CHARACTERIZATION OF THE "BOUQUET" STRAIN OF TOMATO BLACK RING VIRUS FROM IMPORTED SEED-POTATO IN BRAZIL[1] /

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Compendio

Un virus aislado de semilla de papa importada, cv. Sieglinde, fue caracterizado como la cepa Bouquet de la papa, perteneciente al virus del anillo negro del tomate, un nepovirus. El virus fue identificado por medio de plantas hospederas, propiedades físicas, morfología de partículas y ensayos serológicos. La introducción de esta enfermedad en Brasil y su posible subsecuente expansión, es discutida debido a que el país importa semilla de papa y, a que la presencia de nematodos del género Longidorus, *vector natural de algunos nepovirus, ha sido descrita en varias regiones de Brasil.*

Introduction

In the course of routine tests for indexing foreign seed-potato, Silberschmidt (19) described, in seed potato cv. Sieglinde imported from Poland, a virus that induced on diagnostic plants symptoms similar to those caused by potato bouquet virus. Based on only 4 diagnostic host (*Chenopodium amaranticolor, C. quinoa, Nicotiana tabacum* White Burley and *Petunia hybrida*), Silberschmidt suggested the virus was a member of the tobacco ringspot subgroup, possibly the potato bouquet strain. This virus has been maintained in the collection of Instituto Biológico, and no further investigations has been undertaken.

The present work proposes to complement the study of that virus concerning its characterization. Studies on host range, stability in sap, purification, electron microscopy and serological tests were carried out.

Material and methods

The virus isolate used in this study came from dried leaf tissues of *Nicotiana tabacum* L. cv. White Burley stored at low temperature for 4 years (1) and subsequently maintained in inoculated tobacco or *Chenopodium quinoa* Willd.

For host range investigation, mechanical inoculation was performed by grinding 1 g of infected tobacco leaves in 3 ml of 0.5 percent sodium sulfite (20). The inoculum was applied onto carborundum-dusted leaves of the indicator species. All plants were grown in sterilized soil and maintained in a glasshouse at 16°C.

Tests of stability in sap were made according to Ross (16), with *C. quinoa* as source and test plants.

The virus was purified from leaves of experimentally infected *C. quinoa* plants by chloroform clarification (18) and differential centrifugation followed by centrifugation in sucrose density gradient.

Electron microscopic observations of crude juice preparations were made by the leaf-dip method de-

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scribed by Brandes (3) with adaptation according to Hitchborn and Hills (8). Leaf strips of infected tobacco or *C. quinoa* were immersed on a drop of 2 percent sodium phosphotungstate (pH 7.2) placed on a Formvar carbon-coated grid. After 1-2 minutes, excess liquid was removed with a filter paper, and the preparation examined in a EM-200 Philips electron microscope. As control, similar preparations from healthy plants were also examined. Purified virus preparations were mixed with an equal volume of the same stain, placed on a Formvar carbon-coated grid, following the same procedure described for the leaf-dip method.

Virus antisera were prepared by single injection of purified preparations intragangliarly (15) with Freund's complete adjuvant in two rabbits. Rabbits were periodically bled and sera were stored frozen. Antisera to myrobalan latent ringspot virus, raspberry ringspot virus, tomato black ring virus-Bu (bouquet strain) and tomato black ring virus-S (type strain) were kindly supplied by Dr. J. Dunez (Station de Pathologie Végetale, Pont-de-la Maye, France) and antisera to raspberry ringspot virus, tomato black ring virus and arabis mosaic virus were kindly supplied by Dr. A. F. Murant (Scottish Horticultural Research Institute, Dundee, Scotland). Virus preparation and antisera were tested in the Ouchterlony agar double-diffusion test. Purified preparations from extracts of healthy *C. quinoa* plants were assayed as control.

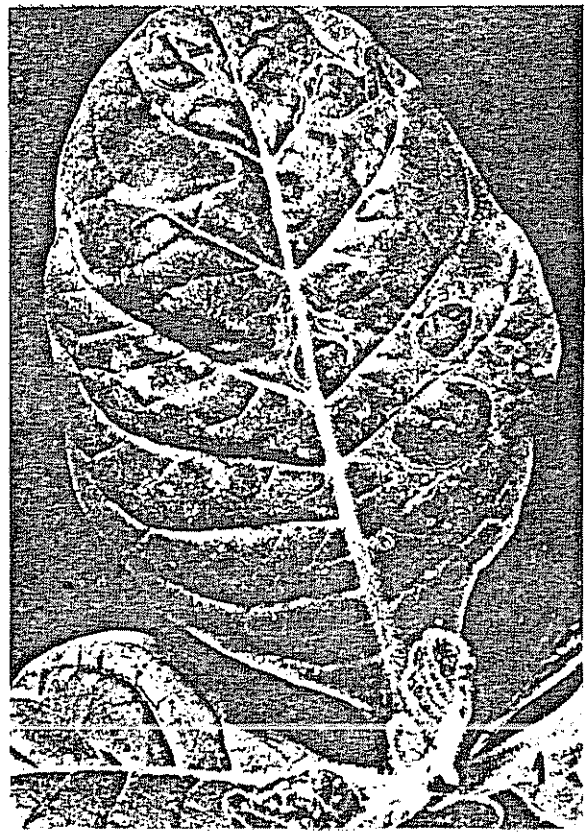

Fig. 1. *Nicotiana tabacum* cv. White Burley: local and systemic symptoms, characterized by necrotic rings and slight leaf deformation.

Results

The symptoms induced by the virus on the diagnostic hosts *Nicotiana tabacum* cv. White Burley (Figure 1), *Gomphrena globosa* (Figure 2), *Petunia hybrida* (Figure 3), *Lycopersicon esculentum* (Figure 4), *Chenopodium amaranticolor, C. quinoa,* correspond to those induced by strains of the tomato black ring virus, which includes the Bouquet strain (7).

Among other inoculated species and cultivars, 7 showed symptons, 2 behaved as latent hosts and 24 were apparently immune (Table 1).

In *Chenopodium quinoa* sap, the virus is inactivated after 10 minutes at 65°C but not at 60°C. The dilution endpoint is between 10^{-2} and 10^{-3}, and the virus retains infectivity for 12 days at room temperature. The virus survives for at least 4 years in leaf tissue stored above $CaCl_2$ at −18°C.

Electron mycroscopy of virus preparations showed the presence of isometric particles of c., 30 nm in diameter (Figure 5).

In double-diffusion tests, virus preparation reacted with the homologous antisera and with antisera to tomato black ring virus, but no reaction was observed with antisera to myrobalan latent virus, raspberry ringspot virus and arabis mosaic virus. In a similar test, no reaction was observed between the antisera and the healthy *C. quinoa* purified preparation.

Discussion

The results obtained confirmed the assumption by Silberschmidt (19) concerning the presence of the Bouquet strain of tomato black ring virus in the seed-potato cv. Sieglinde. The evidences for this conclusion are based on the following characteristics of the virus: particle morphology, stability in sap, host range and serological tests. These data are in agreement with those previously described for the tomato black ring virus (14). The virus under study reacted against the antisera TBRV–S (type strain) and TBRV–Bu (bouquet strain). No reaction was detected against the myrobalan latent ringspot, a strain of the subgroup TBRV, as well as against the

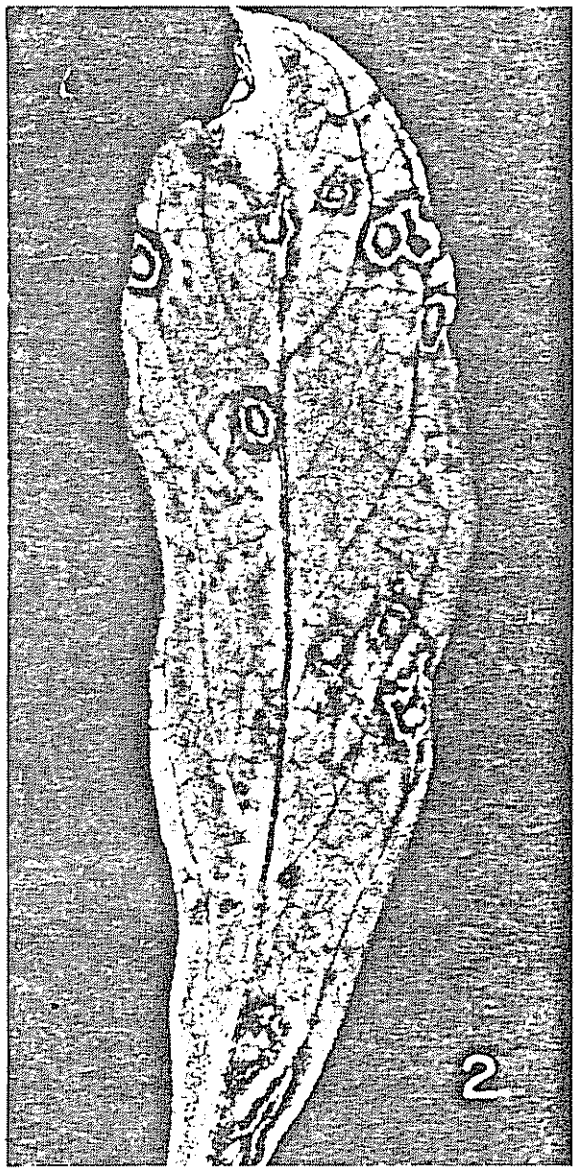

Fig. 2 *Gomphrena globosa* L.: local necrotic rings

arabis mosaic virus and raspberry ringspot virus, also subgroups of the nepoviruses. A previous reported reaction against antiserum to raspberry ringspot virus (11) was not confirmed.

The bouquet disease of potato was first described in Germany under the name "Bukkettkrankheit" (10) and later considered to be caused by one of the strains of TBRV (6, 7) which in Europe may cause important losses on potato crops (2, 17). So far, in Brazil, the Bouquet strain has not been reported in natural conditions, although tobacco ringspot virus – another nepovirus – was described in potato cvs.

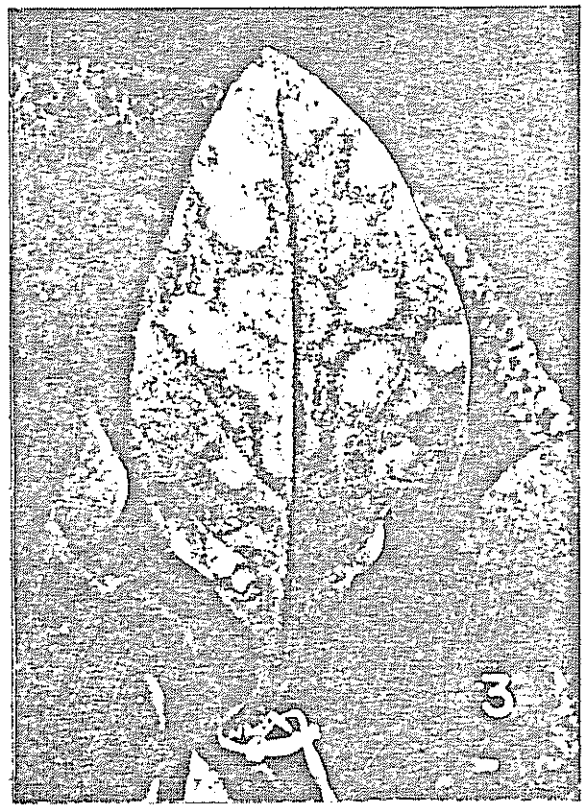

Fig. 3. *Petunia hybrida*: chlorotic spots on inoculated leaf

Olimpia and Anett (5). In Kenya, Kaiser *et al.* (9) detected the beet ringspot strain of TBRV in tubers of c.v. Anett imported from West Germany, and pointed out that affected tubers gave rise to stunted plants. This fact indicates the possible adaptation of

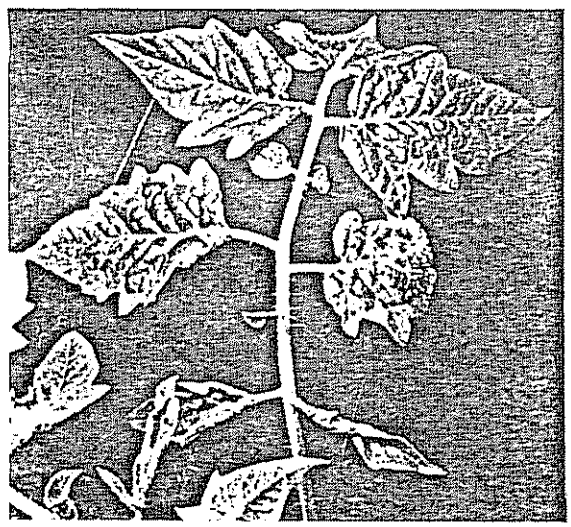

Fig. 4 *Lycopersicon esculentum*: necrotic black rings and distortion of the leaflets

Table 1. Host range and symptomatology of species and cultivars mechanically inoculated with the virus isolated from potato.

Species	Symptoms	
	Local	Systemic
Amaranthaceae		
Gomphrena globosa L.	Foliar necrotic rings	Foliar necrotic rings
Gomphrena decumbens Jacq	(L)	(L)
Chenopodiaceae		
Chenopodium amaranticolor Coste & Reyn.	Chlorotic and necrotic lesions	Downward apical deformation; necrosis
Chenopodium quinoa Willd.	Chlorotic and necrotic lesions (rings)	Downward apical deformation; necrosis and concentric rings
Cruciferae		
Raphanus sativus L.	-	-
Leguminosae		
Phaseolus vulgaris L.		
cv Aete	-	-
Aruana	-	-
Bico-de-Ouro	-	-
Cara suja	-	-
Carioca	-	-
Chuveiro-de-prata	-	-
Franguinho	-	-
Goiano precoce	-	-
Moruna	-	-
Pintado	-	-
Piratã	-	-
Rico-23	-	-
Rosinha	-	-
Rosinha G-2	-	-
Roxão mineiro	-	-
Roxoti	-	-
cv Floresta-5	Chlorotic spots	Slight leaf mottle and necrosis
Jalo	Chlorotic spots	Slight leaf mottle and necrosis
Roxão lustroso	Chlorotic spots	Slight leaf mottle and necrosis
SP 70 S_2	Chlorotic spots	Slight leaf mottle and necrosis
Polemoniaceae		
Phlox drumondii Hook	-	-
Solanaceae		
Datura stramonium L.	-	Leaf mottle and distortion
Lycopersicon esculentum Will cv. S. Cruz	Necrotic black rings	Leaflet distortion; tip blight
Nicotiana clevelandii A. Grey	-	-
N. glutinosa L.	-	-
N. tabacum cv White Burley	Local chlorotic or necrotic rings	Necrotic rings and line patterns; re-entrance of leaf margin
Solanum ciliatum Lam	-	Occasional chlorotic concentric rings; slight mottle and blistering
S. lycocarpum St. Hil.	-	-
S. marmosum L.	-	-
S. nigrum L.	Chlorotic spots	Chlorotic spots
S. palinacanthum Dun	-	-
S. robustum Wendl.	-	-
Petunia hybrida Vilm	Chlorotic and necrotic rings	Chlorotic and necrotic rings; slight mottle
Tropaeolaceae		
Tropaeolum majus L.	(L)	(L)

(–) Plants remained symptomless and no virus was recovered from them

(L) Latent host

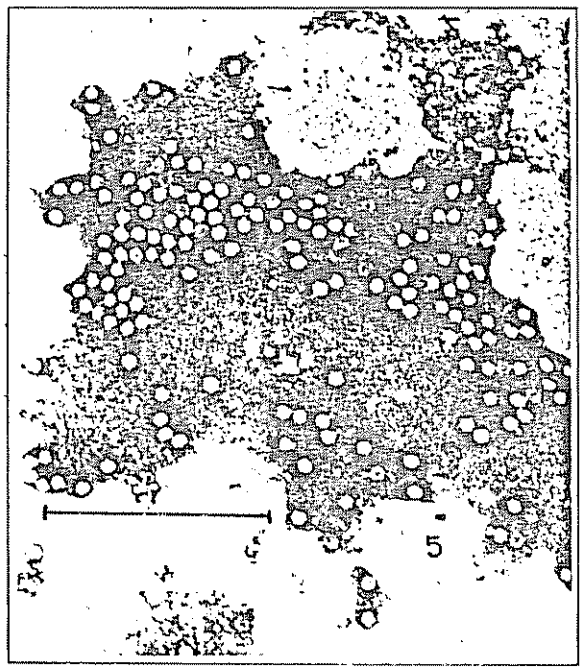

Fig 5. Particles of the Potato Bouquet strain of tomato black ring virus from a partially purified preparation stained with phosphotungstate Magnification mark = 500 nm

nepoviruses from temperate climates to tropical countries.

The consignement of seed-potato cv. Sieglinde infected with the bouquet virus was destroyed (19). However, a possible introduction into Brazil of this virus through imported seed-potato can not be ruled out. If so, in favourable conditions, it could represent a serious problem to our potato crops. This possibility is reinforced by the fact that in several regions of Brazil, nematodes of the genus *Longidorus* – vectors of nepoviruses – have been reported (12, 13, 4).

Summary

A virus isolated from imported seed-potato cv. Sieglinde was characterized as the potato bouquet strain of tomato black ring virus, a nepovirus. The virus was identified by means of diagnostic hosts, stability in sap, particle morphology and serological tests. The possibility of the introduction of the disease into Brazil and its subsequent spreading is discussed, since the country imports seed-potato and the presence of nematodes of genus *Longidorus* – natural vector of some nepoviruses – has been reported in several regions of Brazil.

Literature cited

1. BARRADAS, M. M. Organização de uma coleção de vírus fitopatogênicos em tecidos foliares desidratados com cloreto de cálcio. Biológico, 44(9):221-229, 1978.

2. BODE, O. Die virosen der Kartoffel und des Tabaks. pp. 1-30, **In** M. Klinkowski, ed. Pflanzliche Virologie. Band II, Berlin, 1958. Akademie – Verlag.

3. BRANDES, J. Eine elektronenmikroskopische Schenellmethode zum Nachweiss faden-und stabchenformiger Viren, insbesondere in Kartoffeldunkelkeimen. Nachrichtenbl. Dtsch. Pflanzenschutzd, 9:151-152, 1957.

4. CHOUDHURY, M. M.; MONTEIRO, A. R. e CHOUDHURY, E. N. Ocorrência de fitonematóides em áreas irrigadas do vale do Rio São Francisco. Resumos 3a. Reunião de Nematologia, São Paulo, Brasil, p. 27, 1979.

5. CUPERTINO, F. P. e COSTA, A. S. Ocorrência do vírus da mancha anular do fumo em batata. Bragantia, 28, nota 2:IX-XII, 1969.

6. HARRISON, B. D. Relationship between beet ringspot, potato bouquet and tomato black-ring viruses. Journal Genetic Microbiology, 18:450-460, 1958.

7. HARRISON, B. D. and MURANT, A. F. Nepovirus group. C. M. I./A. A. B. Description of plant Viruses No. 185, 1977.

8. HITCHBORN, S. H. and HILLS, G. I. The use of negative staining in the electron microscopic examination of plant viruses in crude extracts. Virology, 27:528-540, 1965.

9. KAISER, W. J.; BOCK, K. R.; GUTHRIE, E. J. and MEREDITH, G. Occurrence of tomato black ring virus in potato cultivar Anett in Kenya. Plant Disease Reporter, 62(12): 1 088-1 092, 1978.

10. KÖHLER, E. Die Bukkettkranheit eine Viruskrankeit der Kartoffel. Phytopath. Z., 19(3):284-294, 1952.

11. KUDAMATSU, M.; ALBA, A. P. C.; CHAGAS, C. M. e UEDA, M. Purificação parcial e caracterização serológica de um vírus de batata. Ciência e Cultura, 30(7):9, 1978.

12. LEHMAN, P. S.; MACHADO, C. C. e TARRAGO, M. T. Frequência e severidade de doenças da soja nos Estados do Rio Grande do Sul e Santa Catarina. Fitopatologia Brasileira, 1(3):183-193, 1976.

13. LEHMAN, P. S.; ANTONIO, H. e BARKER, K. Ocorrência de nematóides em soja nos Estados de Minas Gerais, Goiás e Mato Grosso. II Reunião de Nematologia, Piracicaba, SP, Brasil, pp. 29-32, 1977.

14. MURANT, A. F. Tomato black ring virus. C. M. I. A. A. B. Descriptions of Plant Viruses No. 38, 1970.

15. OLIVEIRA, A. R. Considerações sobre anti-soros obtidos pela técnica de injeção de antígeno no linfonódulo. Summa Phytopathologica, 1:61-64, 1975.

16. ROSS, A. F. Identification of plant viruses. In M. K. Corbet and H. D. Sisler eds. Plant Virology, Gainesville. University Florida Press, 1964. pp. 68-92.

17. SALZMANN, R. and KELLER, E. R. Krankheiten und Schädlinge der Kartoffel, Bern. Landw. Lehrmittelzentrale Zollikofen, 150 p., 1969.

18. SCHNEIDER, I. R. Solution of tobacco mosaic virus in the aqueous phase of a chloroform-water emulsion and application of this phenomenon in virus assay. Science, 117: 30-31, 1953.

19. SILBERSCHMIDT, K. M. Sobre uma doença de vírus da batatinha veiculada em tubérculos importados. Biológico, 31(8):176-177, 1965.

20. YARWOOD, C. E. Sulfite in plant virus inoculations. Virology, 39(1):74-78, 1969.