Cabrera Mederos et al., J Plant Physiol Pathol 2017, 5:2
DOI: 10.4172/2329-955X.1000163

Journal of Plant Physiology & Pathology

Research Article

A SCITECHNOL JOURNAL

Aggressiveness of Cuban *Papaya ringspot virus* Isolates on *Carica papaya* L. cv. Maradol Roja under Greenhouse Conditions

Dariel Cabrera Mederos[1,2*], Maylin Cruz[3], Claudia F. Nome[2], Fabián Giolitti[2], Orelvis Portal[4*]

Abstract

Carica papaya is a major fruit crop in tropical and subtropical regions; however, its production has several constraints. *Papaya ringspot virus* (PRSV) is the most important pathogen affecting papaya plantations. Symptoms induced by a known PRSV isolate on papaya plants after mechanical inoculation were described to elaborate an evaluation scale under greenhouse conditions. The aggressiveness of 24 Cuban PRSV isolates was determined by assessing symptom severity induced on papaya plants cv. Maradol roja under greenhouse conditions. An evaluation scale of 0 to 6 scores was elaborated based on the observations of PRSV symptom progress. Symptoms induced by Cuban PRSV isolates included vein clearing, mottling and swelling zones on the adaxial leaf surface, slight deformation of young leaves and distortion. PRSV isolates from Nueva Paz, Güines, and San José de Las Lajas in the west, Sancti Spíritus in the center, and Palma Soriano and Puerto Padre in the east of the country were the most aggressive according to their area under the disease progress curve values. Knowledge about the aggressiveness of this virus is crucial for selecting PRSV isolates to implement management strategies and papaya breeding programs in Cuba.

Keywords

Mechanical inoculation; Papaya; PRSV

Introduction

Papaya (*Carica papaya*) is one of the most important fruit crops in tropical and subtropical regions. In Cuba, papaya cv. Maradol roja is widely cultivated due to its high nutritional and digestive values, and is consumed as fresh fruit and as processed products. The favorable edaphoclimatic conditions for the development of this fruit tree, place Cuba within the first 10 papaya producing countries in the world, with annual productions higher than 150 000 t [1].

Papaya ringspot virus (PRSV) is an aphid-transmitted potyvirus that causes a very destructive disease in papaya that is distributed worldwide [2,3]. This virus induces diverse symptoms that include mosaic, leaf distortion, vein clearing and ring spotting on fruits. However, some characterized PRSV isolates differ in epidemiological traits, such as aggressiveness and symptoms [4,5], suggesting a relationship between these traits. In Mexico, Noa-Carrazana et al. [6] showed that genetic diversity among PRSV isolates is correlated with their geographical origin. Accordingly, coat protein analysis of seven Cuban PRSV isolates were grouped into eastern and central-west regions and showed a greater genetic conservation among isolates from nearby regions [7]. According to this result, it is tempting to assume that in Cuba, the symptoms progress and aggressiveness of PRSV isolates could be related to geographical regions, with noticeable differences in agricultural practices, climate and host diversity.

In this study, an evaluation scale was elaborated by assessing symptoms induced by PRSV on papaya plants cv. Maradol roja under greenhouse conditions. Based on the evaluation scale, the aggressiveness of 24 PRSV isolates representing 13 Cuban provinces was determined. Knowledge obtained from epidemiological studies with native PRSV isolates is crucial for the management of this disease in Cuba, either using transgenic or non-transgenic approaches.

Materials and Methods

Description of PRSV symptoms

To describe the symptoms induced by PRSV under greenhouse conditions, papaya plants cv. Maradol roja were inoculated with a characterized Santo Domingo isolate [8]. This PRSV isolate was previously propagated and maintained in papaya plants [9], in an insect-free greenhouse. Nevertheless, to discard the presence of mixed infections, infected tissue was analyzed by electron microscopy, using leaf-dip and inclusion in Spurr resin techniques [10]. Leaf-dips were stained with 2% uranyl acetate (Sigma-Aldrich, USA). Moreover, ultrathin sections were double stained with 0.4% lead citrate (Sigma-Aldrich, USA) and 2% uranyl acetate. Tissue preparations were examined with a JEM 1200 EX II electron microscope (JEOL, Japan).

Fifty papaya plants with 60 days after germination of seeds and seven expanded leaves were mechanically inoculated with the Santo Domingo PRSV isolate. Plants were inoculated with a paintbrush on the first three expanded leaves from the apex of each plant, for which 200 µl of inoculum were applied with carborundum (600 mesh) as an abrasive [5]. Ten plants were mock-inoculated (control group). Inoculated plants were exposed to a photoperiod of 14 h light and 10 h darkness at 26 ± 4°C, which is the optimal range for infection progress [11], and daily watering to maintain relative humidity above 50%. Symptom progress was described daily until no further changes were observed. The experiment was repeated twice under the same conditions. The PRSV symptoms progress was used to elaborate an evaluation scale for other Cuban isolates.

Aggressiveness of PRSV isolates

To determine the aggressiveness of PRSV isolates, papaya leaves with typical symptoms of this disease were collected from representative Cuban regions in the west, center and east, from February 2009 to November 2012. The symptomatic papaya leaves

***Corresponding author:** Dariel Cabrera Mederos, Departamento de Agronomía, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba, Tel: +53-42-207171; E-mail: cabrera.dariel@inta.gob.ar

Orelvis Portal, Departamento de Biología, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba, E-mail: orelvispv@uclv.cu

Received: February 28, 2017 **Accepted:** April 11, 2017 **Published:** April 16, 2017

were collected in 13 out of the 15 provinces in which Cuba is divided, to include different geographic and climatic scenarios (mean temperature and precipitation). Leaves were collected from papaya plantations belonging to the private and public sectors as well as other papaya plants that were in orchards and gardens. In this study, only the Cuban cv. Maradol roja was considered because it is the most widely extended and economically important in the country [1].

The collected symptomatic leaves were used to inoculate papaya plants for the observation of symptoms and the maintenance of putative PRSV isolates in an insect-free greenhouse. A pool of inoculated papaya leaves were analyzed for virus particles observation using the leaf-dip method, as described above. Total RNA was purified from infected leaves using the RNeasy Mini Kit (Qiagen, Germany) and the corresponding cDNA was synthesized with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA), following the manufacturer's instructions. The presence of PRSV was confirmed by the polymerase chain reaction (PCR) amplification of the coat protein (CP) gene, according to Cabrera Mederos et al. [12]. Moreover, the inoculated papaya plants were tested by PCR using *Cucumber mosaic virus* (CMV) specific primers for the CP gene, according to Rizos et al. [13].

PRSV isolates were inoculated using leaves obtained from papaya plants showing typical symptoms of the disease, using the similar method, plant material and experimental conditions to those previously described. Every PRSV isolate was inoculated on 10 papaya plants cv. Maradol roja and the experiment was repeated twice. The symptoms induced for each PRSV isolate were evaluated weekly and the disease index was calculated using the formula described by Townsend and Heuberger [14]. Accordingly, the disease index values corresponding to the first and last evaluation were used to calculate the area under the disease progress curve (audpc), according to that informed for polycyclic foliar pathogens [15]. The audpc was calculated using the formula described by Shaner and Finney [16]. Data were analyzed using the Statistical Package for the Social Science, version 18.0 for Windows.

Results

Description of PRSV symptoms

Electron microscopy observations of leaf-dips from papaya plants infected with the Santo Domingo PRSV isolate under greenhouse conditions revealed the presence of typical flexuous potyvirus particles of approximately 800 × 12 nm (Figure 1a). Pinwheel and scroll cylindrical inclusions were also observed in ultrathin sections (Figure 1b,c). In these observations, particles belonging to other virus families were not detected.

Under greenhouse conditions, the manifestation of PRSV symptoms on papaya plants inoculated with the Santo Domingo isolate started at 14 days post-inoculation (dpi). Eighty seven percent of the plants showed vein clearing (Figure 2a) and the remaining 13% displayed a mild mosaic first, characterized by small light-colored zones distributed on the adaxial leaf surface (Figure 2b). This symptom was secondarily observed in those plants in which vein clearing had started. Subsequently, a mosaic in the form of light and dark-colored zones distributed on the adaxial leaf surface was observed (Figure 2c). Followed by this symptomatology, in 93% of the inoculated plants, dark-colored swelling zones were observed on the adaxial leaf surface (Figure 2d). Furthermore, slight deformation on young leaves began to appear in 98% of the inoculated plants (Figure 2e); the remaining 2% showed leaf distortion (Figure 2f), which was considered the highest disease stage. Based on PRSV symptom progress described for the Santo Domingo isolate, an evaluation scale of 0 to 6 scores was elaborated (Table 1), which will contribute to determine the aggressiveness of Cuban PRSV isolates under greenhouse conditions.

Aggressiveness of PRSV isolates

Papaya plants inoculated with symptomatic leaves, collected from different Cuban regions, developed the typical PRSV symptoms, which were confirmed by the reverse transcriptase-PCR amplification of an expected product of about 850 bp (data not shown). No amplification was observed when CMV specific primers were used in PCR analysis. Moreover, only typical flexuous potyvirus particles were detected in electron microscopy observations of leaf-dips from papaya plants inoculated under greenhouse conditions. The onset of symptoms on papaya plants inoculated with different PRSV isolates was observed between 14 and 21 dpi. The first symptom induced by all isolates was vein clearing (score 1), followed by a mild mosaic on the newly expanded leaves, corresponding to scale score 2. However, papaya plants inoculated with PRSV isolates from Boyeros, Cienfuegos, Rafael Freyre, Niquero, Manzanillo and Bayamo did not develop the mild mosaic. From 21 to 35 dpi, all isolates induced light and dark green zones, and swelling mosaic on the adaxial leaf surface, which were associated to score 3 and 4 of the scale, respectively. From 35 to 51 dpi, papaya plants inoculated with all isolates showed a minor deformation on the newly emerged leaves, associated with score 5. At 63 dpi, papaya plants inoculated with all isolates showed leaf distortion (score 6). At this point, the presence of PRSV was confirmed by enzyme-linked immunosorbent assay using specific PRSV antiserum (Agdia, USA).

The aggressiveness of Cuban PRSV isolates expressed by the audpc values (calculated from disease index) was determined (Table 2). The highest audpc values in western Cuba were obtained for the isolates from Nueva Paz, Güines and San José de Las Lajas, in the central region, for the Sancti Spíritus isolate, and in the east, for the isolates from Palma Soriano and Puerto Padre. In general, isolates

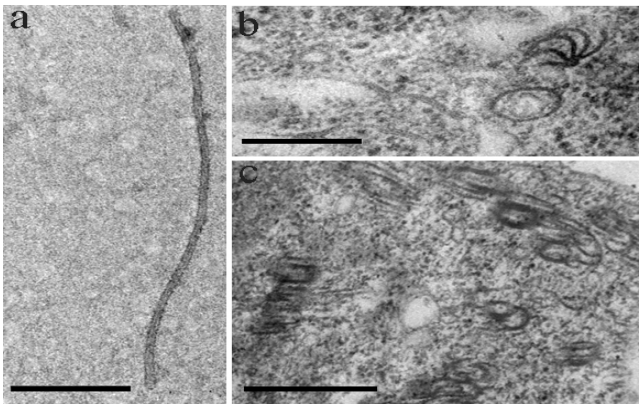

Figure 1: Electron micrographs of virus particles and cytoplasm inclusion bodies induced by Santo Domingo *Papaya ringspot virus* isolate in papaya plants mechanically inoculated in an insect-free greenhouse. **(a)** Flexuous potyvirus-like particle in leaf extracts from a PRSV-infected papaya plant (leaf-dip) (bar=200 nm). Thin sections of PRSV-infected papaya leaf showing cytoplasm inclusions described as **(b)** pinwheel (bar=200 nm) and **(c)** scrolls (bar=500 nm).

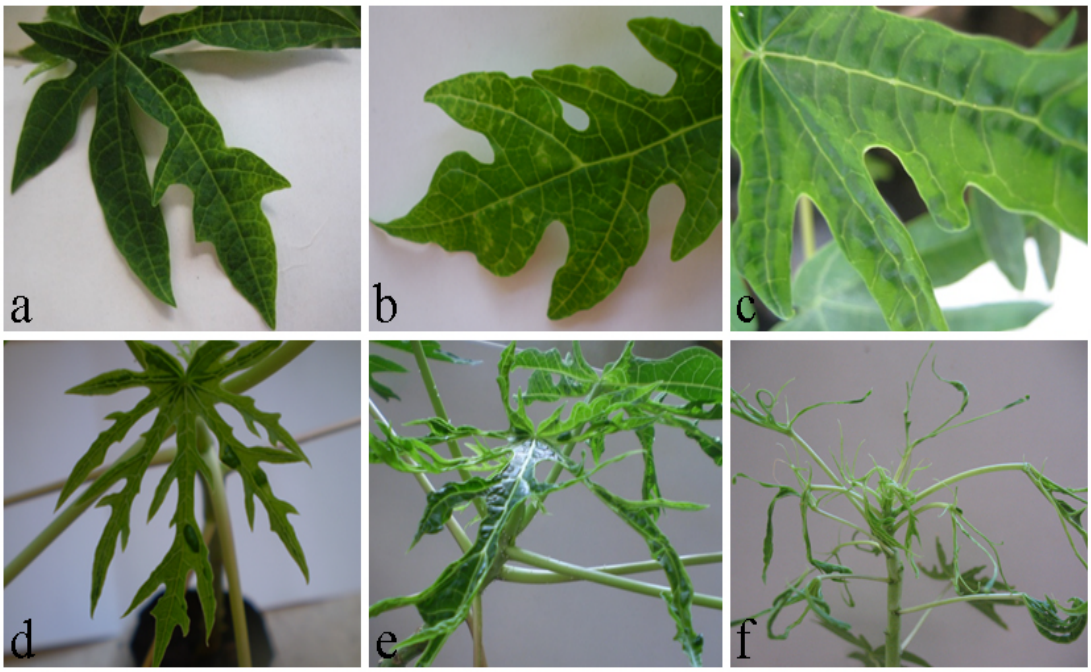

Figure 2: *Papaya ringspot virus* **s**ymptoms induced by the Santo Domingo isolate on papaya plants cv. Maradol roja mechanically inoculated under greenhouse conditions. **(a)** Vein clearing, **(b)** Mild mosaic, **(c)** Clear and intense color zones distributed on the adaxial leaf surface, **(d)** Swelling and intense color zones on the adaxial leaf surface, **(e)** Slight deformation on young leaves, **(f)** Leaf distortion.

Table 1: Evaluation scale of the *Papaya ringspot virus* symptoms on papaya plants cv. Maradol roja mechanically inoculated under greenhouse conditions.

Score	Symptom description
0	Absence of leaf symptoms
1	Vein clearing
2	Light green zones distributed on adaxial leaf surface (mild mosaic)
3	Light and dark green zones distributed on adaxial leaf surface
4	Dark green swelling zones on adaxial leaf surface
5	Slight deformation on young leaves
6	Leaf distortion

from Cienfuegos and Bayamo showed the lowest aggressiveness of all PRSV Cuban isolates.

Discussion

The Santo Domingo PRSV isolate induced pinwheel and scroll inclusions in the cytoplasm of infected papaya leaf cells [4,17]. The virus morphology and cytoplasm inclusions were similar to those caused by other potyviruses infecting papaya [18]; however, only PRSV has been reported in papaya fields in Cuba so far. Moreover, mixed infections of PRSV with BTS phytoplasma and rhabdovirus have been detected [19,20]; since these pathogens are not transmitted mechanically, the symptoms observed in the papaya plants are certainly induced by PRSV.

Symptoms induced by the Santo Domingo isolate coincided with observations of Fitch et al. [21], who found vein clearing on young papaya leaves induced by an early infection of PRSV and the subsequent appearance of leaf mottling and distortion. According to Davis et al. [22] and Tennant et al. [23], the symptoms induced by PRSV in papaya may vary depending on the genotype and the virus isolate present. Furthermore, under greenhouse conditions these symptoms are more differentiated than those observed under field conditions; therefore, scales in both scenarios do not fully coincide [6,22,24]. The PRSV-P is widely adapted to papaya in all regions of the world where it occurs [25], and the symptoms are easily identified. In this study, biological, serological and molecular methods confirmed the wide distribution of PRSV in Cuba, which highlights the importance of establishing efficient management strategies of the disease. For this purpose, a scale was developed and validated to evaluate the aggressiveness of PRSV on papaya cv. Maradol roja under greenhouse conditions (Table 1). The scale describes in detail the evolution of the symptoms induced by Cuban PRSV isolates on papaya cv. Maradol roja.

The symptoms observed on papaya plants inoculated with Cuban PRSV isolates agreed with those reported by Rodríguez et al. [7], who evaluated the symptoms induced by PRSV of seven Cuban and 21 Brazilian isolates on papaya plants cv. Solo and observed vein clearing and mild mosaic as initial symptoms. Preliminary molecular results, including 24 PRSV isolates evaluated in this study, showed genetic differences of the CP gene among Cuban PRSV isolates [26].

The differences in audpc values obtained among the Cuban PRSV isolates (Table 2) suggest the biological diversity of this virus, which might affect the implementation of certain management strategies. Accordingly, variation in the response of transgenic papaya plants to the infection of different PRSV isolates has been reported [5,23]. In Cuba, Rodríguez et al. [24] evaluated PRSV severity in introduced papaya genotypes, but did not include isolates from different regions, which could provide valuable elements for breeding programs and selection of tolerant genotypes.

Table 2: Area under the disease progress curve (audpc) values of papaya plants cv. Maradol roja after mechanical inoculation with Cuban Papaya ringspot virus isolates under greenhouse conditions, for the period between 21-63 days post inoculation. Data were analyzed by a Kruskal Wallis/Mann-Whitney non-parametric test. Different letters in each Cuban region indicate statistical differences at p<0.05.

West PRSV isolates	audpc ± standard error
San Luis	2 187.50 ± 71.26^{c}
San Juan y Martínez	2 205.00 ± 88.33^{c}
San Antonio de Los Baños	2 240.00 ± 39.34^{c}
Boyeros	2 362.50 ± 34.77^{bc}
Bejucal	2 240.00 ± 108.92^{bc}
San José de Las Lajas	2 520.00 ± 32.12^{a}
Melena del Sur	2 187.50 ± 66.58^{c}
Güines	2 590.00 ± 81.89^{a}
Nueva Paz	2 590.00 ± 92.95^{a}
Jagüey Grande	2 467.50 ± 78.16^{ab}
Center PRSV isolates	**audpc ± standard error**
Cienfuegos	1 977.50 ± 38.30^{d}
Corralillo	2 257.50 ± 39.95^{c}
Santo Domingo	2 415.00 ± 66.70^{abc}
Placetas	2 327.50 ± 58.32^{abc}
Sancti Spíritus	2 450.00 ± 50.78^{a}
Trinidad	2 310.00 ± 39.34^{bc}
East PRSV isolates	**audpc ± standard error**
Guáimaro	2 450.00 ± 71.82^{ab}
Las Tunas	2 205.00 ± 62.71^{cd}
Puerto Padre	2 467.50 ± 89.68^{a}
Rafael Freyre	2 100.00 ± 56.78^{d}
Niquero	2 240.00 ± 53.26^{bcd}
Manzanillo	2 345.00 ± 36.80^{abc}
Bayamo	1 592.50 ± 93.21^{e}
Palma Soriano	2 467.50 ± 64.61^{a}

Cuban PRSV isolates with varying degrees of aggressiveness were identified, which could lead to the establishment of differential management strategies for different production areas in Cuba. In addition, having a collection of PRSV isolates could be very useful for epidemiological studies of other papaya genotypes that have been introduced in Cuba so far [24]. Moreover, such collection could serve as the basis for the molecular analysis of the virus, which in turn could assist the implementation of transgenic approaches based on RNA-interference [5,27].

Acknowledgements

This research was supported by the International Foundation for Science, Stockholm, Sweden, through a grant to Dariel Cabrera Mederos (D/5134-1), and by a scholarship from the Food Security Center from the University of Hohenheim, which is part of the DAAD (German Academic Exchange Service) program "exceed" and is supported by DAAD and the German Federal Ministry for Economic Cooperation and Development (BMZ). The authors thank Novisel Veitia (Instituto de Biotecnología de las Plantas, Cuba) and Luke Leroy Theodore Need for the analysis of the data and critical comments on the manuscript, and Yulexis Pino and Danilo Díaz (AGROFAR, Cuba) for support during the collection of papaya leaves across the country.

References

1. FAO (2016) Food and Agricultural Organization of the United Nations. Statistics division.
2. Mora-Aguilera G, Téliz D, Campbell CL, Ávila C (1992) Temporal and spatial development of papaya ringspot in Veracruz, México. J Phytopathol 136: 27-36.
3. Tripathi S, Suzuki JY, Ferreira SA, Gonsalves D (2008) Papaya ringspot virus-P: characteristics, pathogenicity, sequence variability and control. Mol Plant Pathol 9: 269-280.
4. Purcifull DE, Edwardson JR, Hiebert E, Gonsalves D (1984) Papaya ringspot virus: CMI/AAB Descriptions of Plant Viruses No. 292. Wageningen University, The Netherlands.
5. Bau HJ, Cheng YH, Yu TA, Yang JS, Yeh SD (2003) Broad spectrum resistance to different geographic strains of Papaya ringspot virus in coat protein gene transgenic papaya. Phytopathology 93: 112-120.
6. Noa-Carrazana JC, González-de-Leon D, Ruiz-Castro BS, Piñero D, Silva-Rosales L (2006) Distribution of Papaya ringspot virus and Papaya mosaic virus in papaya plants (Carica papaya) in Mexico. Plant Dis 90: 1004-1011.
7. Rodríguez-Martínez D, Geraldino PdS, González J, dos Reis A (2014) Molecular and biological studies of Papaya ringspot virus isolates from Brazil and Cuba. Am J Agric For 2: 209-218.
8. Portal O, Cabrera D, Sánchez A, Darias AL, González JE, et al. (2006) Molecular characterization of two Cuban isolates of the PRSV by means of coat protein analysis. Commun Agric Appl Biol Sci 71: 1203-1205.
9. Yeh SD, Gonsalves D, Provvidenti R (1984) Comparative studies on host range and serology of Papaya ringspot virus and Watermelon mosaic virus 1. Phytopathology 74: 1081-1085.
10. Kitajima EW (1997) Electron microscopy of plant viruses. University of Sao Paulo, Brazil.
11. Mangrauthia SK, Singh Shakya VP, Jain RK, Praveen S (2009) Ambient temperature perception in papaya for Papaya ringspot virus interaction. Virus Genes 38: 429-434.
12. Cabrera Mederos D, Dal Zotto A, Portal O, Giolitti F (2016) First report of Papaya ringspot virus infecting Carica papaya in Argentina. J Plant Pathol 98: 687.
13. Rizos H, Gunn LV, Pares RD, Gillings MR (1992) Differentiation of Cucumber mosaic virus isolates using the polymerase chain reaction. J Gen Virol 73: 2099-2103.
14. Townsend GR, Heuberger JW (1943) Methods for estimating losses caused by diseases in fungicide experiments. Plant Dis Rep 27: 340-343.
15. Jeger MJ, Viljanen-Rollinson SLH (2001) The use of the area under the disease-progress curve (AUDPC) to assess quantitative disease resistance in crop cultivars. Theor Appl Genet 102: 32-40.
16. Shaner G, Finney RE (1977) The effect of nitrogen fertilization on the expression of show-mildewing resistance in knox wheat. Phytopathology 67: 1051-1056.
17. Francki RIB, Milne RG, Hatta T (1985) Atlas of plant viruses. (2nd edtn), CRC Press, Boca Raton, Florida.
18. Bau HJ, Kung YJ, Raja JAJ, Chan SJ, Chen KC, et al. (2008) Potential threat of a new pathotype of Papaya leaf distortion mosaic virus infecting transgenic papaya resistant to Papaya ringspot virus. Phytopathology 98: 848-856.
19. Arocha Y, Horta D, Peralta E, Jones P (2003) First report on molecular detection of phytoplasmas in papaya in Cuba. Plant Dis 87: 1148.
20. Arocha Y, Piñol B, Acosta K, Almeida R, Devonshire J, et al. (2009) Detection of phytoplasma and potyvirus pathogens in papaya (Carica papaya L.) affected with 'Bunchy Top Symptom' (BTS) in eastern Cuba. Crop Prot 28: 640-646.
21. Fitch MMM, Manshardt RM, Gonsalves D, Slightom JL, Sanford JC (1992) Virus resistance papaya plants derived from tissues bombarded with the coat protein gene of Papaya ringspot virus. Nat Biotechnol 10: 1466-1472.
22. Davis MJ, White TL, Crane JH (2004) Resistance to Papaya ringspot virus in transgenic papaya breeding lines. Proc Fla State Hort Soc 117: 241-245.
23. Tennant PF, Gonsalves C, Ling KS, Fitch M, Manshardt R, et al. (1994) Differential protection against Papaya ringspot virus isolates in coat protein gene transgenic papaya and classically cross-protected papaya. Phytopathology 84: 1359-1366.
24. Rodríguez-Martínez D, Tornet Y, Alonso M, Valero L, Peña I, et al. (2011) Severity of the papaya ring spot in different genotypes of the Solo group introduced in Cuba. J Biotec Biodivers 2: 28-36.

25. Gonsalves D (1998) Control of Papaya ringspot virus in papaya: a case study. Ann Rev Phytopathol 36: 415-437.

26. Cabrera Mederos D (2014) Biological and molecular characterization of papaya ring spot virus, epidemiology and management of the disease in Carica papaya L. var. Red Maradol in Cuba. PhD Thesis, Faculty of Agricultural Sciences, UCLV, Cuba.

27. Kung YJ, Bau HJ, Wu YL, Huang CH, Chen TM, et al. (2009) Generation of transgenic papaya with double resistance to Papaya ringspot virus and Papaya leaf-distortion mosaic virus. Phytopathology 99: 1312-1320.

Author Affiliations

Top

[1]*Departamento de Agronomía, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba*

[2]*Instituto de Patología Vegetal (IPAVE), Centro de Investigaciones Agropecuarias (CIAP), Instituto Nacional de Tecnología Agropecuaria (INTA), Av. 11 de Septiembre 4755, X5020ICA Córdoba, Argentina*

[3]*Laboratorio Provincial de Sanidad Vegetal, Santa Clara, Cuba*

[4]*Departamento de Biología, Facultad de Ciencias Agropecuarias, Universidad Central "Marta Abreu" de Las Villas, Santa Clara, Cuba*