Non-transmission of ZYMV and PRSV through Resistant *Cucurbita moschata* Genotypes 'Nigerian Local' and 'Menina'

Magdiel A. Miranda-Vélez and Linda Wessel-Beaver*
Department of Agroenvironmental Science, University of Puerto Rico, Mayagüez, Puerto Rico, USA

Jose Carlos V. Rodrigues
Center for Excellence in Quarantine & Invasive Species, Agricultural Experiment Station, University of Puerto Rico at Mayagüez, Río Piedras, Puerto Rico, USA

**lindawessel.beaver@upr.edu*

Introduction

Surveys conducted in 2001 and 2002 (Paz-Carrasco and Wessel-Beaver, 2002) and in 2006 to 2011 (Rodrigues et al., 2012) found a high incidence of *Zucchini yellow mosaic virus* (ZYMV) and *Papaya ringspot virus* (PRSV) in cucurbits in Puerto Rico. Severe virus and virus vector outbreaks are associated with low yields and limitations to growing cucurbits in Puerto Rico. Overlapping of susceptible cucurbit crops and continuous growing of cucurbit crops throughout the year makes Puerto Rico, an ecologically diverse island with an abundance of alternative hosts and large, year-round populations of insect vectors, an excellent and dynamic environment for plant viruses (Rodrigues et al., 2012). For these reasons, it is very difficult to develop strategies of control that do not consider the use of genetic resistance.

'Nigerian Local' and 'Menina' are two well-known sources of resistance to PRSV and ZYMV in *Cucurbita moschata*. At least five loci are thought to be involved in controlling resistance to ZYMV, and the genes involve vary depending on the source of resistance (Pachner et al., 2011). 'Nigerian Local' carries two dominant genes for resistance (*Zym-0* and *Zym-4*), while resistance in 'Menina' is conferred by *Zym-1*. The inheritance of resistance to PRSV has been less well studied. 'Nigerian Local' appears to carry at least two genes for resistance to PRSV (McPhail-Medina et al., 2012).

Whether the resistance to ZYMV and PRSV in 'Nigerian Local' and 'Menina' is related to impeded replication and/or translocation of the virus in the plant, or some other mechanism, is not known. If resistance is primarily a matter of either reduced replication or minimal translocation of the virus within the plant, we wondered if resistant genotypes could still serve as a sufficiently large reservoir of virus to enable them to infect susceptible genotypes. For resistant genotypes to serve as an effective source of resistance, the answer to this question should be "no." Therefore, the objective of this work was to determine if plants of 'Nigerian Local' and 'Menina', mechanically inoculated with ZYMV and PRSV, produce sufficient virus titer to enable those plants to infect susceptible genotypes of *C. moschata*.

Materials and Methods

Cotyledons of six-days-old plants of resistant genotypes 'Menina' and 'Nigerian Local' were mechanically inoculated with either PRSV or ZYMV. Eighteen days post-inoculation, tissue from these plants (three plants each of 'Menina' inoculated with PRSV, 'Menina' inoculated with ZYMV, 'Nigerian Local' inoculated with PRSV, and 'Nigerian Local' inoculated with ZYMV) was used as inoculum to mechanically inoculate cotyledons of six-day-old plants of highly susceptible *C. moschata* genotypes 'Waltham' and 'Mos166'. For each of the four genotype-virus combinations, five plants were inoculated. At 20 days post-inoculation, the fourth leaf of each inoculated plant was tested with ELISA for the virus used in the inoculation. Readings of <0.400 were consider negative for virus. Data was analyzed as a factorial arrangement (2 genotypes x 2 inoculation treatments) in a one-way analysis of variance with four or five replications (a few plants died during the experiment). Means were compared with Fisher's Least Significant Difference test at the 0.05 level of probability. Plants were evaluated for the presence of any virus symptoms until 25 days post-inoculation.

Results and Discussion

No virus symptoms were observed on either the inoculum source plants ('Menina' and 'Nigerian Local' inoculated with each virus) nor the test plants ('Waltham' and 'Mos166'). Source plants had weakly positive ELISA readings in some cases. 'Menina' source plants inoculated with PRSV or ZYMV

had average ELISA readings of 0.374 and 0.671, respectively, on tissue sampled from the fourth leaf. 'Nigerian Local' source plants inoculated with PRSV or ZYMV had average ELISA readings of 0.462 and 0.360, respectively. Susceptible genotypes 'Waltham' and 'Mos166' had negative ELISA readings when inoculated with fresh inoculum from resistant genotypes 'Menina' and 'Nigerian Local' that had been previously inoculated with either PRSV (Table 1) or ZYMV (Table 2).

In this experiment resistant genotypes 'Menina' and 'Nigerian Local' did not have the capacity to transmit PRSV or ZYMV to susceptible genotypes 'Waltham' and 'Mos166'. 'Menina' and 'Nigerian Local', when mechanically inoculated with PRSV and ZYMV, are not suitable hosts for replication of these two viruses. While this experiment was conducted in the greenhouse, we expect that vector-infected plants of 'Menina' or 'Nigerian Local', or genotypes that carry the same genes for resistance, would also be unable to serve as sources of ZYMV or PRSV inoculum in the field. This research supports of use of 'Nigerian Local' and 'Menina' as excellent sources of resistance to ZYMV and PRSV in *C. moschata*.

Acknowledgement

This work was supported by USDA National Institute of Food and Agriculture, Specialty Crop Research Initiative project 2015-51181-24285, and the Puerto Rico Agricultural Experiment Station. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the USDA or NIFA. This paper is a portion of a thesis submitted by the first author in partial fulfillment of the requirements in a master's degree program.

Literature Cited

1. McPhail-Medina, R., Wessel-Beaver, L., Rodrigues, J.C.V. 2012. Inheritance of resistance to Papaya ringspot virus in tropical pumpkin is controlled by at least two genes. *In:* (Sari, Solmaz and Aras, eds.) Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, pp. 697-701.
2. Pachner, M., H.S. Paris, and T. Lelley. 2011. Genes for resistance to zucchini yellow mosaic in tropical pumpkin. Journal of Heredity. 102(3):330–335. http://doi.org/10.1093/jhered/esr006.
3. Paz-Carrasco, L. and L. Wessel-Beaver. 2002. Survey of cucurbit viruses in Puerto Rico. D. N. Maynard (ed.), Cucurbitaceae 2002: 256-264. American Society for Horticultural Science Press. Alexandria, VA.
4. Rodrigues, J.C.V., I. Quintero-Lopez and L. Wessel-Beaver. 2012. Potyviridae as a major challenge to growing cucurbits in Puerto Rico. *In:* (Sari, Solmaz and Aras, eds.) Proceedings of the Xth EUCARPIA meeting on genetics and breeding of Cucurbitaceae, pp. 786-790.

Table 1. Mean *Papaya ringspot virus* (PRSV) ELISA readings (A_{405nm}) of susceptible genotypes 'Waltham' and 'Mos166' inoculated with sap from plants of 'Menina' and 'Nigerian Local' inoculated with PRSV

Inoculum Source	Tested Genotype	PRSV ELISA Reading
Menina	Waltham	0.310 a
	Mos166	0.275 a
Nigerian Local	Waltham	0.339 a
	Mos166	0.259 a
Mean		0.296
Genotype F-test		0.0723
Source of inoculum F-test		0.8182
Interaction F-test		0.4461
LSD (0.05)		0.090

The fourth leaf was sample at 20 days after inoculation.
LSD=Least Significant Difference at α=0.05 for the inoculum source x tested genotype combination of treatments.
Within a column, means followed by a common letter are not significantly different according to Fisher's Least Significant Difference (α=0.05).

Table 2. Mean *Zucchini yellow mosaic virus* (ZYMV) ELISA readings (A_{405nm}) of susceptible genotypes 'Waltham' and 'Mos 166' inoculated with sap from plants of 'Menina' and 'Nigerian Local' inoculated with ZYMV

Inoculum Source	Tested Genotype	ZYMV ELISA Reading
Menina	Waltham	0.226 a
	Mos166	0.276 a
Nigerian Local	Waltham	0.245 a
	Mos166	0.254 a
Mean		0.250
Interaction F-test		0.4606
LSD (0.05)		0.087
Genotype F-test		0.2974
Source of inoculum F-test		0.9559

The fourth leaf was sampled at 20 days after inoculation.

LSD= Least Significant Difference at α=0.05 for the inoculum source x tested genotype combination of treatments.

Within a column, means followed by a common letter are not significantly different according to Fisher's Least Significant Difference (α=0.05).