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Full Length Research Paper

First report of papaya meleira virus (PMeV) in Mexico

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Papaya meleira virus (PMeV), causal agent of meleira or sticky disease, is a double-stranded RNA (dsRNA) virus which has been previously reported only in Brazil. A study was carried out in order to verify the presence and occurrence of PMeV in Mexico. Latex samples from symptomatic and asymptomatic papaya fruits were collected in Quintana Roo state papaya orchards, where the first symptoms of PMeV were observed, and from 29 different municipalities located in ten papaya producer states in Mexico. A molecular protocol based on nucleic acid extraction was used for the diagnosis and a virus 12 Kb dsRNA distinctive band was observed in all PMeV infected plants. Around 46% of the evaluated plants were positive for this pathogen. Presence of the virus had been confirmed in seven states indicating the potential damage that PMeV could cause in the papaya crop in Mexico. The molecular analysis used allowed the diagnosis of infected plants without symptoms and facilitated the diagnosis in flowers and small papaya fruits also. The early diagnosis of PMeV will allow papaya producers to take appropriate and timely control measures. This is the first report of Papaya meleira virus in Mexico.

Key words: Papaya meleira virus, sticky disease, dsRNA, nucleic acid analysis.

INTRODUCTION

Papaya (*Carica papaya* L.) is an important fruit crop grown widely in tropical and subtropical regions. In Mexico, its importance lies not only for its high consumption rate as fresh fruit but also for its considerable exportation value (SIAP, 2009). Among the pathogens limiting papaya cultivation, infection by viruses invariably leads to a considerable increase in production costs and important losses in productivity. Papaya ringspot virus (PRSV) is by far the most widespread and damaging virus that infects papaya. However, there are other viruses such as Papaya mosaic virus (PapMV), Papaya lethal yellowing virus (PLYV) and Papaya meleira virus that also pose a threat to the production of this crop in Indonesia, Brazil and Mexico, which are the main papaya producing countries (Gonsalves, 1998). Meleira disease was first detected in the late 1980s in Brazil (Lima et al., 2001). During the economic cycle of the culture, meleira typically infects at least 20% of the cultivated area, but sometimes the incidence of the disease can rise up to 100% (Ventura et al., 2004). Its first noticeable symptom is an intense and spontaneous exudation of latex on the surface of green fruits, which soon darkens as it oxidizes (Buss et al., 2011). The exudation of latex also occurs from edges of young leaves in the top of the plant and with oxidation provokes small light-brown necrotic lesions on the leaf tips (Ventura et al., 2004). The diagnosis of Papaya meleira disease is generally based on symptoms observed in the fruit. Thus, an infected plant without fruit or symptoms can remain unnoticed for months in the field, acting as an inoculum source until it is finally detected and eliminated (Araujo et al., 2007).

In Brazil, two diagnostic assays for the disease were developed based on the extraction of nucleic acids from the latex of papaya plants and visualization of the dsRNA

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Abbreviations: PMeV, Papaya meleira virus; **PRSV,** papaya ringspot virus; **PapMV,** papaya mosaic virus; **PLYV,** papaya lethal yellowing virus.

Table 1. PMeV diagnosis results in orchards located in Jose Maria Morelos municipality, Quintana Roo, Mexico.

Orchard	Number of samples	Positive to PMeV	Negative to PMeV	Percentage of disease incidence (%)
Chunhuhub	5	1	4	20
Paraíso Renaciente	46	0	46	0
Quizás	82	60	22	73.1
Much Meyá	188	188	0	100
Total	321	249	72	77.5

on agarose gels (Tavares et al., 2004). This method is suitable for the detection of the virus, because Papaya meleira virus (PMeV) particles are present in high concentrations in fruits (Rodrigues et al., 2005; Ventura et al., 2004; Kitajima et al., 1993) as has been described as the only virus that is located in lactifers (Rodrigues et al., 2011). In 2008, symptoms similar to those of PMeV previously reported in Brazil (Zambolim et al., 2003) were observed for the first time in Quintana Roo State, in the Yucatan Peninsula, Mexico, by personnel of the GeMBio laboratory. The objective of this research therefore, was to verify the presence and occurrence of PMeV in Mexico, in order to understand and eventually prevent the dissemination of this disease.

MATERIALS AND METHODS

Plant materials

Latex samples were taken from *Carica papaya* cv. Maradol in Much Meya, Quizas, Chunhuhub and Paraiso Renaciente orchards, all located in Jose Maria Morelos municipality, Quintana Roo, Mexico. The samples were collected in sterile 2 ml eppendorf tubes using sterile toothpicks to wound the surface of green fruits with and without PMeV symptoms. In order to screen and diagnose the occurrence of PMeV in Mexico between July 2009 and November 2011, 1150 latex samples were also collected from papaya fruits, with and without disease symptoms, from 29 municipalities in ten states within the principal papaya growing regions of this country.

Nucleic acid extraction and analysis for PMeV diagnosis

Nucleic acids were extracted from papaya latex following the protocol 1 previously described by Tavares et al. (2004). Samples were analyzed for purity using an eppendorf BioPhotometer-6131. Subsequently, 10 µl of each sample were analyzed for the PMeV diagnosis. The presence of PMeV was confirmed by the visualization of dsRNA (12 Kb) on an agarose gel (0.8%) in 1× Tris-Borate-EDTA (TBE) which were stained with ethidium bromide and visualized under UV transilluminator. The images were taken with a UVP Biolmaging System. In all cases, a sample from Brazil PMeV infected plant latex was used as positive control, this sample was supplied by SENASICA-SAGARPA, Mexico. Latex from healthy plants was used as negative control. In order to determine the nature of the bands obtained, the nucleic acids were digested differentially with endonucleases DNAse I and RNase A. 10 µl of extracted nucleic acid were treated with 1 µl of DNAse I (1 U/µl) and 1 µl of RNase A (20 mg/ml) and incubated for 30 min at 37°C. Digestion products were analyzed by electrophoresis using agarose gel (0.8%) in 1× TBE which were stained with ethidium bromide and visualized under UV transilluminator. The images were taken with a UVP Biolmaging System.

Pathogenicity test

Koch's postulates were carried out by injecting 500 µl of PMeV infected latex into the stem apex of six-months-old healthy papaya plants, using sterile syringe, under controlled conditions (Rodrigues et al., 2009b). Healthy plants were also inoculated with sterile water as negative controls. All plants were evaluated for PMeV presence every 21 days over 90 days.

PMeV diagnosis in flower and different stages of fruit development

Latex was collected from fruits at different stages of development and flowers in papaya plants with symptoms [small (≤ 13 cm); medium (14 to 26 cm) and large (≥ 27 cm)], the samples were collected in accordance with the extraction protocol. In order to obtain PMeV diagnosis, 10 µl of each sample were analyzed by electrophoresis using agarose gel (0.8%) in 1× TBE which were stained with ethidium bromide and visualized under UV transilluminator. The images were taken with a UVP Biolmaging System.

RESULTS

A total of 321 latex samples were obtained from papaya fruits with and without PMeV symptoms in papaya orchards in Quintana Roo (Table 1). The symptoms observed were similar to those reported for PMeV in Brazil (Zambolim et al., 2003). The most distinct and typical symptom produced by Papaya meleira virus is the spontaneous exudation of translucent and watery latex of an infected fruit (Figure 1a). Other symptoms are: the subsequent oxidation of latex that originates dark scabs on fruit surfaces (Figure 1b), small internal blotches in the pulp of a diseased fruit (Figure 1c) and necrotic spots in petioles (Figure 1d). Figure 2 shows the difference between latex consistency of a diseased fruit (Figure 2a) and a healthy one (Figure 2b). All latex samples were extracted following the protocol 1 described by Tavares et al. (2004). As can be seen in Figure 3, infected plants present four distinctive bands of nucleic acids, one of which, an intense 12 Kb band is also present in the positive control from Brazil, while only one band above

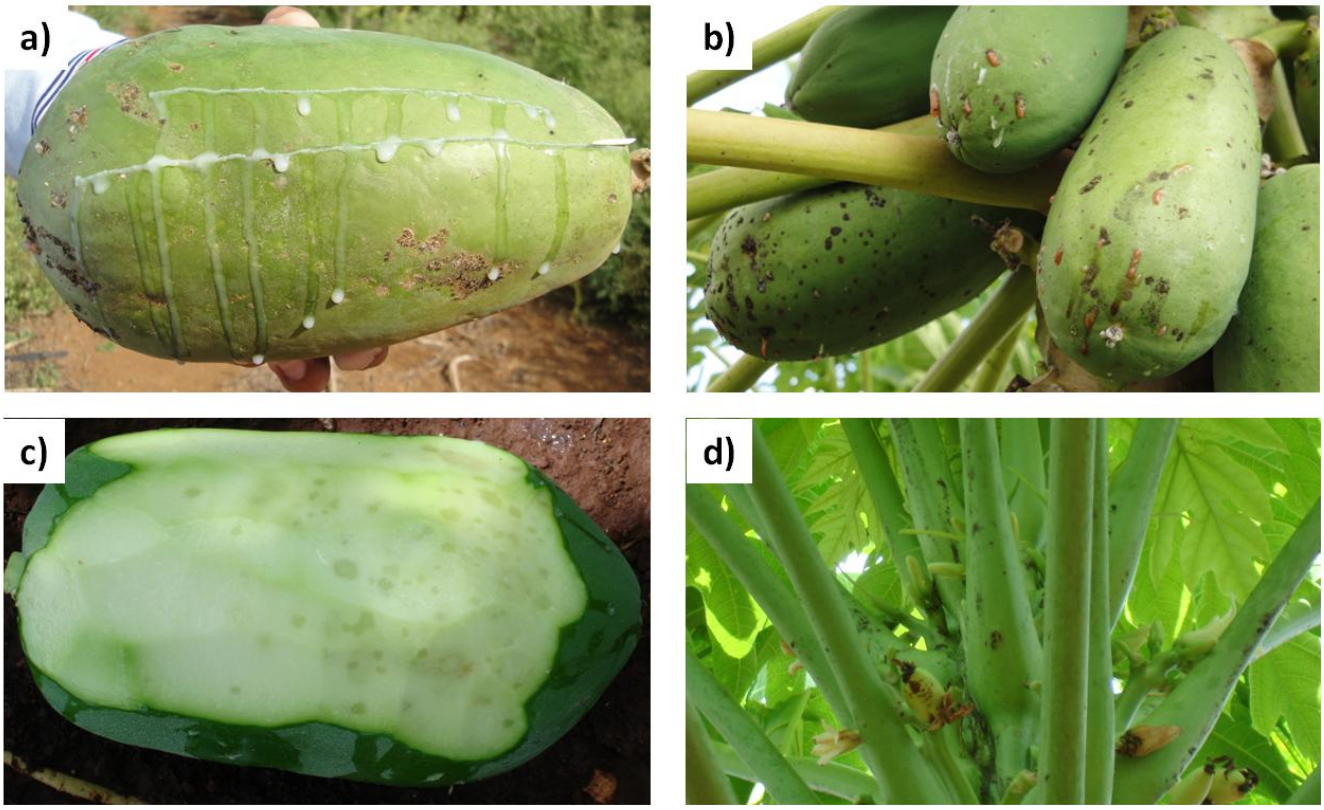

Figure 1. Papaya meleira virus (PMeV) symptoms in *Carica papaya* in Yucatan, Mexico. (a) translucent and watery latex of an infected fruit, (b) dark scabs on diseased fruit surface caused by oxidized latex, (c) small internal blotches in the pulp of a diseased fruit, (d) necrotic spots. in petioles.

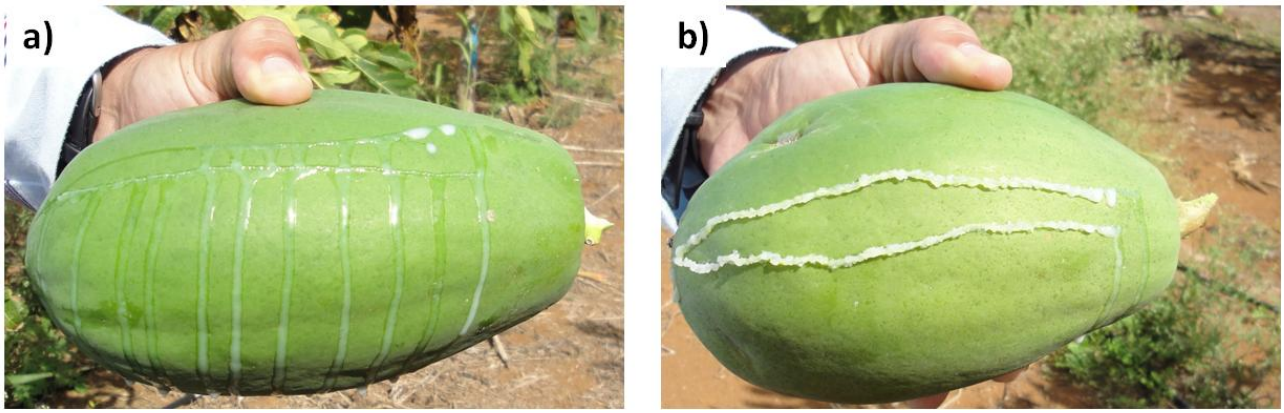

Figure 2. Comparison of latex consistency in (a) infected fruit and (b) healthy fruit.

12 Kb can be observed in the healthy plants.

The analysis of the nature of nucleic acids by differential digestion with the DNAse I and RNAse endonucleases (Figure 4) revealed that, of the four bands resulting from the extraction (lane 1), once the sample was treated with DNAse I (lane 2), only the band above 12 Kb was degraded by the enzyme, while the other bands (12 Kb) remained intact, indicating that the degraded band is DNA. On the other hand, when the sample was treated with RNAse A (lane 3), only the band above 12 Kb was not degraded, demonstrating that the 12 Kb band is RNA. The pathogenicity assay led to the detection of viral dsRNA in the inoculated plants with PMeV infected latex after 21 days. Also, the infected

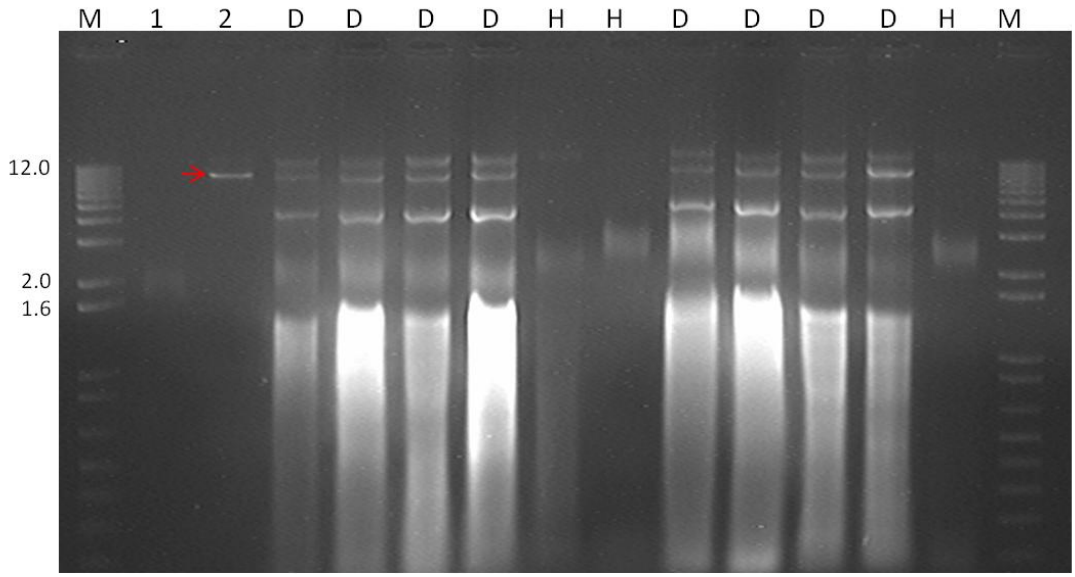

Figure 3. Nucleic acids extracted from latex of papaya fruit (*Carica papaya*) obtained from diseased plants (D) and healthy plants (H). Lane 1, latex from healthy plant (negative control); lane 2, latex from PMeV infected plant (positive control); M: 1 Kb plus DNA ladder maker.

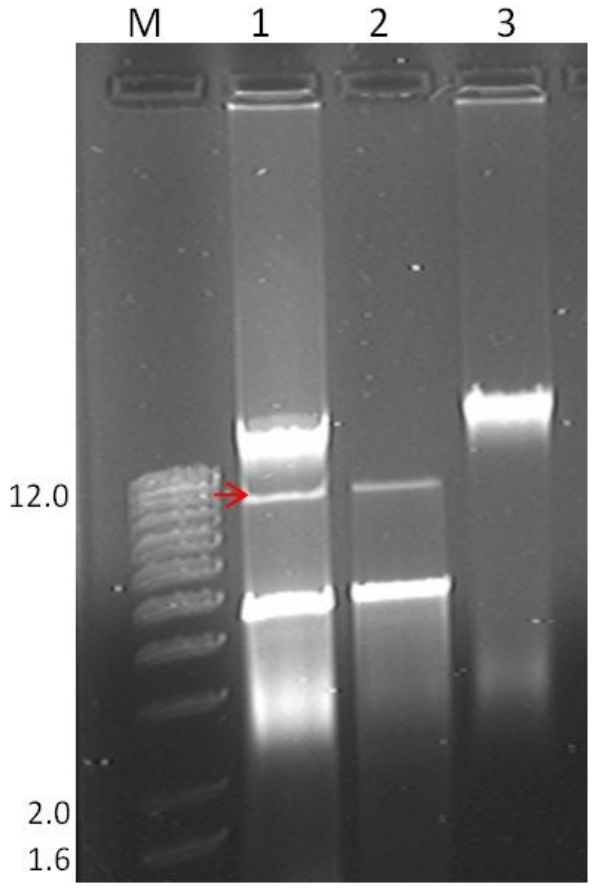

Figure 4. Nature of nucleic acid bands. M: Molecular marker 1 Kb plus DNA Ladder; lane 1, the extract without treatment; lane 2, treatment with DNAse I; lane 3, treatment with RNAse A.

plants showed spontaneous latex exudation 30 days after inoculation.

As can be seen in Table 2 and Figure 5, the PMeV diagnosis carried out on flowers (to our knowledge not previously reported) and fruits at different stages of development (small, medium, large), from plants with symptoms, was positive for PMeV in every case. The results of latex sampling conducted in ten states of Mexico are shown in Figure 6. Of 1150 latex samples collected, 533 (46.55%) were positive to PMeV in seven states (Campeche, Jalisco, Quintana Roo, Oaxaca, Tabasco, Veracruz and Yucatan). The occurrence of PMeV was located mainly in the papaya growing areas of states on the Gulf of Mexico, the Pacific and in the south-southeast region.

DISCUSSION

The diagnostic technique based on the analysis of dsRNA has shown to be reliable for the diagnosis of PMeV, and is being used in many studies all over the world for the detection of this disease (Rodrigues et al., 2009a, b, c, 2005; Araujo et al., 2007; Tavares et al., 2004). The presence of a 12 Kb band and the results obtained with different endonucleases concur with previous reports (Rodrigues et al., 2005; Tavares et al., 2004), in which this band is associated with the dsRNA of the PMeV virus particle, which has a length of approximately 12 000 nucleotides in its genome (Zambolim et al., 2003). The protocol described by Tavares et al. (2004) gave accurate results for all samples tested, thereby verifying the repeatability and

Table 2. PMeV diagnosis in flower and different stages of fruit development.

Sample key	Flower	Fruit size		
		S	M	L
UE3-9	+	+	+	+
UE2-21	+	+	+	+
UE2-24	+	+	+	+
UE2-27	+	+	+	+
UE2-28	+	+	+	+
UE2-32	+	+	+	+
Q-9	+	+	+	+
Q-25	+	+	+	+
Q-28	+	+	+	+
Q-30	+	+	+	+

S: Small (≤13 cm); M: medium (14 to 26 cm); L: Large (≥27 cm).

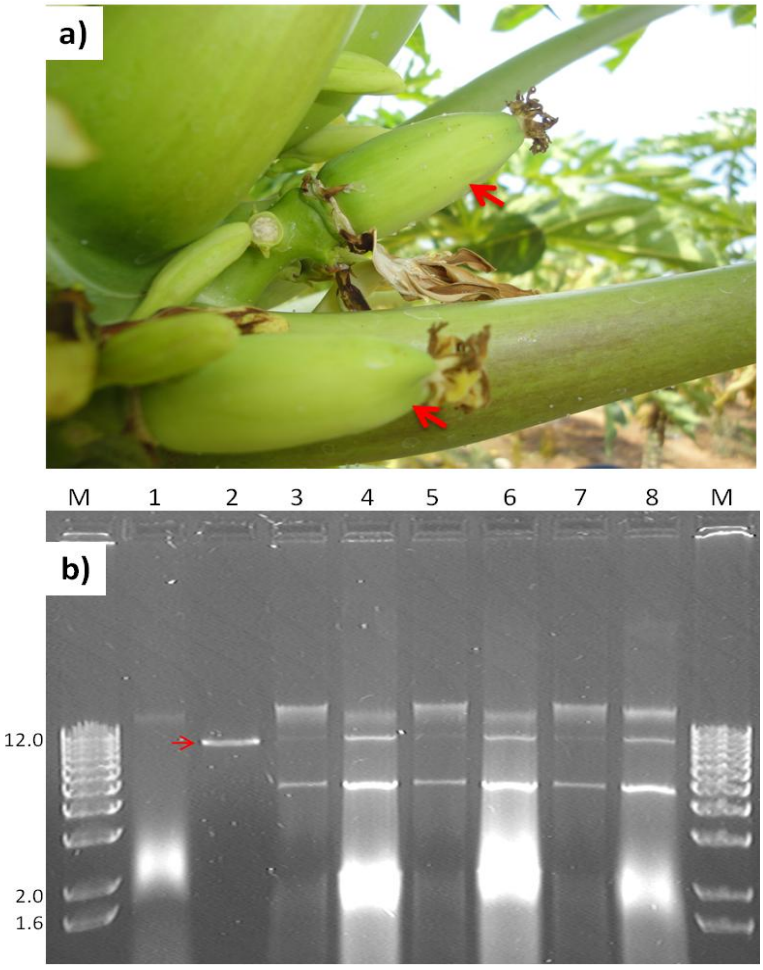

Figure 5. PMeV diagnosis based on symptomatology and nucleic acid extraction: (a) PMeV symptoms from infected papaya flowers; (b) detection of infected papaya flowers and fruits. Lane 1, latex from healthy plant (negative control); lane 2, latex from PMeV infected plant (positive control); lane 3 to 4, flower and fruit of Q-25, respectively; lane 5 to 6, flower and fruit of Q-28, respectively; lane 7 to 8, flower and fruit of Q-30, respectively; lane M, 1 Kb plus DNA ladder maker.

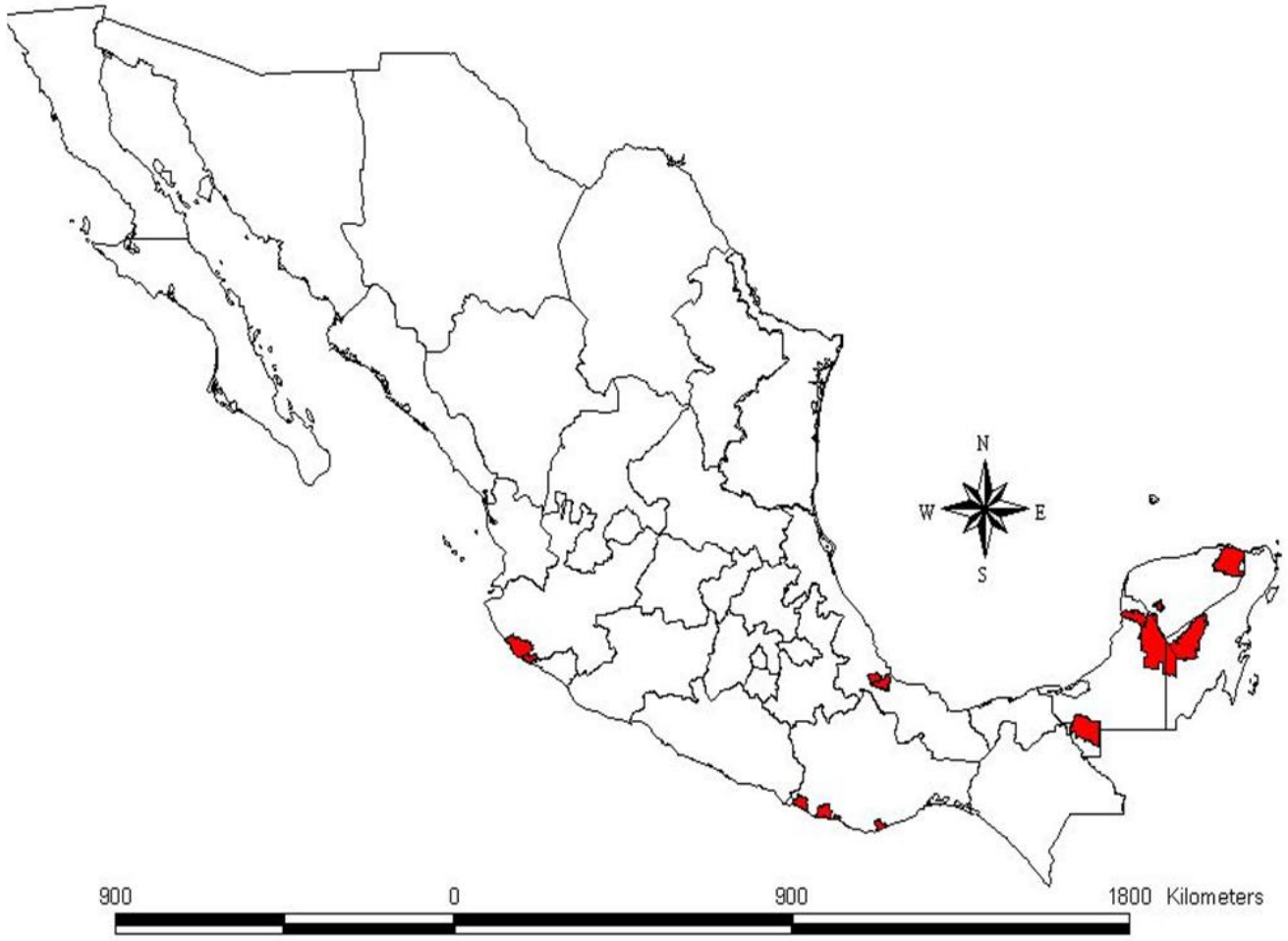

Figure 6. Papaya meleira virus distribution in Mexico. The red zones indicate the areas that gave positive results to PMeV.

solidity of this technique, which is based on the analysis of nucleic acids for the diagnosis of this virus. Koch's postulates confirmed PMeV as the causal agent of meleira disease, which is consistent with the results obtained by Rodrigues et al. (2009b).

Being able to detect PMeV in flowers and small fruits is an enormous advantage in the diagnosis of this virus, facilitating the early detection of the disease in plants with and without symptoms and the subsequent application of control measures, since an asymptomatic plant could continue as an inoculum source in the orchard until its detection and elimination (Vidal et al., 2000). PMeV molecular screening conducted in different papaya growing areas of Mexico revealed the presence of the disease in an extensive area; these findings are similar to sticky disease behavior in Brazil, where first detection was in Bahia state in 1987, followed by Espirito Santo, and by 1989, PMeV had been detected in the rest of the papaya growing regions causing significant yield losses (Lima et al., 2001). These diagnostic results confirmed the presence of the Papaya meleira virus (PMeV) in Mexico and constitute the first report of this disease in this country. All these results indicate the serious phytosanitary risk this pathogen represents to Mexico, and the need for its early detection in order to initiate control measures and avoid the potential risk to the papaya crop.

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