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SHORT COMMUNICATION / *COMUNICAÇÃO*

The wild type of *Momordica charantia* is not infected by potyviruses that cause disease in papaya and cucurbit crops

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ABSTRACT

In the present work, the susceptibility of wild and domesticated plants of *Momordica charantia* to viruses from the genus *Potyvirus* that cause diseases in papaya (*Carica papaya*) and cucurbit crops was evaluated. The plants were subjected to experimental and natural infection with *Papaya ringspot virus* types P and W (PRSV-P and PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV). None of the potyviruses infected the wild type of *M. charantia* through mechanical or aphid inoculation or under field exposition, whereas the domesticated type was only infected by isolates of ZYMV. In addition, both wild and domesticated types of *M. charantia* were not infected in natural conditions by an isolate of *Zucchini lethal chlorosis virus*, genus *Tospovirus,* transmitted by *Frankliniella zucchini*. These data clearly indicated that this wild type of *M. charantia* does not seem to have a role in the epidemiology of the diseases caused by these three potyviruses in Brazil.

Key words: *Carica papaya*, *Cucurbita pepo*, aphid transmission, disease management, potyvirus

Papaya ringspot virus, types P and W (PRSV-P; PRSV-W) and *Zucchini yellow mosaic virus* (ZYMV), family *Potyviridae,* genus *Potyvirus,* cause significant diseases in economically important crops around the world. PRSV-P causes the ringspot disease of papaya (*Carica papaya* L.), which is considered a limiting factor for papaya production everywhere this tropical species is grown (Gonsalves, 1998). In addition to papaya, PRSV-P can also experimentally infect wild species of *Vasconcellea*, which were traditionally included in the genus *Carica* (Conover, 1964; Torres & Giacometti, 1966; Cook & Milbrath, 1971), and some cucurbit species/varieties show variable susceptibility according to the virus isolate (Capoor & Varma, 1958; Sánchez de Luque & Martínez, 1977; Sureka et al., 1977; Yeh et al., 1984). Under natural conditions PRSV-P is not important for cucurbit crops. Isolates of PRSV-W and ZYMV cause the common and yellow mosaic diseases, respectively, in many cucurbit species, dramatically reducing the yield, particularly for the most susceptible species/varieties, including zucchini squash (*Cucurbita pepo* L) (Rezende & Pacheco, 1998; Lecoq et al., 2009). All the three potyviruses are transmitted by several species of aphids in a non-persistent manner (Purcifull et al., 1984; Desbiez & Lecoq, 1997).

The control of these diseases is very difficult throughout the world. Except for the resistant transgenic papaya hybrids commercially grown in Hawaii (Tripathi et al., 2008) and the interspecific hybrid developed by Siar et al. (2011), all varieties of papaya are susceptible to infection by PRSV-P. For more than 25 years, management of papaya ringspot disease in Brazil has been performed through the adoption of several cultural practices that minimise the problems associated with PRSV-P in the commercial orchards. These practices include the following: the use of virus-free seedlings for new plantings; the eradication of old and abandoned orchards before starting new crops; the avoidance of cucurbit plants, which may harbour the virus within the proximity of the orchard; and, in particular, the roguing of diseased plants by means of systematic inspections of the crops by well-trained personnel (Ventura et al., 2004). Although growers are recommended to avoid the planting of cucurbit plants within or near the orchard, recent studies conducted in Brazil by Mansilla et al. (2013) revealed that these species do not have a significant importance on the epidemiology of papaya ringspot disease. The control of PRSV-W and ZYMV relies mainly on the use of resistant/tolerant cucurbit varieties whenever available. In addition, growers are also recommended to eradicate old and abandoned crops before starting new ones and avoid the planting of any other cucurbit plants (wild and/or cultivated) near the crops to reduce the sources of virus inocula (Zitter et al., 1996). Among the wild cucurbit plants that should not be allowed within the vicinity of papaya and cucurbit crops is *Momordica charantia* L. (bitter gourd, balsam pear), a plant that has shown contradictory results of susceptibility to these potyviruses (Adsuar, 1950, Capoor & Varma, 1958; Conover, 1964; Sánchez de Luque & Martínez, 1977; Pearson & Liyanage, 1997; Chin et

al., 2007; Silveira et al., 2009). Therefore, the purpose of this work was to investigate the susceptibility of wild and domesticated types of *M. charantia* to experimental and natural infections with PRSV-P, PRSV-W, and ZYMV. In addition, natural infection with the tospovirus *Zucchini lethal chlorosis virus* (ZLCV) was also evaluated.

Wild and domesticated types of *M. charantia*, also known as 'nigauri' or 'goya', *C. pepo* cv. Caserta and *C. papaya* cv. Golden were used for the experiments. The wild and domesticated types of *M. charantia* are mainly differentiated by the size of their fruits and seeds. The fruits are spindle-shaped, measuring approximately 5 cm long in the wild type population and up to 25 cm long in the domesticated type. The seed size is proportional to the fruit size. The leaves of domesticated type are also larger than those of the wild type population. Seeds of both types of *M. charantia* were obtained from plants naturally grown in the campus Luiz de Queiroz, University of São Paulo, Piracicaba, São Paulo state, Brazil.

ZYMV isolates were obtained in the states of São Paulo (ZYMV-RI), Rio Grande do Sul (ZYMV-RS), Mato Grosso (ZYMV-MT), and Brasilia, DF (ZYMV-Fe, ZYMV-DF). The PRSV-P isolates were from the states of São Paulo (PRSV-P CF), Pernambuco (PRSV-P PE), and Santa Catarina (PRSV-P SC). The PRSV-W isolates were from São Paulo (PRSV-W C) and Brasilia, DF (PRSV-W DF). The experimental transmission tests were performed through mechanical and aphid inoculations. For the mechanical inoculation, infected leaves were ground in 0.02 M phosphate buffer, pH 7.0, containing 0.02 M sodium sulfite, diluted 1:10 (w/v). The inoculum was rubbed on leaves dusted with carborundum. The vector transmission tests were performed using virus-free *Myzus persicae* Sulzer reared on sesame (*Sesamum indicum* L.) and wild radish (*Raphanus raphanistrum* L). Viruliferous aphids were starved for 60 min and then transferred to the source of inoculum for an acquisition access period of 30 min. Afterward, the aphids were transferred in groups of 15 to each test plant. The inoculation access period was 2 h after which the aphids were eliminated with insecticide. For each transmission test, two plants of *C. pepo* were used as control for the infectivity of PRSV-W and ZYMV isolates, whereas two plants of *C. papaya* were used as control for PRSV-P isolates.

Virus infections in the inoculated plants were confirmed by symptomatology, RT-PCR, and plate-trapped antigen enzyme-linked immunosorbent assay (PTA-ELISA). Back inoculation to *C. pepo* for detection of PRSV-W and ZYMV and to *C. papaya* for detection of PRSV-P was done whenever the other assays did not yield conclusive results. Total RNA was extracted from the plant tissues according to Bertheau et al. (1998) and one-step RT-PCR was performed using the PCR Master kit reagents (Fermentas) according to the manufacturer's instructions. The sense ZY2 and antisense ZY3 (Thomson et al., 1995) specific primers, whose amplicon has 1186 base pairs, were used to detect isolates of ZYMV. The PV1 antisense (Gibbs & Mackenzie, 1997) and sense primer designed for the WCIEN motif of the CP gene of potyviruses (Mota et al., 2004), which amplify a fragment of 800 base pairs, were used to detect the PRSV strains. PTA-ELISA (Mowat & Dawson, 1987) was performed using polyclonal antisera raised against ZYMV, PRSV-P, and PRSV-W. Antisera against these potyviruses were from the collection of the Laboratory of Plant Virology, ESALQ/USP, Piracicaba, SP. Primary antisera were diluted 1:1000, whereas secondary antiserum (SIGMA A-8025) was diluted 1:32000. The appropriate positive and negative controls were included in duplicate wells for each assay. The absorbance values (405 nm) were measured at 30 and 60 min after the substrate was added. The samples were considered positive when the average absorbance value was at least three times higher than the average absorbance value of the negative control. Detection of ZLCV was done only by PTA-ELISA using polyclonal antiserum provided by Dr. Alice K. Inoue-Nagata, Embrapa, CNPH, Brasília, DF, following the same procedure described above.

From November 2010 to March 2012, four experiments were conducted in a greenhouse to test the susceptibility of wild and domesticated types of *M. charantia* to different isolates of ZYMV, PRSV-W and PRSV-P by means of mechanical and aphid inoculations. The number of inoculated wild and domesticated *M. charantia* plants differed according to the virus isolate (Table 1). Zucchini squash plants were included in all of the tests as a control for the infectivity of ZYMV and PRSV-W, whereas papaya plants were used as the control for the infectivity of PRSV-P. The plants were maintained in the greenhouse, and 15 days after inoculation, samples from the inoculated leaves were tested by PTA-ELISA, RT-PCR and biological assays for virus detection. The biological recovery test consisted of the mechanical inoculation of zucchini squash or papaya plants with extracts from the *M. charantia* plants inoculated with the different potyviruses. Thirty days later, a systemic infection on the upper leaves of wild and domesticated types was evaluated based on the expression of symptoms, and virus detection was performed as described above.

During March 2011 and April 2012, nine plants of each type of *M. charantia* were evaluated for natural infection with ZYMV, PRSV-W, and PRSV-P, and ZLCV. Control plants of *C. pepo* cv. Caserta and *C. papaya* were planted near *M. charantia* types to serve as the controls for the infection with these viruses. The *C. pepo* plants were renewed three times during that period, with a total of 30 plants. Evaluations were also based on the expression of symptoms, PTA-ELISA, and RT-PCR as described before. Samples from symptomatic *C. pepo* were tested in groups of five plants.

Mechanical and aphid transmission tests performed with all three potyviruses under greenhouse conditions revealed that only the ZYMV isolates were able to infect the domesticated type of *M. charantia*. The ZYMV-MT

TABLE 1 - Reaction of wild and domesticated types of *Momordica charantia* to mechanical and aphid inoculations with *Papaya ringspot virus*, types P and W (PRSV-P; PRSV-W), and *Zucchini yellow mosaic virus* (ZYMV) evaluated by symptomatology, PTA-ELISA, and RT-PCR

Type of *Momordica charantia*	Virus species	No. infected plants/No. inoculated plants					
		Mechanical inoculation				Aphid inoculation	
		Inoculated leaves		Upper leaves		Upper leaves	
		ELISA	RT-PCR	ELISA	RT-PCR	ELISA	RT-PCR
Wild	ZYMV-RI	0/6	0/6	0/6	0/6	1/4[a]	0/4
	ZYMV-RS	0/4	0/4	0/4	0/4	0/4	0/4
	ZYMV-MT	0/4	0/4	0/4	0/4	nt	nt
	ZYMV-Fe	nt[b]	nt	nt	nt	0/4	0/4
	PRSV-W-C	0/6	0/6	1/6 [a]	0/6	0/4	0/4
	PRSV-W-DF	0/4	0/4	1/7 [a]	0/7	nt	nt
	PRSV-P CF	0/6	0/6	0/6	0/6	0/4	0/4
	PRSV-P PE	0/4	0/4	0/4	0/4	0/4	0/4
	PRSV-P SC	nt	nt	0/3	0/3	0/4	0/4
Domesticated							
	ZYMV-RI	0/4	0/4	0/4	0/4	3/4	3/4
	ZYMV-RS	0/4	0/4	1/4	2/4[c]	4/4	4/4
	ZYMV-MT	2/4	1/4	0/4	0/4	nt	nt
	ZYMV-DF	nt	nt	nt	nt	2/4	1/4
	ZYMV-Fe	nt	nt	nt	nt	0/4	0/4
	PRSV-W-C	0/4	0/4	0/4	0/4	0/4	0/4
	PRSV-W-DF	0/4	0/4	0/8	0/8	nt	nt
	PRSV-P CF	0/4	0/4	0/4	0/4	0/4	0/4
	PRSV-P PE	0/4	0/4	0/4	0/4	0/4	0/4
	PRSV-P SC	nt	nt	0/4	0/4	0/4	0/4

[a] Biological recovery tests were negative.
[b] nt: not tested.
[c] Biological recovery test was positive.

isolate was only detected on the inoculated leaf of one plant, but did not move systemically. The ZYMV-RS isolate was able to systemically infect two out of four plants when inoculated mechanically and all four plants when the inoculation was performed by aphids. ZYMV-RI and ZYMV-DF, when inoculated by aphids were detected by PTA-ELISA and RT-PCR on the upper leaves of three and one out of four plants, respectively. None of the wild type of *M. charantia* plants inoculated (mechanically or by aphids) with the three potyviruses was infected (Table 1). Although some plants inoculated with isolates ZYMV-RI, PRSV-W-C, and PRSV-W-DF tested positive by PTA-ELISA, RT-PCR and biological recovery assays did not confirm systemic infections. All *C. pepo* and *C. papaya* plants used for the control of the infectivity of the virus isolates were infected (data not shown). When exposed for 14 months to natural infection in the field, only one domesticated type plant was infected with ZYMV. The zucchini squash plants used as control were infected with PRSV-W and/or, ZYMV and/or, ZLCV, whereas the papaya plants were infected with PRSV-P. None of the *M. charantia* plants exposed in the field were infected with ZLCV, which was detected in plants used as the control (Table 2).

As initially mentioned, reports on the susceptibility of *M. charantia* to PRSV-P, PRSV-W and ZYMV are inconsistent. In Jamaica, Chin et al. (2007) were able to recover PRSV-P from symptomatic *M. charantia* plants present in papaya orchards. Chin et al. (2007) also found that high rates of virus transmission by *A. gossypii* were obtained in tests from *M. charantia* to papaya (77-83%), papaya to *M. charantia* (90-93%), and *M. charantia* to *M. charantia* (60-70%). In Florida, USA, Adlerz (1972) identified *M. charantia* as source of *Watermelon mosaic virus* 1 (WMV-1), presently identified as PRSV-W, but Adlerz et al. (1983) were not able to experimentally infect this species with ZYMV and WMV-2, presently identified as WMV. On Reunion Island, Africa, however, ZYMV was reported as infecting systemically *M. charantia* (Desbiez & Lecoq, 1997). A survey of cucurbit viruses in Western Samoa found *M. charantia* infected with ZYMV but not with PRSV-W (Pearson & Liyanage, 1997). In contrast, *M. charantia* was not infected by mechanical inoculation with PRSV-P, as reported by Adsuar (1950) in Puerto Rico, Conover (1964) in Florida, USA, Pinto (1972) in Venezuela, and Sánchez de Luque & Martínez (1977) in Colombia.

In the present work, the wild-type of *M. charantia* was not infected with any of the tested potyviruses through mechanical and aphid inoculations or under field exposure for 14 months, whereas the domesticated type was only infected with isolates of ZYMV under experimental

TABLE 2 - Reaction of wild (W) and domesticated (D) types of *Momordica charantia* to natural infection with *Papaya ringspot virus*, types P and W (PRSV-P; PRSV-W), *Zucchini yellow mosaic virus* (ZYMV), and *Zucchini lethal chlorosis virus* (ZLCV), evaluated by symptomatology, PTA-ELISA and RT-PCR

Test plant	No. infected plants/No. exposed plants			
	PRSV-P	PRSV-W	ZYMV	ZLCV[a]
Momordica charantia - W	0/9	0/9	0/9	0/9
M. charantia - D	0/9	0/9	1/9	0/9
Cucurbita pepo	nt[b]	6/6[c]	6/6[c]	6/6[c]
Carica papaya	6/6	nt	nt	nt

[a] Only evaluated by PTA-ELISA.
[b] nt: not tested.
[c] Tested as groups of five plants.

and natural conditions. These results are in accordance with previous reports on the reaction of wild type of *M. charantia* to the same potyviruses in Brazil. The absence of natural infection of this cucurbit species with PRSV-P was first reported by Barbosa & Paguio (1982) in a survey in the state of Pernambuco. In an assessment in the states of Pernambuco and Bahia for the natural infection of *M. charantia* with PRSV-W, ZYMV, WMV, and *Cucumber mosaic virus* (CMV), Silveira et al. (2009) did not find any infected plants. Yuki et al. (2000) were not able to infect *M. charantia* experimentally with PRSV-W, ZYMV, WMV, and CMV through mechanical and *M. persicae* inoculations. Altogether, these data clearly indicate that wild type of *M. charantia*, widely distributed in Brazil, does not seem to have importance in the epidemiology of the cucurbit diseases caused by ZYMV and PRSV-W and papaya ringspot disease associated with PRSV-P in Brazil, as based on the high resistance to infection with all three potyviruses. The same is true for the domesticated type of *M. charantia* with regard to the diseases caused by strains of PRSV-P and PRSV-W. Recently, Mansilla et al. (2013) showed the relative importance of some cucurbit species in the epidemiology of papaya ringspot disease in Brazil. These authors recommended that the control of the disease through roguing should focus mainly on diseased papaya trees, a method that has been practiced successfully in the country for many years, and on those cucurbits known to be particularly susceptible to natural infection with PRSV-P.

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