Serological Diagnostic of African Cassava Mosaic by Immuno-Enzymatic Method

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ABSTRACT

African cassava mosaic disease infects more than 95% of cassava plants in the Ivory Coast. The suspected causal agent, cassava latent virus, is a geminivirus. It was purified from systematically infected Nicotiana benthamiana. An antiserum was prepared by injecting rabbits with the purified virus.

Testing of cassava plants by classical serological methods, such as microprecipitin and gel-diffusion precipitin tests, was not possible because the virus level in cassava leaves is very low. It was possible, however, to detect virus in cassava using enzyme-linked immunosorbent assay (ELISA).

Cassava mosaic disease is a major pest of cassava (Manihot esculenta Crantz) in Africa. Most cassava cultivars show severe symptoms in the coastal areas of Ivory Coast, although healthy plants are still found in the middle and the northern parts of the country.

The causal agent is presumed to be a virus, first isolated in East Africa: cassava latent virus (CLV, Bock et al., 1978). The same pathogen was characterized in the Ivory Coast (Walter, 1980) and in Nigeria (Adejare and Coutts, 1982). For all virologists, there is circumstantial evidence that CLV plays a role in the etiology of cassava mosaic disease.

Reported here are trials for serological detection of CLV in cassava plants.

Materials and Methods

Experiments used original isolate of CLV described by Walter (1980) and propagated on Nicotiana benthamiana in insect-proof glasshouses. CLV was purified according the procedure of Walter (1980).

Antiserum to CLV was prepared by injecting two rabbits once a week intramuscularly (using Freund's incomplete adjuvant) during 6 weeks. The antiserum obtained had a reaction end-point of 1/256 in double-diffusion precipitin test with purified CLV. It did not react with sap from virus-free N. benthamiana at a dilution of 1/8.

The method used for enzyme-linked immunosorbent assay (ELISA) was described by Clark and Adams (1977). Globulins are purified from antiserum, and a sample is

conjugated to alkaline phosphatase. Coating globulin was used at 10 ug/ml and enzyme-conjugated globulin was diluted 1/400.

Samples to be tested were prepared by grinding leaves with an Ultra-Turax, in phosphate buffered saline (PBS) pH 7.4; containing 0.05% Tween 20, 2% polyvinyl pyrrolidone and 0.2% ovalbumin.

Absorbance at 405 nm was measured by means of a spectrophotometer Titertek Multiscan.

Results and Discussion

Detection of virus in leaf extracts

By using double-diffusion precipitin tests, it was hazardous to detect CLV in diseased N. benthamiana and it was not possible to obtain positive reaction with diseased cassava, probably because virus concentration in the plant was too low.

In N. benthamiana and cassava, CLV was always detectable by ELISA. The reaction was significantly distinguishable from that of healthy samples at a dilution of 1/2,048 for N. benthamiana extracts, and at a dilution of 1/256 for cassava extracts, using leaves from the apex.

For N. benthamiana, absorbance at 405 nm was regularly decreasing according to the dilution of the extract for cassava extracts, the maximum of absorbance was found at a dilution between 1/12 and 1/16 (g/ml). There were probably inhibitors of the reaction in the cassava extracts. The same type of interference is reported by Sequeira and Harrison (1982). This inhibitory effect of cassava can be removed by treating leaf extracts with organic solvent such as chloroform.

Detection of CLV in mature cassava leaves according to the severity of symptoms

Symptoms on cassava leaves were estimated by Cours (1951) with a scale from 0 to 5. According to this notation, leaves of diseased cassava were collected either from different plants or from the same plant in the field and grouped according to the strength of the symptoms. Six leaves of each category of the scale were tested by ELISA. Positive results were obtained only with categories 3, 4 and 5 (usually younger leaves), namely only with severely infected leaves, suggesting that virus content in leaves noted 1 to 2 (usually older leaves) was very low. Serological test thus seems less sensitive than direct observation of symptoms for diagnosis in the field.

CLV could also be detected from infected leaves of Manihot glaziovii.

ELISA represents a good method for detecting natural hosts of CLV in wild plants; either directly after sample collection in the fields or as a means of control after mechanical inoculation on N. benthamiana.

It appears therefore that an increase of the titre of the antiserum would increase the sensitivity of the test. Sequeira and Harrison (1982) with a different method of purification obtained good yields of CLV and they have prepared antiserum with a titre of 1/500. We are now working to produce an antiserum more specific with a higher titre.

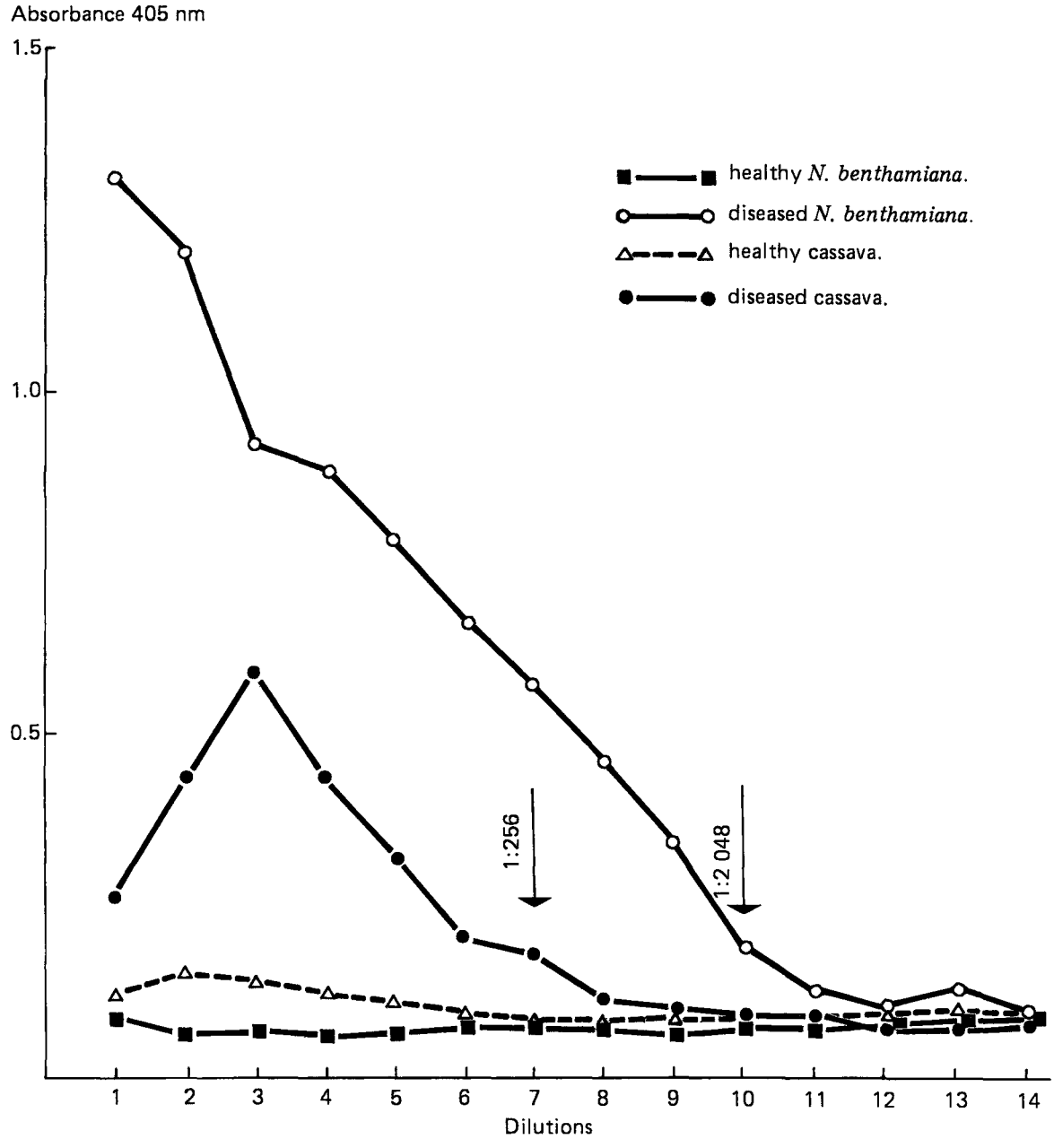

Figure 1. Detectability of cassava latent virus by ELISA in leaf extracts from Nicotiana benthamiana and cassava.

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