# COMPARISON OF METHODS TO DETECT SWEET POTATO FEATHERY MOTTLE VIRUS (SPFMV) IN SWEET POTATOES

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Sweet potato (*Ipomoea batatas* L.), is one of the most important tuber crops worldwide. It has a diverse range of positive characteristics including high yield per unit area, nutritional value, and resistance to several production stresses (Kays, 2005). However, yield of sweet potato is affected by a number of factors including infection by several pathogens (Clark and Moyer, 1988; Moyer and Salazar, 1989). One of these is the sweet potato feathery mottle virus (SPFMV), a major component of the sweet potato virus disease (SPVD; Gibson *et al*., 1998; Gibson and Aritua, 2000) combined with the sweet potato chlorotic stunt virus (SPCSV; Brunt *et al*., 1996). SPFMV has long been recognized to occur wherever sweet potatoes are grown. Although SPFMV may not represent a serious problem individually, it is devastating in SPVD due to the synergistic effect of the viruses, causing significant yield reduction in susceptible varieties (Hanh, 1979).

SPFMV is a type member of the potyviruses. It is transmitted by several genera of aphids including the cotton aphid (*Aphis gossypii*) and the green peach aphid (*Myzus persicae*) in a non-persistent manner (Brunt *et al*., 1996). Symptoms vary according to the cultivated variety and environmental factors that often makes identification of infected plants difficult (Brunt *et al*., 1990). To control spread of virus infection in fields, it is necessary to use sensitive and reliable diagnosis methods for virus detection. Assays should be able to detect viral presence and concentration in different plant organs (Lepoivre *et al*., 1994). Consequently, serological methods are widely used in the diagnosis of viral diseases (Portsmann and Kiessig, 1992). Several other methods have also been applied to detect SPFMV in infected plant materials. These included membrane immunoblotting (Dje and Diallo, 2005), reverse transcription polymerase chain reaction, (RT-PCR; Fenby *et al*., 1998) and nucleic acid spot hybridisation (NASH; Junko *et al*., 2000).

Here, four methods were compared and evaluated for their capacity and reliability to detect SPFMV in infected sweet potato plants. These included immunological and nucleic acid

procedures such as the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), direct ELISA, dot-ELISA on positively charged Nylon membranes, and RT-PCR.

## MATERIALS AND METHODS

An Egyptian isolate of sweet potato feathery mottle virus (SPFMV) was isolated from sweet potato (*Ipomoea batatas* var. Abees) at El Noubaria, Egypt. The virus was identified by mechanical inoculation on the indicator plants *Ipomoea setosa* and *Ipomoea nil* (El Far, unpublished data). Sweet potato plants positive for virus infection were kept under insect free conditions as the source of viral infected plant material for this study.

### *Double Sandwich Antibody ELISA (DAS-ELISA)*

DAS-ELISA was performed as described by Clark and Adams (1977) using a rabbit polyclonal antibody raised against SPFMV (kindly provided by the International Potato Centre, CIP, Peru) and a polyclonal alkaline phosphatase-labelled goat anti rabbit as secondary antibodies (Sigma, USA). Results were documented using Bio-Rad microplate reader 3550 at 405 nm.

### *Direct ELISA*

Healthy and SPFMV-Infected sweet potato leaf tissues were extracted in 10 volumes phosphate buffer saline, PBS, (173 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4$-$7H_2O$, 1.4 mM $KH_2PO_4$, pH 7.4) supplemented with 0.01% Tween-20 or in 15 mM sodium carbonate-bicarbonate buffer pH 9.6. Aliquots of 200 µl tissue extracts were loaded into each well of the ELISA plate. Plates were placed in plastic bags and incubated at 37°C for 2 hours. After washing three times, 3 minutes each, with PBS supplemented with 0.05% Tween 20, 200 µl of 5% bovine serum albumin in PBS was added to each well and incubated at 37°C for 1 hour. After washing as above, 200 µl SPFMV antibodies diluted in PBS-T according to the recommended dilution by CIP were added and plates incubated overnight at 4°C. After washing as above, 200 µl of the polyclonal alkaline phosphatase-labelled goat anti rabbit antibody (Sigma, USA) diluted in PBS buffer supplemented with 0.01% Tween-20 as recommended by the manufacturer were added. Plates were incubated at 37°C for 4 hours. After washing as above, 200 µl of substrate (3mM ρ-nitrophenyl phosphate, Sigma, USA, in 1 M diethanolamine substrate buffer pH 9.8, Sigma USA,) was added. Results were documented using Bio-Rad microplate reader 3550 at 405 nm 15 minutes post incubation with substrate.

### *Dot ELISA*

The method described by Gultierrez *et al*. (2003) was used for the dot ELISA with a modification of replacing the nitrocellulose membrane by the positively charged membrane as a supporting material for the samples as follows:

Healthy and SPFMV-Infected sweet potato leaf tissues were ground in a mortar in 10 volumes of Tris buffer saline, TBS, (20 mM Tris-Cl pH 8.0, 150 mM NaCl) supplemented with 0.01% Tween 20. After grinding, samples were clarified by centrifugation at 14,000 rpm in microfuge tubes at 4°C for 10 minutes. An aliquot of 10 µl was spotted on positively charged Nylon membranes (Roche, USA). Membranes were blocked for 1 hour at room temperature in TBS-T (TBS and 0.05% Tween 20) supplemented with 5% non-fat dry milk. After blocking, SPFMV antibodies were added to the blocking buffer according to the recommended dilution by CIP. Membranes were further incubated overnight at 4°C. Membranes were washed 3 times 10 minutes each at room temperature in an elevated volume of TBS-T. Membranes were incubated for one hour at room tempreture with the alkaline phosphatase-labelled goat anti rabbit antibodies (Sigma, USA) diluted in blocking buffer following the manufacturer's instructions. Filters were washed as described above. A final wash for 10 minutes was carried out using the NIP/BCIP substrate buffer (100 mM Tris-HCl pH 9.5; 100 mM NaCl and 5 mM MgCl2). Signals were visualised by incubating the filter in 10 ml substrate buffer containing 66µl of 100 mg/ml stock solution p-nitro blue trizolium, NBT, in dimethylformamide and 35 µl of 100 mg/ml stock solution 5-bromo-4-chloro-3-indolyl phosphate-toluidine, BCIP, in dimethylformamide for 15-60 minutes in the dark. The filters were washed with distilled water and photographed.

***RT-PCR***

Total RNA was extracted from healthy and SPFMV-infected sweet potato plants using Tri-Pure kit (Roche, USA) according to the manufacturer's standard protocol. One µg of RNA was denatured at 70°C for 5 minutes in the presence of 100 pmol of primer P-SPFMV-R (5'-AAGAGGTTATGTATATTTCTAGTAA-3'). Samples were chilled on ice for 2 minutes. RNA was reverse-transcribed for 1 hour at 42°C in the presence of 1 × reverse transcriptase buffer, 20 mM dNTPS, and 200 U of M-MuLV reverse transcriptase (Promega, USA). After incubation, PCR was carried out using 5 µl of cDNA in 1 × PCR buffer, 10 pmol of primer P-SPFMV-R, 10 pmol of primer P-SPFMV-F (5'-CTTCAGTGACGTTGCTGAAGC-3'), 1.5 mM $MgCl_2$, 10 mM dNTPs, and 2 units of *Taq* DNA polymerase (Fermentas, USA). Samples were subjected to 30 cycles of PCR with 15 seconds of denaturing at 94°C, 15 seconds of annealing at 60°C, and 30 seconds of extension at 72°C. The series of cycles was preceded by 3 minutes initial denaturing at 94°C. Five µl of the PCR products were analysed on a 1% agarose gel in TAE buffer, visualized by ethidium bromide staining, and photographed (Ausubel *et al*., 1995).

## RESULTS AND DISCUSSION

SPFMV is one of the common viruses affecting the production of sweet

potatoes worldwide. Symptoms of virus infection in cultivated varieties of sweet potato are phenotypically difficult to observe, reflecting the problem to diagnose the viral-infected plants in cultivated sweet potato fields. The availability of a reliable system to detect the virus in the field is therefore mandatory to improve the productivity of sweet potato crop. To accomplish this task, different methods were implemented and their efficacy to distinguish between healthy and infected plant material were compared to find the most suitable method for this objective**.** These assays included the standard DAS-ELISA, direct ELISA, dot-ELISA as serological methods and RT-PCR as molecular based method. When the standard DAS-ELISA (Clark and Adams, 1977) technique was used to distinguish between healthy and SPFMV-infected sweet potato plants, results indicated that differences between values of healthy samples readings (0.411) and infected samples readings (0.473) were not sufficient to distinguish between healthy and infected samples (Table 1). Whereas, modifications by applying the samples directly to the plates as in direct ELISA followed by washing steps improved the efficiency of the method and resulted in clear cut distinguishing between healthy and infected samples (Table 2). Infected sample readings (1.399) were approximately 3 times higher than the healthy sample readings (0.468) when sodium carbonate-bicarbonate buffer pH 9.6 was used as sample extraction buffer and infected samples readings (1.015) were about 2.5 times as high as the healthy sample readings (0.410) when PBS pH 7.4 was used as sample extraction buffer. Results obtained from the dot-ELISA also indicated a desirable level of efficiency to distinguish between healthy and SPFMV-infected sweet potato plants (Fig. 1). Since the viral protein is attached directly to the supporting material during the washing and blocking steps, removal of inhibitory factors present in the sweet potato extracts is improved, resulting in better specific binding of the virus to the antibodies against SPFMV. In this study, nitrocellulose membranes were replaced by positively charged membranes because they are relatively easy to handle.

With regard to the results obtained from DAS-ELISA, direct ELISA and dot-ELISA carried out on the same healthy and SPFMV-infected samples for viral detection with a serological back ground, Cadena-Hinjosa and Campbell (1981) described the loss of sensitivity of SPFMV detection by DAS-ELISA to the low concentration of virus and its irregular distribution in the sweet potato plants. However, by comparing DAS-ELISA and direct ELISA results obtained from this study, we could exclude that the low concentration of the virus in infected tissues is responsible for loss of sensitivity of DAS-ELISA to detect the virus, in addition to the presence of several inhibitory factors such as phenols, latex, and other inhibitors present in the sweet potato extracts which are responsible for interfering with specific binding between the virus and its specific antiserum thus resulting in not being able to distinguish

between healthy and viral infected plant material (Aritua *et al*., 2005). Meanwhile, with direct ELISA and dot ELISA, the interaction between antibodies and virus present in the infected samples is carried out post to the removal of sweet potato inhibitors by washing processes and prior to the addition of antibodies, resulting in efficient binding between viral particles and their specific antibodies and improving the efficiency to detect the virus in the infected samples. The dot ELISA procedure is preferred for the routine detection of the virus in the fields of sweet potato because it is simple and low cost (Salazar and Fuentes, 2000). However, the implementation of direct ELISA for viral detection has an advantage in possible estimation of viral concentration since it is a method for quantitative evaluation while dot-ELISA results are only qualitatively evaluated and can not estimate viral concentration in values.

RT-PCR was very efficient in distinguishing between healthy and SPFMV-infected plants. Primers used in this study were specifically designed based on the conserved regions present in all viral strains obtained from the NCBI database. Table (3) shows the accession number and the geographical allocations of the viral isolates compared in this study. The advantage of utilizing these specific primers lies in that they are universal primers that can detect any SPFMV-infected sweet potato material regardless of the viral isolate. The resulting amplification product is 300 nucleotides (Fig. 3) and present only in infected plants. There were no additional dominant bands detected in either the healthy or infected plant materials (Fig. 2). The Try Pure kit resulted in extracting sufficient quantities and satisfactory qualities of total RNA when used as an extraction method. Several RNA extraction kits were also applied to remove inhibitors from RNA preparations of sweet potato plants (Fenby *et al*., 1998). On the other hand, the Try Pure extraction kit is less sensitive to amounts of the starting material since it is a solution based method which makes it easier to handle more than the column based methods to extract RNA. Unfortunately, RT-PCR is an expensive process and considered laborious since it includes an RNA extraction step, which does not make it the method of choice in the routine detection of the virus in sweet potato fields. However, because of its high sensitivity, RT-PCR is recommended to be used as a method for detection of the viral infection in the parental mother plants that will be used to distribute healthy cuttings to farmers for the further cultivation of sweet potato or to detect viruses in plant materials following thermotherapy treatments.

## SUMMARY

Sensitive analytic techniques including double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA), direct ELISA, dot ELISA and reverse transcription polymerase

chain reaction (RT-PCR) were compared and evaluated for their capability to reliably distinguish between healthy and sweet potato feathery mottle virus (SPFMV) infected sweet potato plants. Here we show that DAS-ELISA is not adequate for the detection of SPFMV since almost identical ELISA values were obtained for healthy and infected plants. In contrast, an accurate and reliable detection of SPFMV could be performed by either direct or dot ELISA with quantitatively and qualitatively clearly distinguishable values (direct ELISA) or signals (dot ELISA) for infected and non-infected material. Similar results were observed with RT-PCR as a non-immunological method. However, this method is quite laborious and expensive and therefore, not recommendable for the routine detection of SPFMV in cultivated sweet potato fields.

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## REFERENCES

Aritua, V., R. Gibson, and J. Vetten (2005). Serological analysis of sweetpotatoes affected by sweet-potato virus disease in east Africa. In: Whitefly and Whitefly-borne Viruses in the Tropics: Building a knowledge base for global action. Anderson, P.K. and Morales, F.J with the collaboration of Jones, A.L and. Markham, R.H (Eds.), p. 83-88. Cali, Colombia.

Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. D. Seidman, J. A. Smith and K. Struhl (Edt.) (1995). Current Protocols in Molecular Biology. (New York: John Wiley and Sons).

Brunt, A., K. Crabtree and A. Gibbs (Eds.). (1990). Viruses of tropical plants: Descriptions and lists from the VIDE database. CAB International, Wallingford, UK.

Brunt, A. A., K. Crabtree, M. J. Dallwitz, A. J. Gibbs, L. Watson and E. J. Zurcher (Eds.) (1996). Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version: 20th August 1996.' URL http://biology.anu.edu.au/Groups/MES/vide/.

Cadena-Hinojosa, M. A. and R. N. Campbell (1981). Serological detection of feathery mottle virus strains in sweetpotatoes and Ipomoea incarnate. Plant Diseases, 65: 412-414.

Clark, M. F. and A. N. Adams (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology, 34: 475-483.

Clark, C. A. and J. W. Moyer (1988). Compendium of Sweet Potato Diseases. APS Press, St. Paul, pp. 74.

Dje, Y. and H. A. Diallo (2005). Detection and distribution of sweetpotato feathery mottle virus in sweetpotato using membrane immunobinding assay. African Journal of Biotechnology, 4: 717-723.

Fenby, N. S., S. Seal, R. W. Gibson and G. D. Foster (1998). RNA extraction from virus-diseased sweet potato for reverse transcriptase polymerase chain reaction analysis. Molecular Biotechnology, 10: 187-190.

Gibson, R. W., I. Mpembe, T. Alicai, E. E. Carey, R. O. M. Mwanga, S. E. Seal and H. J. Vetten (1998). Symptoms, aetiology and serological analysis of sweet potato virus disease in Uganda. Plant Pathology, 47: 95-102.

Gibson, R. W. and V. Aritua (2000). Sweetpotato virus disease in Africa. African Potato Conference Proceeding, 5: 373-377.

Gutierrez D. L., S. Fuentes and L. F. Salazar (2003). Sweetpotato virus disease (SPVD): Distribution, incidence, and effect on sweetpotato yield in Peru. Plant Diseases, 87: 297- 302.

Hanh, S. K. (1979). Effects of viruses (SPVD) on growth and yield of sweet potato. Experimental Agriculture, 15: 253-256.

Junko, A. J., A. Watanabe and K. N. Watanabe (2000). Application of NASH (Nucleic Acid Spot Hybridization) in Diagnosis of SPFMV Infection to Sweet-potato Genetic Resources. p. 99-101. In: Nakazawa, Y., and K. Ishiguro (Eds). International Workshop on Sweetpotato Cultivar Decline Study, Proceedings, Kyushu National Agricultural Experiment Station, Miyakonjo, Japan.

Kays, S. J. (2005). Sweetpotato production worldwide: assessment, trends and the future. Acta Horticulturae, 670: 19-25.

Lepoivre, P., J. Kummert, D. Colinet, O. Duterme and C. Anceau (1994). Tech. moléculaires de détection et d'identification des agents pathogènes. Cahiers Agric., 3: 217-225.

Moyer, J. W. and F. L. Salazar (1989). Virus and virus-like diseases of sweet potato. Plant Diseases, 73: 451-455.

Porstmann, T. and S. T. Kiessig (1992). Enzyme immunoassays techniques. An overview. Journal of Immunological Methods, 150: 5-21.

Salazar, L. F. and S. Fuentes (2000).

Current knowledge on major virus diseases of sweetpotatoes. p 14-19 in: Nakazawa, Y., and Ishiguro, K., (Eds). International Workshop on Sweetpotato Cultivar Decline Study, Proceedings, Kyushu National Agricultural Experiment Station, Miyakonjo, Japan.

Table (1): DAS-ELISA values of healthy and SPFMV-infected sweet potato samples obtained after 15 minutes of incubation with the substrate. The average of all individual values is indicated by an asterisk.

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Healthy | 0.420 | 0.406 | 0.424 | 0.439 | 0.424 | 0.377 | 0.403 | 0.395 | 0.411* |
| Infected | 0.473 | 0.492 | 0.468 | 0.451 | 0.451 | 0.480 | 0.481 | 0.488 | 0.473* |

* Significant at 5%

Table (2): Direct-ELISA values of healthy and SPFMV-infected sweet potato samples obtained 30 after minutes of incubation with the substrate. The average of all individual values is indicated by an asterisk.

| Healthy in Na-Carbonate-Bicarbonate buffer | Infected in Na-Carbonate-Bicarbonate buffer | Healthy in PBS+0.1% Tween-20 | Infected in PBS+0.1% Tween-20 |
|---|---|---|---|
| 0.417 | 1.397 | 0.403 | 0.978 |
| 0.498 | 1.390 | 0.420 | 1.000 |
| 0.497 | 1.372 | 0.411 | 1.085 |
| 0.474 | 1.382 | 0.410 | 1.022 |
| 0.482 | 1.404 | 0.436 | 1.094 |
| 0.444 | 1.403 | 0.428 | 1.014 |
| 0.469 | 1.405 | 0.392 | 1.004 |
| 0.468 | 1.444 | 0.381 | 0.928 |
| 0.468* | 1.399* | 0.410* | 1.015* |

* Significant at 5%

Table (3): Accession numbers of SPFMV strains in the NCBI database. Alignment of all sequences was performed for optimal RT-PCR primer design.

| Accession Number | SPFMV Isolates and Strains |
|---|---|
| AF015540 | Korean strain 1 |
| AF015541 | Korean strain 2 |
| AF016366 | Zimbabwe |
| AF439637 | United States |
| AF439638 | United States |
| AY459591 | Kenya: Kakamega strain C |
| AY459592 | Kenya: Kakamega strain EA |
| AY459593 | Kenya: Kisii strain EA |
| AY459594 | Kenya: Kisumu strain C |
| AY459595 | Uganda: Arua strain O |
| AY459596 | Uganda: Namulonge strain C |
| AY459597 | Madagascar strain EA |
| AY459598 | Tanzania strain RC |
| AY459599 | Portugal strain EA |
| AY459600 | Spain, Canary Islands strain EA |
| AY459601 | South Africa strain C |
| AY459602 | China strain RC |
| D16664 | ordinary strain, SPFMV-O |
| D38543 | Strain Severe |
| S43450 | Strain RC |
| S43451 | Strain C |
| S69825 | China isolate CH |

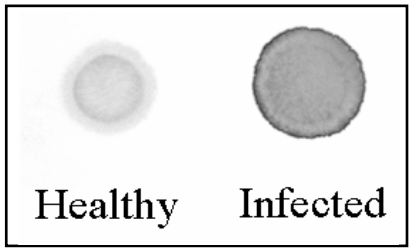


Fig. (1): Dot-ELISA of healthy and SPFMV-infected sweet potato plants.

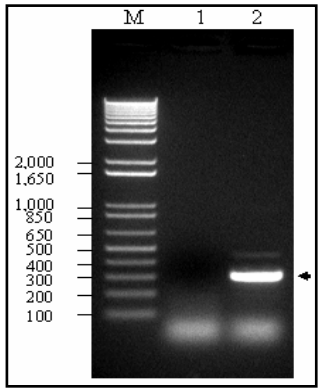


Fig. (2): Detection of SPFMV by RT-PCR. Healthy (1) and SPFMV-infected (2) sweet potato plants. (M) Molecular weight marker. PCR product is indicated by an arrow (MW 300 nt).