only at two banana farms in the state of Hawaii, induces severe mosaic and leaf distortion symptoms. CMV-Oahu shares 91% and 93% nucleotide and amino acid sequence identity, respectively, with both CMV-C and CMV-Hawaii. A reverse transcription–polymerase chain reaction (RT-PCR) assay was developed for detection of both CMV subgroups from banana samples. The RT-PCR product (~750 bp) was also labeled as a probe to detect CMV in dot blot hybridization tests. PCR is a more sensitive assay than either dot blot or ELISA. The dot blot assay was 100 times more sensitive than ELISA. The distribution of CMV within banana plants was uneven. CMV concentrations were higher in younger leaves than in older ones. The CMV-Oahu isolate was not detected in banana by ELISA using antibodies to both CMV subgroups I and II, but was positive in dot blot and RT-PCR tests.

Cucumber mosaic virus (CMV) has a tripartite, positive-sense, single-stranded RNA genome (12,18). The virus genomic RNAs, designated RNAs 1, 2, and 3, are 3.4, 3.0, and 2.2 kb in length, respectively. RNA 1 and RNA 2 encode putative replicase proteins (16); RNA 3 encodes a movement protein and a coat protein (CP) (3,18). A subgenomic RNA, RNA 4, is a duplication of the 3′ half of RNA 3, and is not required for infectivity. CMV has a large number of strains that can be classified into two major subgroups (5,17,18). CMV is transmitted in a nonpersistent manner by more than 60 aphid species. It has the largest host range of any virus, infecting more than 800 species. The virus can cause devastating diseases in tomato and many other crops worldwide (18).

CMV causes chlorosis, mosaic, and heart rot in bananas and has been found in most banana-growing areas of the world (15). In general, CMV infection does not have a major impact on banana production. The virus induces mild mosaic symptoms and has little effect on plant growth. However, severe diseases have been observed in CMV-infected banana in Morocco (1). In China, CMV infection incidence in some banana fields was from 40 to 90%, with yield losses as high as 100% (13).

As recommended by Niblett et al. (15), the most effective strategy for control of CMV in bananas is using virus-free plants. In recent years, the banana industry in Hawaii has been expanding using former sugarcane land. Use of banana plantlets produced by tissue culture has been increasing. Enzyme-linked immunosorbent assay (ELISA) has been used to index banana mother plants for CMV infection prior to tissue culture propagation (25). Recently, samples were collected from symptomatic banana plants in the field on the island of Oahu and tested for CMV by ELISA. Some of the plants from which samples were collected showed severe mosaic and leaf distortion symptoms; however, all the samples were negative in ELISA tests with anti-CMV subgroup I antiserum. Because of the mosaic symptoms and lack of detection by ELISA, we suspected that the ELISA-negative banana plants might be infected by CMV subgroup II isolates. This incident led us to re-evaluate indexing systems for detection of CMV from bananas and to examine the molecular relatedness of CMV isolates infecting bananas in Hawaii. Dot blot hybridization and reverse transciption–polymerase chain reaction (RT-PCR) assays were compared with ELISA for detection of CMV from banana samples.

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MATERIALS AND METHODS

CMV isolates. Two CMV isolates from banana were used in this study. The first isolate (CMV-Hawaii) was obtained from an infected Cavendish banana (cultivar Williams, AAA genotype) on the island of Hawaii. This CMV isolate is common in banana plants in the state of Hawaii and induces typical mosaic symptoms. The second isolate (CMV-Oahu) was collected from the banana cultivar Brazilian (AAB genotype) on the island of Oahu. This isolate was first found on Oahu and was referred to as CMV-Oahu. Banana plants infected by the CMV-Oahu isolate exhibited severe mosaic and leaf distortion symptoms. The CMV isolates were inoculated and propagated in zucchini squash plants (*Cucurbita pepo* L.) and purified as described elsewhere (13).

RT-PCR. Total nucleic acid samples were extracted from the CMV-infected banana plants for PCR and cloning of the CP gene using a procedure described by Hadidi et al. (8) with minor modifications. Approximately 0.1 g of leaf tissue was homogenized in 1.5-ml microcentrifuge tubes with a glass rod in 0.5 ml of extraction buffer (0.1 M glycine-NaOH, pH 9.0; 50 mM NaCl; 10 mM EDTA; 2% sodium dodecyl sulfate [SDS]; 0.2% sodim diethyldithiocarbamate [DIECA-Na]; and 1% sodium lauryl sarcosine). Homogenates were then emulsified with phenol/chloroform/isoamyl alcohol (25:24:1). Total nucleic acids were recovered by precipitation with ethanol and dissolved in 100 µl of TE (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). Two oligonucleotide primers were designed for the amplification of the CMV CP gene based on a comparison of known CMV CP gene sequences of both CMV subgroups (20). The primers were designed for cloning and amplification of the CP gene of both subgroups of CMV. The upstream primer 93-309 (5′-CATCGACCATGGACAAATCTGAATCAAC), is identical to the 5′ terminus of the CMV CP gene, and includes 8 nucleotides upstream of the starting codon of CMV-C RNA 3. The downstream primer 93-359 (5′-CTCTCCATGGCGTTTAGTGACTTCAGCAG), is complementary to the 3′ end of CMV-C RNA 3, about 80 nucleotides after the stop codon of the CP gene. The predicted length of the amplified DNA product is approximately 745 bp. An *Nco*I restriction site was included at the 5′ termini of both primers to facilitate cloning into

plasmid vectors.

Thermostable rT*th* DNA Polymerase (Perkin-Elmer Cetus, Norwalk, Conn.) was used for RT-PCR. For reverse transcription, 75 pmoles of the downstream primer (93-359) and 7.6 µl of master mix (master mix contains 2 µl of 10× Reverse Transcription Buffer [Perkin-Elmer Cetus], 2 µl of 10 mM $MnCl_2$ solution, 0.4 µl of each 10 mM dNTP, and 2 µl of rT*th* DNA Polymerase) were added to 1 µl (~200 ng) of total nucleic acids from banana. Deionized, ultrafiltered water was added to give a final volume of 20 µl. The mixture was incubated in a DNA Thermal Cycle 480 (Perkin-Elmer Cetus) at 70°C for 5 min, 60°C for 15 min, and 50°C for 10 min. For PCR, the 20-µl RT mixture was transferred to a tube containing 15 pmoles of the upstream primer (93-309), 8 µl of 0.25 mM $MgCl_2$, 8 µl of 10× Chelating Buffer (Perkin-Elmer Cetus), and sufficient deionized, ultrafiltered water to give 100 µl. The reactions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 1 min at 94°C and 1 min at 50°C, and a final extension of 7 min at 50°C.

Cloning and sequence analysis of CMV CP gene. The RT-PCR products were directly loaded and separated on a 1.2% agarose gel in Tris-acetate-EDTA buffer (TAE) (14). The CP gene fragment was electroe-

A

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Hawaii    ATGGACAAATCTGAATCAACCAGTGCTGGTCGTAACCATCGGCGTCGTCCGCGTCGTGGT  60
C         ............................................................
Oahu      ......................C.............G......................

Hawaii    TCCCGCTCCGCCCCCTCCTCCGCGGATGCTAACTTTAGAGTCTTGTCGCAGCAGCTTTCG 120
C         ............................................................
Oahu      .........CGTT..............................C...T...A.........

Hawaii    CGACTTAATAAGACGTTAACAGCTGGTCGTCCAACTATTAACCACCCAACCTTTGTAGGG 180
C         ..................G.........................................
Oahu      .....C............G.................C..........G........CG..T

Hawaii    AGTGAACGCTGTAGACCTGGGTACACGTTCACATCTATTACCCTAAAGCCACCAAAAATA 240
C         ............................................................
Oahu      ........A....A.T.C......G.............C.....G..A.....G......

Hawaii    GACCGTGAGTCTTATTACGGTAAAAGGTTGTTACTACCTGATTCAGTCACGGAATATGAT 300
C         ............................................................
Oahu      ..T..ACG.........T......G..........T........G.....T..G.TC...

Hawaii    AAGAAGCTTGTTTCGCGCATTCAAATTCGAGTTAATCCTTTGCCGAAATTTGATTCTACC 360
C         ............................................................
Oahu      ............................C.......................C........

Hawaii    GTGTGGGTGACAGTCCGTAAAGTTCCTGCCTCCTCGGACTTATCCGTTGCCGCCATCTCT 420
C         ............................................................
Oahu      ..............T................T...CA.......................

Hawaii    GCTATGTTCGCGGACGGAGCCTCACCGGTACTGGTTTATCAGTATGCCGCATCTGGAGTC 480
C         ............................................................
Oahu      ....................................C........C........C......

Hawaii    CAAGCCAACAACAAACTGTTGTTTGATCTTTCGGCGATGCGCGCTGATATAGGTGACATG 540
C         ............................................................
Oahu      ..............GT......AC.........................T..C.C....

Hawaii    AGAAAGTACGCCGTCCTCGTGTATTCAAAAGACGATGCGCTCGAGACGGACGAGCTAGTA 600
C         ............................................................
Oahu      ..............T.......................T...........T.....G...

Hawaii    CTTCATGTTGACATCGAGCACCAACGCATTCCCACATCTGGAGTGCTCCCAGTCTGA     657
C         ...........................................................
Oahu      ........C.....T.....................A..G...........T...
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B

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Hawaii    MDKSESTSAGRNHRRRPRRGSRSAPSSADANFRVLSQQLSRLNKTLTAGRPTINHPTFVG  60
C         ..............................................A.............
Oahu      ............R..........RS..........L..........A........R..A.

Hawaii    SERCRPGYTFTSITLKPPKIDRESYYGKRLLLPDSVTEYDKKLVSRIQIRVNPLPKFDST 120
C         ............................................................
Oahu      ....KS..A.............R.....G.........F.....................

Hawaii    VWVTVRKVPASSDLSVAAISAMFADGASPVLVYQYAASGVQANNKLLFDLSAMRADIGDM 180
C         ............................................................
Oahu      ..........F.N.................................Y.............

Hawaii    RKYAVLVYSKDDALETDELVLHVDIEHQRIPTSGVLPV                       218
C         ....................................
Oahu      ............V.......................
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Fig. 1. Nucleotide **(A)** and predicted amino acid **(B)** sequences of the coat proteins of CMV-Hawaii, CMV-C (D00462), and CMV-Oahu. Dots (...) indicate residues identical to CMV-Hawaii. GenBank accession numbers are U31219 and U31220 for CMV-Hawaii and CMV-Oahu strains, respectively.

luted from the agarose gel (14), ethanol-precipitated and dissolved in 20 µl of distilled water (14). The gel-isolated CP gene fragments were digested with *Nco*I and ligated into *Nco*I-digested vector pBI 525 (obtained from William Crosby, Plant Biotechnology Institute, Saskatoon, Saskatchewan, Canada). Clones were sequenced by the dideoxynucleotide chain-termination method and the primer-directed dideoxynucleotide sequencing method (10,22,24). Sequence data were compiled and analyzed using PC/Gene (Intelligenetics, Inc.) and the University of Wisconsin Genetics Computer Group (GCG) computer program, available from GCG, Inc., Madison, Wis. (4). The CMV banana isolate CP sequence was compared with that of eight subgroup I and four subgroup II CMV strains, whose sequences were available in the literature and in the GenBank. The GenBank sources of data sequence were from the following accession numbers: subgroup I strains C (D00462), FC (D10544), FNY (D10538), Korea (X77855), O (C00385), PR (M98501), Y (D12499), China (X65017), and subgroup II strains Kin (Z12818), TRK-7 (L15336), WL (D00463), and Q (J02059).

Dot blot hybridization. Sap extracts were prepared by vortexing ground plant tissue (0.1 g) in 0.5 ml of TE buffer (pH 8.0) containing 1% SDS, 0.2% DIECA-Na, and 0.5 ml of phenol/chloroform (1:1), followed by clarification with chloroform. Extracts were precipitated with ethanol and resuspended in 1 ml of TE (pH 8.0). Aliquots of 100 µl were loaded onto Zeta-Probe membrane using a Bio-Dot blotting manifold (Bio-Rad Laboratories, Richmond, Calif.). The agarose gel-isolated RT-PCR product of CMV-Hawaii isolate was ^{32}P-labeled as a probe using the random primed DNA labeling kit (Boehringer Mannheim, Indianapolis, Ind.). Zeta-Probe membranes were prehybridized and hybridized following the procedures provided by the vendor (Bio-Rad).

ELISA. Polyclonal antibodies against CMV-C strain (CMV subgroup I) or CMV-WL strain (CMV subgroup II) were produced previously by D. Gonsalves at Cornell University (5,7) and used in double antibody sandwich ELISA for detection of CMV in banana as described previously, with minor modifications (2,9). Microtiter plates were coated with specific antibody at a concentration of 1 µg per ml, 100 µl per well. The plates were then incubated overnight at 4°C and washed three times (2). The plant samples (0.1 g) were prepared with 1 ml of extraction buffer (2) containing 0.2% DIECA and added to the plate at 100 µl per well. The plates were again incubated overnight at 4°C and later washed three times. Alkaline phosphatase-labeled specific antibody in enzyme conjugate buffer (2) at a dilution of 1:2,000 was added and incubated for 4 h at 30°C. After washing the plates as before, substrate (p-nitrophenyl phosphate at 1 mg per ml) in 100 µl of substrate buffer was added to each well and incubated 2 h at room temperature. Absorbance at 405 nm was measured with a Model 450 Microplate Reader (Bio-Rad). Controls with virus extraction buffers, healthy samples, and virus-infected samples were included in all tests. A reaction was considered positive only if the absorbance was >0.1, which was at least three times the mean absorbance value of the healthy control.

CMV distribution in banana plants. Two CMV-Hawaii–infected individuals of Williams, Brazilian, or Valery banana plants were tested by ELISA to assess variations in virus titer within individual plants. Two samples (about 0.1 g per sample) were collected from each leaf, one from the midrib tissue and one from leaf lamina. In other tests, ELISA, dot blot and RT-PCR assays were compared to evaluate their sensitivity and reliability in detecting virus in CMV-Hawaii–infected banana tissues.

RESULTS

CP gene sequence analysis. The CP genes of two CMV isolates infecting banana plants were amplified in RT-PCR, cloned, and sequenced. Computer-aided analyses of the 657 base pair (bp) sequence revealed the presence of a single long open reading frame capable of encoding 218 amino acids (Fig. 1). Computer-aided comparisons of the predicted CP sequence of CMV-Hawaii and CMV-Oahu isolates with that of other CMV strains revealed that the banana isolates are more closely related to CMV strains in subgroup I (Table 1). The CMV-Hawaii isolate was almost identical in nucleotide and predicted amino acid sequences to CMV-C (99%), whereas the CMV-Oahu isolate shares 91% nucleotide and 93% predicted amino acid sequence identity with both CMV-C and CMV-Hawaii, respectively (Fig. 1, Table 1). The CMV-Hawaii isolate shares 92 to 99% predicted amino acid and nucleotide identities with the eight other subgroup I strains, but only 77 to 81% predicted amino acid and 77% nucleotide sequence identity with the four subgroup II strains (Table 1). CMV-Oahu is different in that it shares 91 to 95% amino acid and 90 to 92% nucleotide sequence identity with the other subgroup I strains, and 74 to 79% amino acid and 76 to 77% nucleotide sequence identity with the four subgroup II strains tested (Table 1). The fidelity of nucleotide incorporation of rT*th* DNA polymerase did not appear to be a problem in cloning, based on the high percent sequence identity of CMV-Hawaii with CMV-C (only one nucleotide difference).

Virus detection. The RT-PCR assay amplified an approximately 750-bp DNA fragment from banana plants infected by

Table 1. Coat protein nucleotide and amino acid sequence identities between subgroup I and subgroup II strains of cucumber mosaic virus (CMV) and CMV-Hawaii and CMV-Oahu isolates[a]

	Subgroup I										Subgroup II			
CMV strains	**Hawaii**	**C**	**FNY**	**FC**	**O**	**PR**	**Y**	**Korea**	**China**	**Oahu**	**WL**	**Q**	**Kin**	**TRK-7**
Hawaii		99.5	98.2	97.7	96.8	97.3	95.4	95.4	92.7	92.2	80.3	77.1	80.7	78.9
C	99.7		98.6	98.2	97.3	97.7	95.9	95.9	92.7	93.1	80.8	77.5	81.2	79.4
FNY	99.2	99.5		99.5	98.6	99.1	97.3	97.3	94.0	94.0	78.4	82.1	82.6	80.7
FC	98.3	98.6	99.1		98.2	98.6	97.7	96.8	93.6	93.6	78.0	81.7	82.1	80.3
O	97.4	97.7	98.2	98.0		98.6	96.8	96.8	93.6	93.6	78.4	78.4	83.0	81.2
PR	97.4	97.4	97.9	97.7	97.3		98.2	98.2	95.0	95.0	78.4	82.6	83.0	81.2
Y	97.0	97.3	97.7	97.9	97. 1	98.0		97.3	93.1	93.1	81.7	77.5	82.1	80.3
Korea	93.8	94.1	94.5	93.9	93.9	93.9	94.4		94.0	94.0	77.5	81.7	82.1	80.3
China	92.1	92.4	92.8	92.8	92.5	92.5	92.7	94.2		90.8	78.4	74.8	78.9	77.7
Oahu	90.6	90.4	91.0	90.7	90.4	90.7	90.3	92.2	90.8		78.0	74.8	78.9	78.4
WL	76.8	76.8	77.3	77.4	7 6.8	78.0	77.7	77.8	77.3	76.4		92.7	98.2	96.3
Q	76.9	76.8	77.3	77.4	76.8	78.0	77.7	77.8	77.3	76.5	98.2		93.6	92.2
Kin	76.7	76.7	77.1	77.3	76.6	77.8	77.6	77.8	77.1	76.7	98.2	99.1		97.3
TRK-7	76.2	76.2	76.7	76.8	76.2	77.4	77.1	77.2	76.7	76.1	97.7	98.5	98.5	

[a] The GenBank sources of data sequence were from the following accession numbers: subgroup I strains C (D00462), FC (D10544), FNY (D10538), Korea (X77855), O (C00385), PR (M98501), Y (D12499), China (X65017), and subgroup II strains Kin (Z12818), TRK-7 (L15336), WL (D00463), and Q (J02059). Comparisons above the diagonal refer to coat protein amino acid sequences; figures below the diagonal are from coat protein gene nucleotide sequence comparisons.

CMV-Hawaii or CMV-Oahu strains, whereas no band was amplified from healthy banana samples (data not shown). The RT-PCR product of CMV-Hawaii was labeled as a probe in dot blot hybridization for detection of CMV-Hawaii and CMV-Oahu from banana samples. The probe did not react with healthy banana samples (data not shown). The sensitivity of the dot blot assay was compared with that of ELISA and RT-PCR using crude sap extracts and purified CMV-Hawaii. When crude samples were used, CMV was detected at dilutions equivalent to 100 µg, 1 µg, and 10 ng banana leaf tissue in ELISA, dot blot, and PCR respectively (Fig. 2). RT-PCR was negative when undiluted sample was used. This was likely due to inhibitory materials in the banana tissue and could be overcome by diluting the samples (Fig. 2). When purified CMV-Hawaii virus was used, CMV could be detected at 100 ng, 1 ng, and 0.1 pg in ELISA, dot blot, and PCR, respectively (data not shown).

Usually, virus could be readily detected by ELISA from banana plants infected by the common CMV-Hawaii isolate. In our sensitivity comparison tests, these samples were ELISA positive when the samples were diluted at 1:100 (Fig. 2). However, the samples collected from banana plants infected by the CMV-Oahu isolate were negative in ELISA using antibodies to both CMV subgroup I and II. A total of 28 banana plants were collected from a banana farm on the island of Oahu from which the severe isolate was identified.

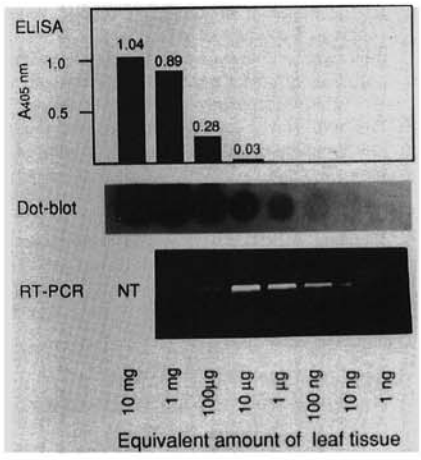

Fig. 2. Sensitivity comparisons of enzyme-linked immunosorbent assay, dot blot, and reverse transcription–polymerase chain reaction (RT-PCR) for detection of the CMV-Hawaii isolate. Ten-fold dilutions of infected banana leaf tissue samples corresponding to 10 mg, 1 mg, 100 µg, 10 µg, 1 µg, 100 ng, 10 ng, and 1 ng of leaf tissue were used. There were no reactions to healthy control samples in dot blot and RT-PCR tests. The RT-PCR assay amplified an approximately 750-bp DNA fragment from banana plants infected by CMV-Hawaii or CMV-Oahu strains. The mean absorbance value of the healthy control was below 0.03. NT = not tested for RT-PCR.

Seven of these plants had severe symptoms, 11 had mosaic symptoms, and 10 displayed no symptoms. All 28 samples were negative in ELISA tests; however, 23 were positive in dot blot tests with CMV-Hawaii probe, including 9 samples collected from asymptomatic plants. When this CMV isolate was transmitted mechanically into squash plants, it was positive in ELISA tests using antibodies against CMV subgroup I (data not shown). Another banana sample collected from the island of Hawaii also showed similar severe symptoms, and again it was negative in ELISA tests and positive in dot blot tests. The CMV-Oahu collected from both Oahu and Hawaii was positive in RT-PCR tests.

CMV-Hawaii could be detected by ELISA in both leaf laminae and midrib samples of the symptomatic banana plants tested (Table 2); however, the relative concentration of CMV in infected banana plants varied considerably. CMV was undetectable in some banana leaves exhibiting strong symptoms (Table 2). Absorbance values, however, were generally higher in extracts of midrib tissue than of leaf laminae tissue and were also generally higher in extracts of younger leaves than in those of older ones (Table 2). CMV was readily detected from all three banana cultivars infected with CMV-Hawaii isolate (Table 2).

DISCUSSION

Strains of CMV are characterized into two subgroups on the basis of serological relationships, host range, peptide mapping of the viral CP, and nucleic acid hybridization (5,6). In addition, sequence comparison results show that CMV strains within each subgroup share 91 to 99% amino acid and nucleotide sequence identity, whereas strains between subgroups share 76 to 84% sequence identity (17,20, 21,23, and this report). Based on nucleotide and amino acid sequence comparisons, both CMV isolates infecting banana plants in the state of Hawaii belong to the CMV subgroup I. The CMV-Oahu isolate is more unique in that it is less closely related to other strains in subgroup I than is the CMV-Hawaii isolate. Pappu et al. (19) sequenced CMV strains in banana from Puerto Rico and also found that all the strains belong to CMV subgroup I. Singh et al. (23) showed that all four CMV isolates in banana in Western Australia shared 98% nucleotide sequence identity with that of CMV subgroup I strains. Thomas, however, has detected both CMV subgroups infecting bananas in Australia (25).

The sensitivity of the dot blot hybridization, ELISA, and RT-PCR assays was compared for detection of CMV-Hawaii from banana samples. As expected, RT-PCR is the most sensitive assay among the three and might be used to confirm samples that are inconclusive in ELISA and dot blot tests. As noted by Singh et al. (23), dilution of extracts of banana leaf samples improved reliability of RT-PCR assay for detection of CMV in banana. For routine tests of large numbers of samples, ELISA and dot blot assays are more convenient and practical. The dot blot assay was 100 times more sensitive than ELISA for detection of the CMV-Hawaii strain in banana.

In this study, all 28 samples collected from banana plants infected by the CMV-Oahu strain were negative in ELISA tests, regardless of symptom expression. How-

Table 2. Distribution of the cucumber mosaic virus (CMV)–Hawaii isolate in infected banana plants as determined by enzyme-linked immunosorbent assay[a]

	Cultivar					
	Williams		Brazilian		Valery	
Tissue/Number	Plant 1	Plant 2	Plant 1	Plant 2	Plant 1	Plant 2
Leaf lamina						
1	0.611 ++[b]	0.876 +	1.247 +	1.725 +	1.341 +	0.497 +
2	0.276 +++	1.319 ++	0.841 ++	0.574 +	1.202 +++	0.255 ++
3	0.242 +++	0.608 +++	0.790 ++	0.421 +++	0.727 +	0.275 +++
4	0.009 +++	0.594 +++	0.617 +++	0.204 ++	0.751 +	0.214 +++
5	0.006 +++	0.235 ++	0.747 +++	0.020 +++	0.751 +++	0.137 +++
6	0.004 +++	0.350 +++	0.433 +++	0.189 +++	0.720 +++	0.066 +++
Leaf midrib						
1	0.850	1.796	0.987	1.416	0.784	0.976
2	0.753	1.407	0.879	2.003	0.707	0.078
3	0.438	1.010	0.559	0.027	0.582	0.524
4	0.549	0.946	0.047	0.032	0.562	0.038
5	0.092	1.003	0.775	0.020	0.720	0.054
6	0.236	1.421	0.399	0.614	0.978	0.084
Healthy CK						
Leaf	0.006	0.008		0.009		
Midrib		0.008				

[a] Symptomatic banana plants were tested for CMV distribution within individual plants. Leaf/midrib 1 was the youngest whereas leaf/midrib 6 was the oldest. Values are means of four absorbance readings for the leaf samples, recorded 60 min after substrate was added.

[b] Symptoms exibited on the leaves: + = very mild mosaic, ++ = mild but clear mosaic, +++ = strong mosaic.

ever, 23 out of 28 samples were positive in dot blot tests, including nine samples collected from asymptomatic plants. It is clear that lack of detection of CMV-Oahu strain in banana plants by ELISA is not due to heterologous antiserum, because CMV-Oahu was readily detected by ELISA with anti-CMV subgroup I antibody when the virus was transmitted into squash plants. Similarly, another banana sample collected from the island of Hawaii, also showing similar severe symptoms, was negative in ELISA tests and positive in dot blot and RT-PCR tests. It appears unlikely that different banana cultivars are responsible for differences in symptom expression and detection by ELISA, because virus was readily detected by ELISA from all three banana cultivars infected by the CMV-Hawaii isolate. From a virus-indexing point of view, lack of detection of CMV-Oahu strain in banana by ELISA is a potential problem. ELISA negative samples collected from fields where some banana plants show severe symptoms should be re-tested by dot blot hybridization and/or RT-PCR to test for the presence of CMV-Oahu or similar isolates.

Previously, Thomas found that CMV concentrations can vary between plants and even between leaves on the same plant (25). Our results confirm that the distribution of CMV in infected banana plants varies considerably. However, it appears that the virus titer is higher in the midrib tissue of young leaves. Thus, the most reliable detection of CMV from newly infected plants or from symptomless plants is from midrib tissue of young leaves. Previously we found that the best place for detection of banana bunchy top virus (BBTV) is also from midrib tissue of young leaves (11). Therefore, it will be convenient to use one set of samples for the detection of both CMV and BBTV from bananas in indexing banana planting materials prior to tissue culture propagation.

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