

# Inheritance of resistance to *Papaya ringspot virus* in tropical pumpkin is controlled by at least two genes

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## Abstract

Tropical pumpkin (*Cucurbita moschata*) in Puerto Rico is often infected with viruses of the *Potyviridae* family. One of the most common is *Papaya ringspot virus* (PRSV). A previous study concluded that the African landrace ‘Nigerian Local’ (NL) carries a single recessive gene for resistance to PRSV. The study used the susceptible cultivar ‘Waltham’, and classified the F<sub>2</sub> into two phenotypic classes. When using NL to introgress PRSV resistance into cultivars of tropical pumpkin, we observed more than two classes of disease severity. Thus, the purpose of this study was to re-investigate the inheritance of PRSV resistance from NL. ‘Waltham’ and three susceptible tropical pumpkin cultivars were crossed with NL. Plants in the parental, F<sub>1</sub> and F<sub>2</sub> populations were classified for PRSV symptom severity on a 0 (no symptoms) to 3 (severe symptoms) scale. The four phenotypic classes (0, 1, 2 and 3) were grouped in several ways, and 1-gene and 2-gene models were tested. As in the previous study, a single recessive gene model (3:1 susceptible:resistant) fit the data from ‘Waltham’ x NL when the resistant class consisted of plants with a severity of 0 or 1 and the susceptible class combined plants with a severity rating of 2 or 3. But no single method of class grouping resulted in good fits of this 1-gene model to the tropical pumpkin F<sub>2</sub> populations. A 13:3 (susceptible:resistant) model (dominant suppression epistasis) provided the best overall fit of the data for both ‘Waltham’ and the tropical pumpkin populations. Plants with severity = 0 were classified as resistant, and plants with severity = 1, 2 or 3 were classified as susceptible. These results suggest that PRSV resistance in both ‘Waltham’ and tropical pumpkin cultivars is controlled by at least two genes: a dominant gene for resistance together with an epistatic dominant suppressor gene.

## INTRODUCTION

Resistance to *Papaya ringspot virus-watermelon strain* (PRSV-W=*Watermelon mosaic virus-1*=WMV-1) has been identified in *Cucurbita moschata* (Duchesne) (Costa 1974; Brown 2003), *C. maxima* (Maluf et al. 1984, 1985, 1986, 1997), *C. ecuadorensis* (Provvidenti et al. 1978) and *C. foetidissima* (Provvidenti et al. 1978; Maluf et al. 1986). Studies with *C. maxima* and *C. ecuadorensis* conclude that the inheritance of resistance within these sources is polygenic (Herrington et al. 1989; Maluf et al. 1985, 1997). Oliveira et al. (2003) also reported quantitative variation in a F<sub>2</sub> population when attempting to introgress genes from resistant *C. moschata* ‘Duma’ (a Brazilian cultivar) into *C. pepo*. In contrast to these previously mentioned studies, Brown et al. (2003) concluded that resistance from *C. moschata* ‘Nigerian Local’ (an African landrace) is controlled by a single recessive gene. Their study used *C. moschata* ‘Waltham’ (a butternut squash adapted to temperate areas) as the susceptible parent, and F<sub>2</sub> progeny were classified into only two phenotypes: resistant and susceptible. They suggested *prv* as the symbol for this source of resistance.

While attempting to introgress PRSV resistance from ‘Nigerian Local’ into tropical pumpkin cultivars, we observed more than just two phenotypic classes in F<sub>2</sub> populations, and deviations from the 3:1 (susceptible:resistant) segregation expected of *prv* as proposed by Brown et al. (2003). This led us to the objective of this research which was to re-investigate the inheritance of PRSV resistance from ‘Nigerian Local’ using both ‘Waltham’ and several tropical cultivars as susceptible parents.

## MATERIALS AND METHODS

F<sub>2</sub> populations were developed from crossing PRSV-resistant ‘Nigerian Local’ with the susceptible parent ‘Waltham’ (as previously tested in Brown et al. (2003)) and three susceptible tropical pumpkin cultivars from the University of Puerto Rico tropical pumpkin breeding program: ‘Soler’, ‘Taína Dorada’ and ‘Verde Luz’. ‘Nigerian Local’ was derived from seed originally provided by R. Provvidenti (formally of the New York Agricultural Experiment Station, Geneva).

The cotyledons of 7 day old seedlings were dusted with carborundum and rubbed with a mixture of PRSV infected tissue and buffer (1 g fresh wt. of inoculum per 10 ml of phosphate buffer). The PRSV isolate was collected in Puerto Rico and maintained on seedlings of ‘Waltham’. Plants were kept in a greenhouse in Mayaguez, Puerto Rico with no artificial lighting. The experiment took place in late December through January when days are generally sunny. Daytime greenhouse temperatures were from 32 to 35 °C. The symptoms on each leaf were noted beginning on the 3<sup>rd</sup> true leaf at 7 days post-inoculation and continuing about every 3 days until 28 days post-inoculation (6<sup>th</sup> or 7<sup>th</sup> true leaf). Symptom severities

reported are from 28 days post-inoculation and were classified on a 0 to 3 scale (no symptoms to severe symptoms) as described in the footnote in Table 1. At day 28, each plant was tested with commercial ELISA (enzyme-linked immunosorbent assay) kits from Agdia (Elkhart, Indiana, USA) to confirm that plants were infected with PRSV and not with *Zucchini yellow mosaic virus* (ZYMV), the other common potyvirus in Puerto Rico and a potential contaminant.

Data from the four phenotypic classes of severity (0, 1, 2, 3) were grouped in various ways (for example, class 0 vs. classes 1+2+3) to test gene models with 2 or 3 phenotypic classes. A total of 11 models were tested using chi-square ( $\alpha=0.05$ ): two 1-gene models (3:1, 1:2:1) and nine 2-gene models (9:3:3:1, 9:4:3, 9:6:1, 7:6:3, 12:3:1, 15:1, 13:3, 11:5, 9:7).

## RESULTS AND DISCUSSION

Symptoms began to appear on the 3<sup>rd</sup> true leaf about 7 days post-inoculation. Severity ratings in Table 1 are from 28 days post-inoculation. ELISA tests detected the presence of PRSV in most plants and did not detect contamination by ZYMV (data not shown). All plants of ‘Nigerian Local’ remained free of symptoms throughout the study and their ELISA tests were negative. However, some F<sub>2</sub> plants had positive ELISA readings despite severity ratings of 0. Plants of the susceptible cultivars had ratings greater than 0, and most plants had ratings from 2 to 3. Plants in the F<sub>1</sub> populations were susceptible (ratings > 0), with the exception of ‘Taína Dorada’ x ‘Nigerian Local’. In the F<sub>2</sub>, a 3:1 (susceptible:resistant) model fit ‘Waltham’ x ‘Nigerian Local’ (p=0.078) when plants were grouped into severity class = 0 vs. severity class = 1+2+3 and had an even better fit (p=0.609) when grouped into severity class = 0+1 vs. severity class = 2+3. However, these groupings did not always result in a good fit for the other populations. Of the eleven models tested, the best overall model was 13:3 (susceptible:resistant) (p=0.495, 0.415, 0.095, and 0.227 for the ‘Waltham’, ‘Soler’, ‘Taína Dorada’ and ‘Verde Luz’ F<sub>2</sub> populations, respectively). In this case, plants were grouped into severity=0 as the resistant class and severity=1+2+3 as the susceptible class. The results in the F<sub>1</sub> populations, with the exception of ‘Taína Dorada’ x ‘Nigerian Local’, also fit the 13:3 model.

The 13:3 model is known as “dominant suppression epistasis.” A possible explanation for this 2-gene model is a dominant gene for resistance working together with a dominant suppressor gene negating the dominant resistance. Resistance would only be expressed when at least one dominant gene for resistance is present together with the homozygous recessive state at the suppressor locus.

In practical terms, selection for PRSV resistance has proven successful in our breeding program at the Agricultural Experiment Station at the University of Puerto Rico, Mayaguez. We have been able to develop PRSV-resistant F<sub>4</sub> families with good horticultural attributes by crossing our best tropical pumpkin cultivars with ‘Nigerian Local’ followed by several generations of backcrossing and selfing.

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Table 1. Number of plants in each severity class in parental, F<sub>1</sub> and F<sub>2</sub> populations of *Cucurbita moschata* inoculated with *Papaya ringspot virus*.

Population	No. of plants	Severity <sup>z</sup>			
		0	1	2	3
<i>Parents:</i>					
Nigerian Local	16	16	0	0	0
Waltham	18	0	0	0	18
Soler	20	0	2	11	7
Taina Dorada	19	0	0	2	17
Verde Luz	19	0	0	1	18
<i>F<sub>1</sub>:</i>					
Waltham x Nigerian Local	6	0	0	0	6
Soler x Nigerian Local	20	0	0	12	8
Taina Dorada x Nigerian Local	14	3	6	3	2
Verde Luz x Nigerian Local	16	0	7	7	2
<i>F<sub>2</sub>:</i>					
Waltham x Nigerian Local	53	8	4	9	32
Soler x Nigerian Local	130	28	21	37	44
Taina Dorada x Nigerian Local	124	16	14	25	69
Verde Luz x Nigerian Local	124	18	15	31	60

<sup>z</sup> The degree of symptom severity was rated 28 days post-inoculation on the 3<sup>rd</sup> to the 7<sup>th</sup> true leaf of seedlings using the following 0 to 3 scale: 0 = absence of virus symptoms on all leaves; 1 = small chlorotic flecks or spots on <10% of the leaf area with symptoms diminishing on newer leaves; 2 = large chlorotic flecks extending to complete vein clearing and/or coalescing chlorotic spots (mottling) on 10 to 25% of leaf surface, symptoms similar in each new leaf; 3 = mosaic, vein clearing, blistering and/or leaf distortion on more than 25% of the leaf area, new leaves with similar or more severe symptoms.

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