EVALUATION OF FILTER PAPER SERO-ASSAY (FIPSA) TO DETECT THREE VIRUSES

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Introduction

The application of serological detection methods for the plant viruses requires the development of rapid, simple and sensitive immunoassays that employ the specific relationship between an antigen and its homologous antibody. The adaptation and improvement of rapid immunofilter assay has steadily been developed and could easily be used for virus diagnosis with considerable high sensitivity. The filter paper sero-assay (FiPSA) protocol is analogous to the indirect ELISA (dot-immunobinding assay) of Hawkes et al. (1982) that utilised filter paper instead of nitrocellulose membrane. Haber and Knapen (1989) had demonstrated the usefulness of the modified technique for the detection of several viruses. In this project, the sensitivity of FiPSA is examined, tested and compared in three different plant viruses; *Clitoria* yellow vein virus (CYVV), *Hibiscus* chlorotic ringspot virus (HCRV) and tomato mosaic virus (ToMV).

Materials and Methods

The CYVV, HCRV and ToMV isolates and the antisera were as described by Abdul-Samad (1994), Abdul-Samad and Mat (1995) and Abdul-Samad et al. (1996), respectively.

Whatman No. 1 filter paper disks (7 mm diameter) were heated at 150° C for 1 hr and stored in the dessicator until used. Albumin from rabbit normal serum was used as blocking solution. The dried disks were soaked for 15 min in the blocking solution, suction-dried and stored in the dessicator.

The purified viruses were diluted to a series of dilution in Tris-buffered saline (TBS). Leaf extract from infected plants were prepared simply as crude plant materials which were free of particulate matter and a serial dilution was prepared in spotting buffer. Two microlitres of sample were spotted in the centre of each disk and transferred to individual cylindrical vial and dried at 40° C for 10 min. Antisera were diluted 75 x in TBS; normal serum diluted similarly as control. Three hundred microlitres of each serum was applied to the disk and shaken gently for 60 min after which the serum was aspirated from the vial. The disks were washed in 1 ml of TBS for 10 min with shaking and aspirated. The disk was then immersed in 300 µl protein A-peroxidase at 2 µg/ml in TBS and incubated with shaking (60 rpm) for 30 min. The disks were removed and washed again as above. They were incubated in the substrate [5 parts TBS/1 part 4-chloro-1-napthol (3 mg/ml in methanol)/0.018 parts 3% hydrogen peroxide] for 1 min in the dark in a volume just sufficient to ensure immersion. They were then separated from the substrate and immersed in water for 10 min for further colour development and air-dried at 40° C.

Results and Discussion

Positive reactions appeared as violet-blue centres against white or light backgrounds. The green colour sap on the spots did not interfere with the results. False positives were controlled by using antigen-free samples, as well as by incubating duplicates of samples with normal or non-specific serum. From the results obtained, the use of blocking solution to preblock the disks was an important factor in increasing the sensitivity of detection to reduce nonspecific binding to acceptable amounts (Haber and Knapen, 1989).

FiPSA was sufficiently sensitive to detect purified viruses and sap extracts of CYVV, HCRV and ToMV present in low concentration. For CYVV, the colour intensity could be detected up to 3.0 ng/ml for purified virus and 1:10,000 dilution for sap extract; for HCRV, 15 ng/ml for purified virus and 1:4,800 dilution for sap extract; and for ToMV, up to 5.0 ng/ml and 1:8,000 dilution for purified virus and sap extract, respectively by using a densitometer. However, by using a quick visual detection the values were 50 ng/ml and 1:6,000, 130 ng/ml and 1:1,200 and 90 ng/ml and 1:3,500 for purified and sap extract of CYVV, HCRV and ToMV, respectively.

Conclusions

Ultimately, it is hoped that this rapid immunoassay will be used as a tool for quick virus detection rather than for accurate quantification.

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