Received December the 9th, 1981

Detection of CM 112 Latent Grapevine Virus by the Passive Haemagglutination Test *(PHT)*

by

AMARILIS DE VARENNES

Assistant of Agricultural Chemistry

and

ANA CARLA MADEIRA

Assistant of Agricultural Mesology and Meteorology

RESUMO

A Técnica de Hemaglutinação Passiva (PHT) foi utilizada para detectar o Vírus CM 112 da Videira directamente de folhas infectadas de videira (*Vitis vinifera* L. cv Borraçal).

Determinaram-se as condições de extracção, volumes de amostra, concentração de eritrócitos e de γ-globulina. Foram detectados 25ng.ml^{-1} de vírus, em preparações purificadas diluídas em tampão, e 50ng.ml^{-1} em presença de extractos brutos de videira sã.

SYNOPSIS

Passive Haemagglutination Test (PHT) was used to detect CM 112 Latent Grapevine Virus directly from grapevine (*Vitis vinifera* L. cv Borraçal) infected leaves. Extraction conditions, sample volumes, erythrocytes and γ-globulin concentrations were established. The test was able to detect 25ng.ml^{-1} of virus, from purified preparations diluted in buffer, and 50ng.ml^{-1} when healthy grapevine crude extracts were present.

INTRODUCTION

Madeira (1978) and Madeira & Sequeira (1980) used the Passive Haemagglutination Test (PHT) to detect CM 112 Latent Grapevine Virus from herbaceous hosts and found it had a sensitivity 30 times and 800 times greater than the Latex and the Bentonite Flocculation tests, respectively.

In this paper the results of experiments made in order to detect CM 112 virus directly from grapevine (*Vitis vinifera* L.) infected leaves by PHT, are described.

MATERIAL AND METHODS

The infected grapevine (*V. vinifera* cv Borraçal) plants and the CM 112 rabbit antiserum were the same used by Varennes & Sequeira (1980).

The purification of CM 112 virus was performed according to Ferreira (1970). Dilutions were made from purified preparations previously adjusted to A260 = 1.0.

Sheep erythrocytes were obtained from a slaughterhouse and were sensitized as described by Abu Salih, Murant & Daft (1968), after treatment with formalin according to Madeira & Sequeira (1980). Coated erythrocytes were kept frozen at – 20°C and used whenever required. The concentration of erythrocytes was evaluated using a Neubauer chamber.

The test was performed in microtitre U-form plates (Cooke). In all the plates, controls made from healthy material were included (buffer, or extracts made from healthy *V. vinifera* cv Borraçal — red variety — and/or cv Vital — white variety). Infected and healthy material with unsensitized erythrocytes was also included as control.

RESULTS AND DISCUSSION

Quantity of antibody used to sensitize the erythrocytes

Best results were obtained when tanned erythrocytes were sensitized with 2mg of anti-CM 112 γ-globulin. Lower sensitivities were obtained when 1 or 3mg of γ-globulin were used.

Preparation of the test samples

Extracts from grapevine gave raise to non-specific reactions strong enough to mask those due to the virus. To reduce these non-specific reactions, several reagents were added to the extraction buffer (phosphate-buffered saline, 0.02M, pH 7.3 — PBS)): polyvinyl pyrrolidone at 2.0% (w/v) plus egg albumin at 0.2% (w/v); nicotine at 0.5, 1.0 and 2.5% (v/v); aluminium oxide at 2.5, 5.0 and 10% (w/v); nicotine 2.5% (v/v) plus aluminium oxide at 10% (w/v); 2-mercaptoethanol 0.02M; sodium diethyldithiocarbamate 0.02M; ascorbic acid 0.2% (w/v); caffeine 0.4% (w/v); sodium thioglycollate 0.02M; activated charcoal 0.5, 1.0 and 2.5% (w/v).

Extracts were prepared 1:25 or 1:50 in the above-mentioned buffer plus additives.

Best results were obtained with a 1:25 extraction in nicotine 2.5% plus aluminium oxide 10%.

Before testing, samples had to be further diluted. Dilutions tested were 1:1 and 1:3 in PBS plus one of the following additives: bovine serum albumin 0.5, 1.0 or 2.0% (w/v); normal sheep serum 0.5, 1.0 or 2.0% (v/v); normal rabbit serum 0.5, 1.0 or 2.0% (v/v).

Best results were obtained with a 1:3 dilution in normal rabbit serum. Results obtained with 1.0 and 2.0% normal rabbit serum concentrations did not show any improvement on sensitivity when compared with the 0.5% concentration. Therefore, the 0.5% concentration was adopted in this work. The same concentration was used by Saito & Iwata (1964).

Volume of samples

Madeira & Sequeira (1980) used 0.2ml of sample in this test. Smaller volumes of samples were tried in order to spare erythrocytes which are of time consuming elaboration. Volumes tested were 0.20, 0,15, 0.10, 0.090 and 0.075ml.

The 0.075ml sample volume yielded lower sensitivity, being the 0.090ml volume sufficient to obtain the same sensitivity when compared with higher sample volumes.

Concentration of coupled erythrocytes

Test negative controls were not sharply defined when using erythrocytes concentrations below a certain value. Twenty five µl of

erythrocytes were tested in the concentrations of 2×10^9, 3×10^9, 5×10^9, 7×10^9, and 8×10^9 cells per mililiter.

The concentration of 7×10^9 cell per ml was sufficient to obtain neatly defined negative controls. The results suggested that a lower concentration of erythrocytes might be used when testing white grapevine varieties.

Detection of CM 112 virus; PHT sensitivity

Results showed that CM 112 virus could be detected directly from grapevine infected leaves, using PHT (Plate I). The same

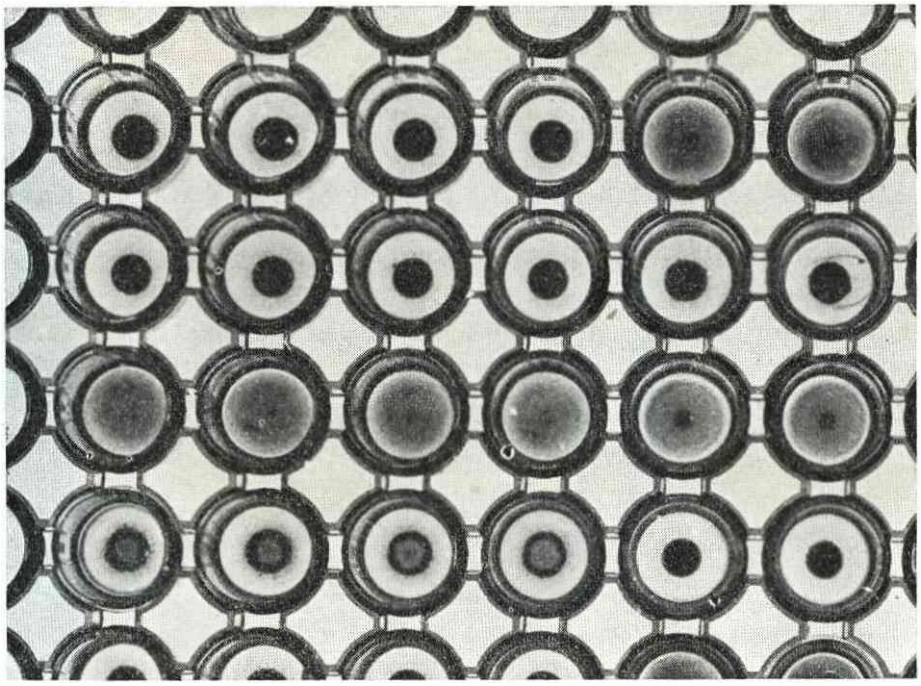

PLATE I — Detection of CM 112 virus by PHT directly from infected grapevine leaves (*Vitis vinifera* L. cv Borraçal).

1st. and 3rd. rows — sensitized erythrocytes; 2nd. and 4th. rows — unsensitized erythrocytes.

1st and 2nd. rows:

healthy grapevine extracts (*Vitis vinifera* cv Vital) — 1st. and 2nd. wells; healthy grapevine extracts (*Vitis vinifera* cv Borraçal) — 3rd. and 4th. wells; infected grapevine extracts (*V. vinifera* cv Borraçal) — 5th. and 6th. wells.

3rd. and 4th. rows:

infected grapevine extracts (*Vitis vinifera* cv Borraçal).

material yielded values within the range of 17 — 40 μg of virus per gram of leaf, whent tested by ELISA (unpublished results).

Using dilutions of purified CM 112 virus in 0.5 % normal rabbit serum in PBS, PHT could detect 25ng.ml^{-1} of virus (Plate II). With

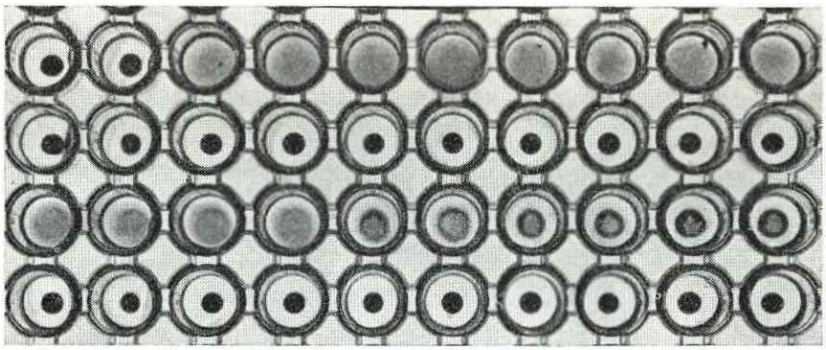

PLATE II — Detection of CM 112 virus by PHT using a purified virus preparation .

1st. and 3rd. rows — sensitized erythrocytes; 2nd. and 4th. rows — unsensitized erythrocytes.

Purified virus concentration (ng.ml^{-1}):

1st. and 2nd. rows:

zero = buffer — 1st and 2nd. wells;
200 — 3rd and 4th. wells;
100 — 5th. and 6th. wells;
67 — 7th. and 8th. wells;
50 — 9th. and 10th. wells.

3rd. and 4th. rows:

33 — 1st. and 2nd. wells;
25 — 3rd. and 4th. wells;
17 — 5th. and 6th. wells;
12,5 — 7th. and 8th. wells;
11 — 9th. and 10th. wells.

dilutions of purified virus in the extraction buffer and these dilutions used to homogeneize healthy grapevine leaves, PHT detected 50ng.ml^{-1} of virus corresponding to 5 μg of virus per gram of leaf.

The test sensitivity proved to be about 6 times lower than ELISA. Chief advantages are the use of a cell marker with no biological risks, lower costs and quicker execution.

ACKNOWLEDGEMENTS

The authors thank the Director of Estação Agronómica Nacional for making this work in this research center possible and Eng. Agrón. O. Amaro de Sequeira of the Phytopathology Department for making the facilities to carry out this work available.

The authors thank also Mrs. Custódia Alves for her kind help in laboratory work, Eng. Agrón. J. Gonçalves Passos for photographic work and Eng. Agrón. J. Diniz for helpful criticism of style.

REFERENCES

ABU SALIH, H. S., MURANT, A. F. & DAFT, M. J.

1968 — Comparison of the Passive Haemagglutination and Bentonite Flocculation Tests for Serological Work with Plant Viruses. *J. Gen. Virol. 2*: 155-166.

FERREIRA, A. A.

1970 — Um Novo Vírus de Videira. Relat. Final do Curso de Engenheiro Agónomo, Lisboa, 139 pp. (mimeog.)

MADEIRA, A. C. de A.

1978 — Estudo de Técnicas Imunológicas na Detecção de Infecções Virais em Plantas. Relat. de Actividades do Aluno Estagiário do Curso de Engenheiro Agrónomo, Lisboa, 50 pp. (mimeog.)

MADEIRA, A. C. & SEQUEIRA, O. A. de

1980 — A Hemaglutinação Passiva na Detecção de Infecções Virais em Plantas. Comunic. I Congr. Português de Fitiatria e Fitofarmacologia, Lisboa, Portugal.

SAITO, Y. & IWATA, Y.

1964 — Hemagglutination Test for Titration of Plant Virus. *Virology 22*: 426-428.

VARENNES, A. de & SEQUEIRA, O. A. de

1980 — A técnica ELISA («Enzyme-linked Immunosorbent Assay») na Detecção de Vírus em Plantas. Comunic. I Congr. Português de Fitiatria e Fitofarmacologia, Lisboa, Portugal.