Indexing System for Sweet Potato Feathery Mottle Virus in Sweet Potato Using Enzyme-Linked Immunosorbent Assay

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ABSTRACT

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Enzyme-linked immunosorbent assay was adapted for detection of sweet potato feathery mottle virus in sweet potato (*Ipomoea batatas*). Detectable virus levels were highest in shoots 5–8 wk after emergence. There were highly significant differences in detectable virus levels between leaf positions sampled from shoots infected with feathery mottle virus. Leaves near the proximal portion of the shoot were the most consistent source of detectable virus. Enzyme-linked immunosorbent assay detected 100% of the plants (ie, a root and all shoots produced by that root) infected with feathery mottle virus by sampling two shoots per plant, using a composite sample of three leaves from each shoot.

Sweet potato feathery mottle virus (FMV) (6,14) is widespread in commercial sweet potato (*Ipomoea batatas* (L.) Lam.) plantings (13) and may exist as several strains (5–7,13). Losses resulting from FMV strains may be qualitative because of loss in market value from the russet crack disease or quantitative because of loss of plant vigor associated with chronic infections with mild transient foliar symptoms. A possible control strategy is incorporation of virus indexing into existing certification programs.

Sweet potatoes are currently indexed for FMV by graft transmission to indicator plants (7). Graft transmission is a reliable method of detection (13) but requires large quantities of *I. setosa* Ker. seedlings, propagation of indicator plants, greenhouse space, insect control, and labor. Serological indexing methods such as microprecipitin and immunodiffusion tests lack the sensitivity required for routine detection of FMV in sweet potato tissue. An enzyme-linked immunosorbent assay (ELISA) technique detected FMV in partially purified preparations and in symptomatic leaves of *I. batatas* and *I. incarnata* Choisy (4). In addition, similar reactions were reported for homologous and heterologous combinations of antiserum and virus for the four isolates investigated.

Our experience (13,14) is consistent with the reports of others (2,4) on the variable concentration of FMV and/or ability to detect the virus in sweet potato tissue. This suggested that tissue selection and timing of the assay may be of critical importance. The objective of this study was to improve ELISA for use as an indexing tool for FMV-infected sweet potato.

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MATERIALS AND METHODS

Virus culture and indexing procedure. FMV-infected sweet potato plants were obtained by sprouting naturally infected commercial roots of cultivars Porto Rico and Jewel and by inoculating healthy plants. Healthy Porto Rico and Jewel plants were obtained by meristem tip culture, maintained in screen cages, and periodically indexed by grafting. All graft indexing was done using a modified cleft graft (7) with 5-wk-old *I. setosa* indicator plants.

ELISA. Antisera to the common (C-FMV) and russet crack (RC-FMV) strains of FMV were prepared as previously described (6,14). The double-antibody sandwich form of ELISA was used throughout this investigation (8). The virus extraction buffer was amended with 0.01 M sodium diethyldithiocarbamate (DIECA). Spectrophotometric measurement of the reaction mixture was done by combining 0.2-ml samples from two wells in a microcuvette (No. 108B-OS, Hellma Cells, Inc., Box 544, Borough Hall Station, Jamaica, NY 11424) and reading the absorbance at 405 nm with a Pye Unicam SP8-100 spectrophotometer. Healthy and infected samples were processed randomly and placed into the coated plate in order of extraction. Samples were arranged in a randomized complete block; the reported absorbance is a mean of at least two replicates per extract. Extracts from healthy sweet potato plants accounted for at least 15% of the samples in each test. Samples giving an absorbance reading greater than three standard deviations above the mean of the healthy controls in the same test were considered positive.

A preliminary comparison of virus extraction by mortar and pestle and by leaf press (11) indicated no significant differences in virus detection when leaf tissue was extracted in 30 volumes of buffer with the mortar and pestle and with four volumes of buffer with the leaf press (optimum buffer:tissue [v/w] ratio for each method). Most leaf samples were extracted with a leaf press, and the mortar and pestle method was used for other tissues.

Homologous and heterologous ELISA reactions were compared between C-FMV and RC-FMV strains. Porto Rico cuttings were graft inoculated with *I. nil* plants infected with C-FMV or RC-FMV. Three leaves from each of 15 plants were assayed for each strain with each antiserum. Homologous and heterologous reactions were also measured against purified virus diluted with extracts from healthy plants. Virus concentrations ranged from 5 to 0.01 μg/ml. Antiserum to C-FMV was used in all other tests.

Virus distribution. Leaf, stem, and root tissues were assayed in vegetatively propagated, greenhouse-grown Porto Rico plants. A 0.5-g sample of each tissue was extracted with a mortar and pestle as previously described. Comparable tissues from five healthy and five infected plants were assayed. In a separate experiment, leaf tissue was selected as a source. The influence of leaf position and cultivar was tested by assaying four leaves (designated A–D) equally distributed between the distal and proximal ends of the shoot: A, a young leaf near the distal end of the shoot; D, the oldest leaf at the proximal end of the shoot; and B and C were evenly spaced between A and D.

Single-leaf samples (corresponding to position D) from shoots produced by 60 infected Jewel and Porto Rico roots were collected at 4-wk intervals beginning 1 wk

after emergence. Variation between leaves in detectable virus suggested that a three-leaf composite sample should be taken from each shoot. Composite samples were taken for 2 additional weeks. A similar experiment was conducted under field conditions, simulating commercial sweet potato growing practices. Four bushels of FMV-infected Jewel and Porto Rico roots were bedded according to standard practices (9). Healthy roots of both cultivars were planted at one end of the bed. Sixty plants (a plant was defined as all shoots produced by a single root) were randomly selected and tagged. A composite leaf sample from a single shoot from each plant was assayed at 3, 5, and 8 wk after emergence; each sample consisted of one-half of each of three adjacent leaves from the proximal half of the vine. The remaining middle leaf-half with the petiole and node was indexed by grafting onto *I. setosa*.

To test the possibility that all shoots on each plant were not infected, composite samples from three infected Porto Rico plants (47 shoots) and six infected Jewel plants (40 shoots) were assayed by ELISA and grafting.

The composite sampling method was evaluated under field conditions, with the junior author collecting and coding the samples and the senior author performing the assays. Three 8-wk-old shoots were collected from each of 10 FMV-infected plants and five healthy plants. A composite sample from each shoot was numbered, randomized, and assayed with ELISA.

RESULTS

ELISA. Initial trials demonstrated that the basic ELISA technique (8) detected FMV in sweet potato. The influence of interfering compounds present in sweet potato homogenates was minimized by the addition of 0.01 M DIECA to the extraction buffer and 0.2% ovalbumin to the virus buffer and to the rinse buffer following incubation of the coating antibody. Ovalbumin in the virus buffer eliminated nonspecific reactions associated with certain tissues.

There were no significant differences in absorbance values when leaf extracts of different ages from Porto Rico and Jewel were used as diluent for purified C-FMV nor between homologous and heterologous combinations of antisera and virus for C-FMV and RC-FMV in ability to detect virus.

Virus distribution. FMV was detected in all tissues assayed except fibrous roots and apical shoot tissue but was not detected in every sample assayed from any of the tissues (Table 1). Mature leaves on the proximal half of the shoot gave the most consistent results. Analysis of variance of absorbance values for infected leaves (A–D) revealed that subsamples from the same extract on the same plate did not vary significantly, suggesting that the number of subsamples could be reduced. Between-leaf variance was highly significant ($P < 0.01$), suggesting that in future studies sampling should consist of more leaves per shoot. Similar analysis of absorbance values for healthy leaves indicated no significant differences between leaves within a trial or between subsamples of the same extract. Healthy leaves used to establish baseline levels in further experiments were selected without regard for position.

Leaves A–D from 12 (6 Jewel, 6 Porto Rico) infected, 8-wk-old shoots collected in the field were assayed by grafting and by ELISA. Grafts from all 48 leaf positions were positive. ELISA detected 2 of 12, 7 of 12, 9 of 12, and 9 of 12 positives at leaf positions A–D, respectively (Table 2). One shoot was negative at all four leaf positions. Results indicated that FMV occurred in leaves of infected plants at concentrations approaching the limits of ELISA detection using standard methods. Samples from Porto Rico gave 15 positives with a mean absorbance of 0.602; Jewel samples gave 12 positives with a mean absorbance of 0.420. Symptoms were also recorded at the time of assay; there were 14 instances where there were no visible symptoms and ELISA was positive.

Virus multiplication. Shoot age was a significant factor in FMV detection using ELISA. Single-leaf samples from the proximal half of an FMV-infected shoot were collected from 60 greenhouse-grown plants, and ELISA detected FMV in 27, 52, 40, and 29% of the plants assayed 1, 2, 3, and 4 wk after emergence, respectively. A three-leaf composite sample assayed during weeks five and six resulted in 60 and 74%, respectively, of the infected samples being detected.

Field plants, which tested positive by grafting, were assayed by ELISA using three-leaf composite samples per plant at various intervals. Eighteen, 70, and 90% of the shoots tested positive with ELISA 3, 5, and 8 wk, respectively, after emergence.

To test the hypothesis that all shoots on each plant were infected, even though the virus was not detected in every leaf assayed, all shoots produced by three infected Porto Rico roots and six infected Jewel roots grown in the field were assayed by ELISA and grafting. Of 87 shoots from FMV-infected plants assayed, 86 shoots (99%) were positive when assayed by ELISA (83%) and by grafting (93%).

A final experiment was conducted to determine the number of shoots to sample per plant to ensure detection of an

Table 1. Distribution of sweet potato feathery mottle virus detectable by enzyme-linked immunosorbent assay in tissues of 20-wk-old Porto Rico plants

	Number of positive samples[a]									
	Apex	Leaf A[b]	Internode	Leaf B	Internode	Leaf C	Internode	Leaf D	Fleshy root	Fibrous root
Infected	4[c]	3	0	3	1	4	2	4	3	0
Healthy	5[c]	0	0	0	0	0	0	0	0	0

[a] Samples with absorbance values three standard deviations above the mean of healthy control samples, from a total of five samples tested.
[b] Expanded leaves equally distributed between the distal and proximal end of the shoot designated A–D: A, young, expanding leaf near the distal end of the shoot; D, the oldest leaf at the proximal end of the shoot.
[c] These false positives were the result of nonspecific reactions. Further tests revealed that the addition of 0.2% ovalbumin to the extraction buffer eliminated false positives.

Table 2. Distribution of virus detectable by enzyme-linked immunosorbent assay in sweet potato leaf samples collected 8 wk after emergence

	Absorbance A_{405}			Positive samples[a] (no.)
	Mean	SD	Range	
Leaf A[b]				
Jewel	0.022	0.006	0.016–0.030	0
Porto Rico	0.051	0.047	0.019–0.138	2
Leaf B				
Jewel	0.194	0.258	0.015–0.663	4
Porto Rico	0.316	0.554	0.020–1.427	3
Leaf C				
Jewel	0.423	0.839	0.019–2.131	4
Porto Rico	0.595	0.446	0.019–1.117	5
Leaf D				
Jewel	0.242	0.252	0.018–0.634	4
Porto Rico	0.581	0.534	0.029–1.399	5

[a] Samples with absorbance values three standard deviations above the mean of the healthy control samples ($\bar{x} + 3$ SD = 0.031), from a total of six samples tested.
[b] Expanded leaves equally distributed between the distal and proximal end of the shoot designated A–D: A, young, expanding leaf near the distal end of the shoot; D, the oldest leaf at the proximal end of the shoot. Grafts from all leaf positions were positive.

infected plant using ELISA. Composite samples were assayed from three shoots from each of 10 infected plants. Assay of a single shoot per plant resulted in 80% detection, whereas assay of two or three shoots per plant resulted in 100% detection of the infected plants.

DISCUSSION

The basic ELISA protocol (8) was similar to that used by others for detection of viruses in plant extracts. The addition of DIECA and ovalbumin to the virus buffer (1,10,12) prevented non-specific reactions observed during preliminary experiments. The narrow strain specificity of the double-antibody sandwich method of ELISA for other rod-shaped viruses such as TMV has been cited as a disadvantage of the method for indexing (15). However, the strains of FMV investigated here and by others (4) reacted similarly in heterologous and homologous combinations, as did citrus tristeza (3).

Sweet potatoes are vegetatively propagated, and viruses are widespread in commercial production (13). Limited indexing programs have relied on infectivity assays by grafting. Shoots produced from roots are transplanted to fields for approximately 120 days, at which time the tuberous roots are harvested; 10% of the roots are retained for propagation of the succeeding crop. The shoots produced from 1,000–2,000 kg of roots are required for each acre of sweet potato production. Thus, a practical virus-indexing program for sweet potatoes will require a relatively efficient assay system such as ELISA.

The variability in FMV concentration in sweet potato shoots has been well documented (4,13). A previous study (4) and this one have demonstrated that FMV is often present below the limits of detection by ELISA. However, it is proposed that FMV-infected, field-grown sweet potato plants can be reliably detected by proper timing of the assay. The virus was most easily detected in shoots 5–8 wk following emergence, which is about the time of transplanting. Sampling three leaves from each of two or three shoots produced by each plant gave 100% detection of infection. Each plant (ie, all shoots produced from a single root) yields 10–20 shoots on the first harvest of transplants and a lesser number on successive harvests. Three samples assayed can therefore be representative of 30 or more transplants, making this an efficient method of indexing.

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LITERATURE CITED

1. Adams, A. N. 1978. The detection of plum pox virus in *Prunus* species by enzyme-linked immunosorbent assay (ELISA). Ann. Appl. Biol. 90:215-221.
2. Alconero, R. 1973. Mechanical transmission of viruses from sweet potato. Phytopathology 63:377-380.
3. Bar-Joseph, M., Garnsey, S. M., Gonsalves, D., Moscovitz, M., Purciful, D. E., Clark, M. F., and Loebenstein, G. 1979. The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. Phytopathology 69:190-194.
4. Cadena-Hinojosa, M. A., and Campbell, R. N. 1981. Serologic detection of feathery mottle virus strains in sweet potatoes and *Ipomoea incarnata*. Plant Dis. 65:412-414.
5. Cadena-Hinojosa, M. A., and Campbell, R. N. 1981. Characterization of isolates of four aphid-transmitted sweet potato viruses. Phytopathology 71:1086-1089.
6. Cali, B. B., and Moyer, J. W. 1981. Purification, serology, and particle morphology of two russet crack strains of sweet potato feathery mottle virus. Phytopathology 71:302-305.
7. Campbell, R. N., Hall, D. H., and Mielinis, N. M. 1974. Etiology of sweet potato russet crack disease. Phytopathology 64:210-218.
8. Clark, M. V., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
9. Covington, H. M. 1976. Growing and marketing quality sweet potatoes. N.C. Agric. Ext. Serv. Publ. AG-09.
10. Flegg, C. L., and Clark, M. F. 1979. The detection of apple chlorotic leafspot virus by a modified procedure of enzyme-linked immunosorbent assay (ELISA). Ann. Appl. Biol. 91:61-65.
11. Hastings, R. C., Kucera, H. L., and Tool, E. 1971. North Dakota State University Leaf "Squeezer." Am. Potato J. 48:33-34.
12. McLaughlin, M. R., and Barnett, O. W. 1979. The influence of plant sap and antigen buffer additives on the enzyme-immunoassay of two plant viruses. (Abstr.) Phytopathology 69:1038.
13. Moyer, J. W., Cali, B. B., Kennedy, G. G., and Abou-Ghadir, M. F. 1980. Identification of two sweet potato feathery mottle virus strains found in North Carolina. Plant Dis. 64:762-764.
14. Moyer, J. W., and Kennedy, G. G. 1978. Purification and properties of sweet potato feathery mottle virus. Phytopathology 68:998-1004.
15. van Regenmortel, M. H. V., and Burckard, J. 1980. Detection of a wide spectrum of tobacco mosaic virus strains by indirect enzyme-linked immunosorbent assays (ELISA). Virology 106:327-334.