

SUSCEPTIBILITY ASSESSMENT OF WATERMELON AND PUMPKIN CULTIVARS TO POTYVIRUS IN TOCANTINS

AVALIAÇÃO DA SUSCEPTIBILIDADE DE CULTIVARES DE MELANCIA E ABÓBORA A POTYVIRUS OBTIDOS DO ESTADO DE TOCANTINS

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ABSTRACT: Cucurbits cultivation has great importance in the food industry. However, in the main producing regions of cucurbits, the increase of cultivation areas has been limited by viral infections. Among the most prevalent viruses there is the Watermelon Mosaic Virus (WMV), Papaya Ringspot Virus (PRSV-W) and Zucchini Yellow Mosaic Virus (ZYMV). The aim of this study was to evaluate the reaction of watermelon genotypes (Crimson sweet and Charleston Gray) and pumpkin (Jacarezinho and Caserta) to isolate watermelon strains in the following cities: Palmas (PYP-12), Gurupi (PYG-14 and PYG-19), Formoso do Araguaia (PYF-6), and Porto Nacional (PyPn-36 and PyPn-39). The experiment was performed in a greenhouse, in a completely randomized design, with 5 testing plants for each repetition to measure the response of these strains to cucurbit genotypes. The inoculated strains were obtained from leaves of watermelon cv Crimson sweet with symptoms. The plants were rated by severity of viral symptoms in intervals of four days during 32 days. It was verified that the cucurbit genotypes showed different susceptibility to the viral strains. However, Caserta and Charleston gray were the ones which had greater sensitivity due to their high severity variation among the tested strains. Thus, it can be observed that the reaction among strains depends on the host and this reaction can be antagonistic or synergistic, depending on the mixed infection. Charleston gray watermelon was the most susceptible to most of the viral strains. Thus, this cultivar should be avoided in these regions.

KEYWORDS: *Citrullus lanatus*. *Cucurbita pepo*. Viral diseases.

INTRODUCTION

Among the cucurbits cultivated in Tocantins, watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) and pumpkin (*Cucurbita pepo*) (AGUIAR et al., 2015) have an important place (DA COSTA et al., 2013). However, the cucurbit production in this region has shown high susceptibility to several diseases. Among these diseases, there are some caused by viruses which have been responsible for relevant production losses (BESERRA et al., 2007).

The genus *Potyvirus* has three relevant species for the cucurbit cultivation: *Papaya ringspot virus* strain watermelon (PRSV-W) (RAZEAN HAIREEN; DREW, 2014), *Watermelon mosaic virus* (WMV) (ELBESHEHY et al., 2015) and *Zucchini yellow mosaic virus* (ZYMV) (LECOQ et al. 2009). These viruses are transmitted in a non-persistent way through several species of aphids, including *Myzus persicae* and *Aphis spp.* (STOBBS et al., 2015). The infected plants may show symptoms such as mosaic, reduction of the foliar

limb and leaf deformation which significantly reduce crop yield and also affect the fruit quality (REZENDEZ et al., 1999).

These diseases severity can vary according to the pathogen, host, vector and environment interaction (KOTZAMPIGIKIS et al., 2010). Moreover, the occurrence of mixed infection, in which different viruses strains infect the same plant, can result in more complex symptoms, leading to production losses. The viruses can infect cucurbit in all the development stages. However, the losses are larger when the infection occurs during seedling phase (VAN MUNSTER et al., 2003).

In several locations worldwide, most of the economic losses in cucurbit production are associated to viral diseases. The location has been associated to the virus predominance, since there are different strains of viruses in each region. Thus, in Brazil, PRSV and ZYMV were predominant in the region of São Francisco valley (SILVEIRA et al., 2009), in the state of São Paulo (REGATIER et al., 2008) and Maranhão state (MOURA et al., 2001). According to Halfeld-Vieira et al. (2004), in

Roraima, there are PRSV-W, WMV and ZYMV in simple and mixed infections.

The aim of the present study was to evaluate the reaction of watermelon and pumpkin genotypes to isolates with single, double and triple infections, obtained from watermelon crops in the regions of Formoso do Araguaia, Porto Nacional, Gurupi and Palmas.

MATERIAL AND METHODS

The experiment was performed in a greenhouse at the Federal University of Tocantins – Gurupi in 2010-2011. The viral samples were collected in the producer regions: Palmas (PyP-12), Gurupi (PyG-14 e PyG-19), Formoso do Araguaia (PyF-6) and Porto Nacional (PyPn-36 e PyPn-39). The leaves were placed in kraft paper bags and conditioned in coolers under the temperature of -4°C, at the phytopathology laboratory.

The identification of the viruses was performed by the serological test of Dot-ELISA, with specific antisera for WMV-II, ZYMV and PRSV-W, according to Aguiar et al. (2015). The samples were macerated and prepared according to the procedures used by Banttari & Goodwin (1985). All the results obtained from the samples were compared to the results obtained from the controls (healthy plants with symptoms of the respective viruses).

The cultivars were sown in Styrofoam trays and after the emergence of four definitive leaves, they were transplanted into five-liter pots with substrate. The mechanic inoculations were performed during the cotyledonary phase. The first one was performed in the phase with two definitive leaves and the second, three days after the first inoculation. Infected leaf tissues of Caserta pumpkin in a monophasic and biphasic phosphate buffer 0,01 M, pH 7,0 and sodium sulfite were used as inoculum in both procedures. The viral solution was obtained by maceration in the proportion of 1g of infected leaf tissue and 2 mL of inoculation buffer. The inoculations were performed through friction of gauze soaked with the viral suspension in the presence of carborundum (400 mesh), previously sprayed on the leaves (MORENO et al., 2012). After inoculation, the plants were maintained in a greenhouse. The study was carried out in a completely randomized design with 5 replications per isolated viruses, for two watermelon cultivars (Crimson sweet and Charleston Gray) and two pumpkin cultivars (Caserta and Jacarezinho).

The evaluations started eight days after the first inoculation and the symptoms were assessed

every four days, based on a severity rating proposed by Rezende (1996) where: (1) plants without mosaic symptoms on the leaves; (2) plants with few leaves with mosaic symptoms on the edges; (3) plants with mosaic symptoms in many leaves; (4) plants with mosaic symptoms in most leaves, many bubbles and/or leaves presenting slight deformation; and (5) plants with intense mosaic symptoms, presence of bubbles and severe deformation. The means were submitted to regression analysis for the function $Y = a + bx$, then the means of the regression parameters a and b were compared by Tukey test with 5% of probability using the software Sisvar 5.8 (FERREIRA, 2011).

The symptoms caused by the viral infections were determined according to a classification system in which: M: Mosaic; Re: ruffled edges; Bl: bubbles; Sr: spur; Ln: leaf narrowing; Ld leaf deformation; Lr: leafroll; Ud: underdevelopment; Ef: early flowering; Sl: shoelace; MI: Mottled; Ns: No symptoms. Data were obtained in a period of 32 days after the second inoculation. The symptoms of the disease were based on phenotypic standards of leaves of watermelon and pumpkin, according to Desbiez (2010).

RESULTS AND DISCUSSION

Through ELISA serological identification method, all the potyviruses that attack cucurbit were identified. WMV was identified as the one with the highest prevalence in all samples. Besides, variation between regions was also observed (Table 1), where samples of PyPn of-36 (WMV, ZYMV) PYP-12 (WMV), PYF-6 (WMV), PYG-14 (WMV), PyPn-39 (WMV; PRSV-W) and PYG-19 (WMV; PRSV-W, ZYMV) showed single, double and triple infections (Table 1). Visual symptoms in Crimson sweet, Charleston Gray, Caserta and Jacarezinho manifested in variable forms when they were inoculated with viral strains for each cucurbits.

Based on the visual evaluations in Caserta, the PyG-19 strain, which was positive for WMV; PRSV-W; ZYMV, reached maximum severity according to Figure 1. (A, C and D), showing plants with more severe symptoms of virus, such as leaf deformation (Lf), underdevelopment (Ud), mosaic (M) and leaf narrowing (Ln). During the evaluations, these factors were not observed in other strains which did not get grades higher than 4, showing less sensitivity, especially for PyG- 14, which showed less severity when inoculated with potyvirus (Figures 1. B, C).

Table 1. Serological identification of *Papaya ringspot* Watermelon-virus (PRSV-W), *Watermelon mosaic virus* (WMV) and *Zucchini yellow mosaic virus* (ZYMV) in leaf samples of watermelon species in production areas in Tocantins.

Strains	PRSV-W	WMV	ZYMV
PyPn-36	-	+	+
PyP-12	-	+	-
PyF-6	-	+	-
PyG-14	-	+	-
PyPn-39	+	+	-
PyG-19	+	+	+
Control	-	-	-

* (-) negative for viral species (+) positive for viral species.

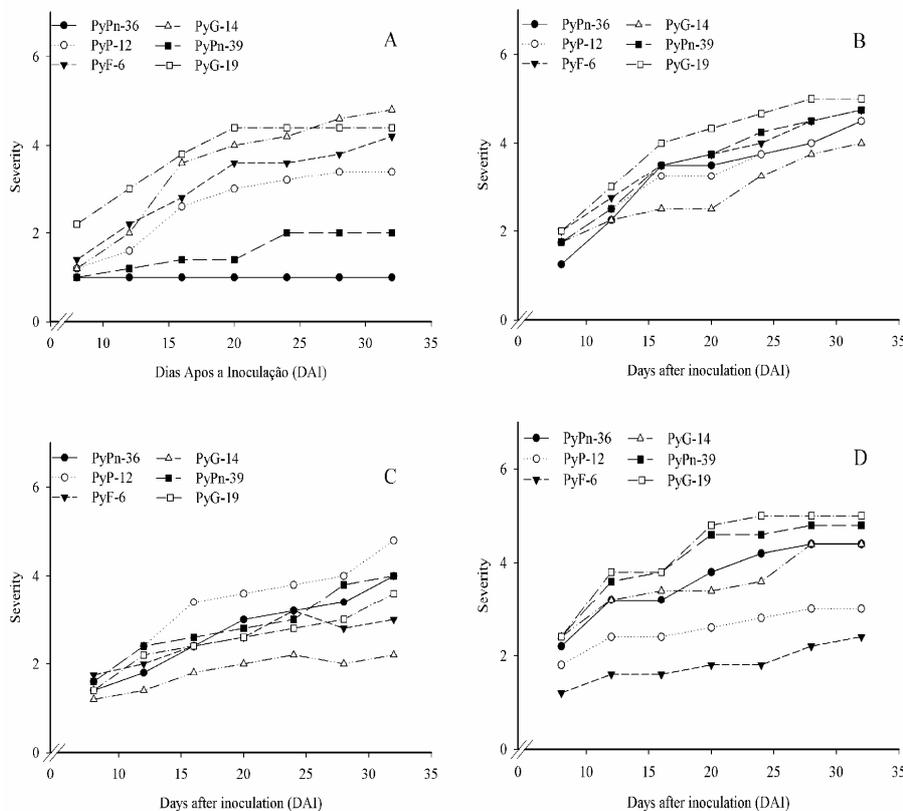


Figure 1. Average severity as a function of time for different strains: Palms (PyP-12), Gurupi (PyG-14 and PyG-19), Formoso Araguaia (PyF-6) and National port (PyPn-36 and PyPn-39). A - Caserta pumpkin, B- Jacarezinho pumpkin, C - Crimson sweet watermelon and D - Charleston Gray watermelon.

However, there were no significant differences among the strains compared to the means by Tukey test (Table 2). In the Charleston gray cultivar, the higher susceptibility was observed in PyG-19 strain where the presence of the three analyzed viral strains (PRSV-W, WMV and ZYMV) was observed, with maximum severity on the 24th day after inoculation (DAI). PyF-6 strain

was positive only for WMV and demonstrated the lowest severity (less than two). The most severe symptoms were represented by plants with bubbles, mosaic and underdevelopment. Unlike what was found in Crimson watermelon, the strain PyP-12 was the least pathogenic from the beginning to the end of the evaluation (Figure 1 C).

Table 2. Summary of linear regression analyzes ($Y = b + ax$) of the susceptibility of cucurbit genotypes to different viral strains obtained from four cities in Tocantins.

		Parameters					
Genotypes	Strain	<i>a</i>	<i>b</i>	GL	F	R ²	P
Caserta	PyPn-36	0.000c	1.000bc	5	-----	1.00	-----
Caserta	PyP-12	0.160a	0.830bc	5	40.89	0.93**	0.0019
Caserta	PyF-6	0.170a	1.470ab	5	71.33	0.95**	0.0007
Caserta	PyG-14	0.140a	2.010a	5	49.03	0.93**	0.0018
Caserta	PyPn-39	0.040bc	1.050bc	5	58.97	0.94**	0.0011
Caserta	PyG-19	0.060b	1.820a	5	42.01	0.87**	0.0095
Average		0.095A	1.363A				
CV%		21.00	20.94				
Jacarezinho	PyPn-36	0.120ab	1.270a	5	32.83	0.93**	0.0023
Jacarezinho	PyP-12	0.110ab	1.370a	5	99.50	0.97**	0.0002
Jacarezinho	PyF-6	0.090b	1.730a	5	108.15	0.97**	0.0001
Jacarezinho	PyG-14	0.120ab	1.530a	5	116.05	0.97**	0.0001
Jacarezinho	PyPn-39	0.120ab	1.400a	5	73.06	0.96**	0.0004
Jacarezinho	PyG-19	0.130a	1.770a	5	37.69	0.93**	0.0017
Average		0.115A	1.511A				
CV%		12.84	23.10				
Crimson Sweet	PyPn-36	0.110a	1.060a	5	200.05	0.98**	0.0001
Crimson Sweet	PyP-12	0.090ab	1.420a	5	58.54	0.95**	0.0006
Crimson Sweet	PyF-6	0.050bc	1.370a	5	22.30	0.90**	0.0052
Crimson Sweet	PyG-14	0.040c	1.240a	5	23.82	0.90**	0.0045
Crimson Sweet	PyPn-39	0.090ab	1.400a	5	93.88	0.97**	0.0002
Crimson Sweet	PyG-19	0.080ab	2.310b	5	68.48	0.96**	0.0004
Average		0.076A	1.466A				
CV%		26.69	14.05				
Charleston Gray	PyPn-36	0.100b	2.690a	5	46.64	0.95**	0.0010
Charleston Gray	PyP-12	0.070b	1.480a	5	44.47	0.94**	0.0011
Charleston Gray	PyF-6	0.170a	1.320a	5	66.48	0.96**	0.0005
Charleston Gray	PyG-14	0.050b	1.850a	5	44.66	0.94**	0.0011
Charleston Gray	PyPn-39	0.060b	2.770a	5	22.83	0.90**	0.0050
Charleston Gray	PyG-19	0.080b	2.810a	5	23.65	0.92**	0.0067
Average		0.088A	2.153B				
CV%		33.08	25.95				

* and ** significant by the F test of the regression at 1% and 5% probability, respectively; Means of the regression parameters followed by the same lowercase letter for strains and upper case for genotypes do not differ statistically by Tukey test at 5% probability.

Among the pumpkins genotypes, the cultivar Caserta was highly sensitive to PyF-6, PyPn-36, PyP-12, PyG-14 and PyG-19 strains, with symptoms such as mild mosaic in the first evaluation. Thus, over the evaluation period, there was a trend of severity evolution for watermelon and pumpkin genotypes reaching top marks on the

24th day after inoculation, with a total deformation of leaves and appearance of severe symptoms such as shoelace, spur and enation. On the other hand, it was observed that PyPn-36 strain showed multiple virus (ZYMV and WMV), in which no viral symptoms were observed. This low severity of symptoms may be associated with the antagonistic

relationship. The difference between the evaluated strains is evident through the means test performed in the regression parameters (Table 2).

Jacarezinho pumpkin showed susceptibility to all inoculated strains, and it was observed a gradual increase in severity after inoculation, with early symptoms reaching severity grade of 2 in the 8th day after inoculation. However, the PYG-39 strain that presents interaction between the two viral strains (PRSV-W + WMV) was more severe in all evaluations, where the symptoms were already visible on the 5th DAI with mosaic on the edges and small bubbles. Overall, samples showed symptoms such as bubbles, leaf deformation, mosaic, shoelace and spurs. Comparing Caserta and Jacarezinho, it is verified a difference in the severity among strains (Table 1). The results observed in this study corroborate with other results of viral infection in

cucurbits (ROMAY et al., 2014), demonstrating that the virus severity in species of cucurbit is very dynamic, varying according to virus and strains, climate condition, population, vectors migration, vegetal variety and natural reservoirs of predominant virus (AGUIAR et al., 2015).

In the assessment of the isolates collected in Gurupi, the most severe mixed infections were observed in the pumpkins genotypes. Caserta and Charleston gray had greater susceptibility in PyG-19 strain, where the presence of the three viral strains (PRSV-W, WMV and ZYMV) was observed. For simple infection, PyG-14 strain (WMV) showed the most severe symptoms only for Caserta pumpkin plants, showing symptoms such as mosaic, deformed leaf, leaf narrowing and bubbles (Figure 2., Table 3).

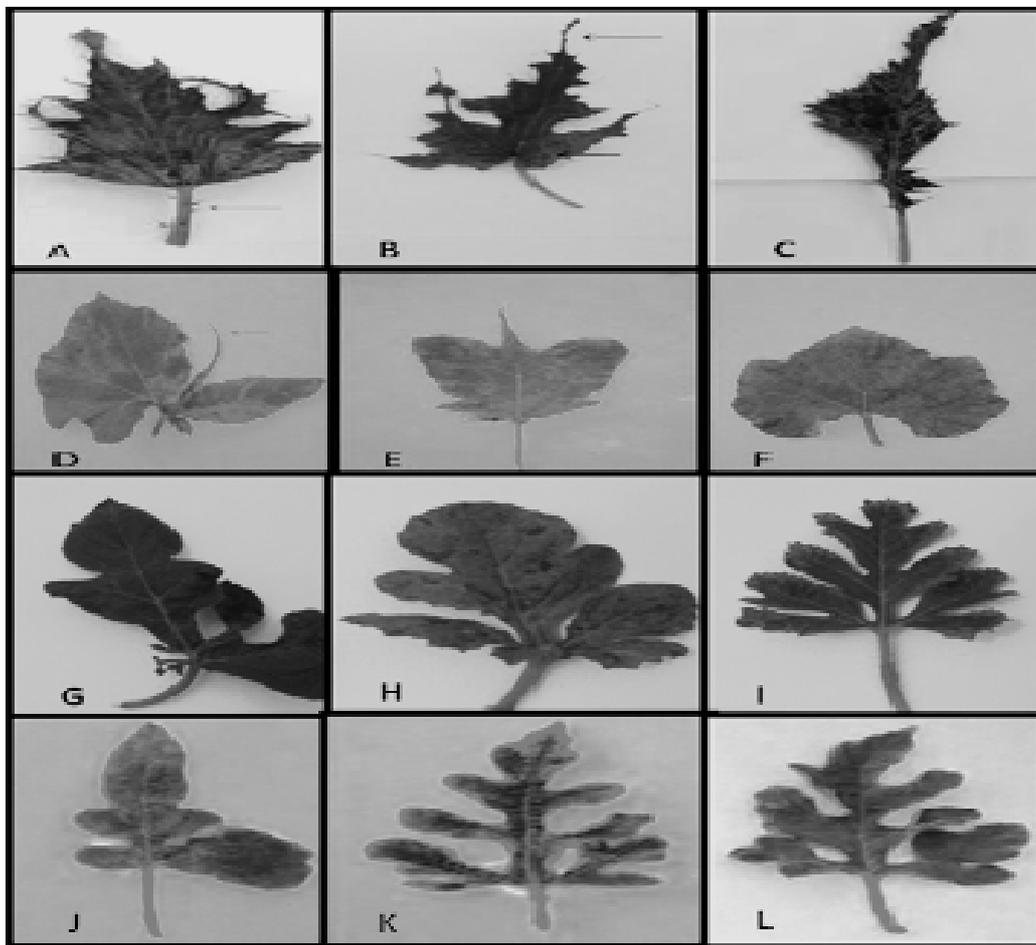


Figure 2. Phenotypic symptoms observed in Caserta Pumpkin: **A**, Spur (Sr) and Mosaic (M); **B**, Shoelace (Sl); **C**, Bubble (Bl). Leaf deformation (Ld) and Leaf narrowing (Ln). Symptoms observed in Jacarezinho pumpkin: **D**, Shoelace (Sl) and Leaf deformation (Ld); **E**, Spur (Sr) and Leaf narrowing (Ln); **F**, Mosaic (M) and Bubble (Bl). Symptoms observed in Charleston Gray watermelon: **G**, Leaf deformation-Ld; **H**, Bubble-BI, Mosaic (M) and Leaf narrowing (Ln); **I**, Light mosaic (Lm). Symptoms observed in Crimson Sweet watermelon: **J**, Leaf deformation (Ld); **K**, Mosaic (M) and Bubble (Bl); **L**, Light mosaic (Lm).

Table 3. Symptomatology of the strains inoculated in two watermelon and pumpkin genotypes

Strain	Symptomatology			
	Crimson sweet	Charkeston gray	Caserta	Jacarezinho
PyPn-36wz	Lm, Bl, Ld, Ud.	M, Bl, Ld Ud.	Ns.	Lm, Bl, Ld.
PyP-12w	M; Lr; Ld; Ud.	M; Df; MI.	M; Re;Lr; Bl; Ld; Ud.	M, Re; Sl, Ld.
PyF-6w	M, Re, Lr, Sl, Ld.	M, Re, Ln, Sl, Ld.	M; Bl; Ld; Ud; MI.	M, Re; Sl, Ld.
PyG-14w	M, Re, Ln, Sl, Ld.	M, Re, Ln, Sl, Ld.	M; Ln; Ld; Ud.	M, Bf.; Sl, Ld.
PyPn-39pw	M; Re; Ln; Ld; Ud.	M; Lr; Re; Bl; Ld; Ud.	M; Ln; Re; Bl; Ld; Sr; S.l; Ud;	M; Lr; Ef; Ud.
PyG-19wzp	M; Re; Bl; Ln; Ld; Ef; Ud; MI.	M; Re; Bl; Ld; Ef; Ud; MI	M; Re; Bl; Ld; Ef; Ud; <u>Sr;Sl</u> ; MI	M; Bl; Ld; Ef; Ud; MI.
Control	Ns	Ns	Ns	Ns

*Symptoms: M: Mosaic; Re: ruffled edges; Bl: bubble; Sr: spur; Ln: leaf narrowing; Ld: leaf deformation; Lr: leaf roll; Ud: underdevelopment; Ef: early flowering; MI= Mottled; Sl = Shoelaces; Ns: no symptoms.

In Gurupi, the mixed infections have been very severe for pumpkin plants, totally compromising the plant growth. According to Mandakombo et al. (2013), in most cases, the symptoms caused by the interaction of two viruses in mixed infection are much more severe and different from those found in single infections, which indicates synergism between these viruses, increasing viral replication capacity and systemic dissemination in the infected plant.

In the evaluation of the isolates in Porto Nacional, two strains (PyPn-36 and PyPn-39) with mixed infections, more severe in pumpkin plants were WMV + PRSV-W and another featuring many less severe symptoms WMV + ZYMV, totally compromising the plant growth (Table 3). According to Aguiar et al. (2015) who studied mixed infections of PRSV + ZYMV in Charleston Gray watermelon and Caserta pumpkin, an increase in symptoms severity and symptoms of plant and leaf underdevelopment, mosaic, bubbles, leaf deformation and shoelace were observed. In Crimson Sweet, the symptoms presented by plants with double infection were much more severe than those presented by PRSV (single), and with greater intensity than those presented by ZYMV (single).

Results in synergistic increases of plant diseases are the product of the expression of the HC-Pro gene, associated with a non-coding RNA sequence. They were sufficient to enhance viral replication (HASIOW-JAROSZEWSKA et al.,

2014). Even so, data of this study showed similar results for the purposes of double infected plants with TuMV P1 and LIYV in transgenic host with HC-Pro. These data suggest that the TuMV activity probably mediates increased accumulation of LIYV in the plant protoplast, which can be one of the factors that help to limit infections for phloem tissue (WANG et al., 2015). However, studies have shown an eight times increase in cells infected in phloem, shown to Potato leaf roll virus (PLRV) in Potato virus (PVA) in transgenic plants HC-Pro (TU et al., 2015).

The occurrence prevalence of single and mixed infections of potyvirus PRSV-W, ZYMV and WMV in the State of Tocantins, differed between regions (ALVES et al., 2014). Several studies have shown that PRSV-W has a high incidence in cucurbit crops in the Northeast of Brazil (DO NASCIMENTO et al., 2012.). For some authors, this prevalence may be influenced by biotic and abiotic factors and the high activity of the potyvirus for cucurbits (IBABA et al., 2015). Studies conducted in different regions of Brazil have shown high prevalence of PRSV-W and ZYMV in cucurbits, followed by Olarte Castillo et al. (2011).

According to Yakoubi et al. (2008), the predominance of PRSV-W was probably due to its virulence and plant x vector interaction. Several studies have reported differences in the interactions between viruses in laboratory and field conditions associated with the host plant and the interaction

between the insect and vector. Crop residues, presence of cultivars susceptible to potyvirus group, and inefficient control practices may have favored the prevalence of potyvirus in watermelon production areas in Tocantins (AGUIAR et al., 2015).

When the virus is installed in the field, it is a very difficult problem to solve because there are no measures to control the virus. The earlier the plant is

infected, the more severe will be the symptoms and the losses in production will be greater.

CONCLUSION

Charleston gray watermelon was the most susceptible to most of the viral strains. Thus, this cultivar should be avoided in these regions.

RESUMO: O cultivo de cucurbitáceas tem grande importância no que diz respeito à alimentação, mas o aumento das áreas de plantio tem sido limitado por infecções virais nos principais pólos produtores de cucurbitáceas. Entre os mais prevalentes destacam-se o vírus do mosaico da melancia (WMV), o vírus da mancha anelar do mamoeiro estirpe melancia (PRSV-W) e o vírus do mosaico amarelo da abóbora (ZYMV). Este estudo teve como objetivo avaliar a reação dos genótipos de cucurbitácea Melancia Crimson sweet e Charleston gray; Abóbora: Jacarezinho e Caserta, para isolar variedades de safra de melancia nas cidades de Palmas (PYP-12), Gurupi (PYG-14 e PYG-19), Formoso do Araguaia (PYF-6) e Porto Nacional (PyPn-36 e PyPn-39). O experimento delineamento inteiramente casualizado (DIC), foi conduzido em estufa e foram utilizadas 5 plantas de teste para cada repetição para medir a resposta dessas cepas aos genótipos de cucurbitáceas. A inoculação dos vírus em plantas realizada a partir de folhas com a infecção de melancia Crimson sweet. As plantas inoculadas foram avaliadas por graus de severidade em quatro dias de intervalo durante 32 dias após a inoculação. Verificou-se que os genótipos de cucurbitáceas analisadas apresentaram susceptibilidade diferente às cepas virais testadas. No entanto, o genótipo de abóbora de Caserta e Charleston gray foram as que apresentou maior sensibilidade, devido à sua alta variação de severidade entre as cepas. Assim, podemos ver que a reação entre as estirpes depende do hospedeiro e a reação pode ser antagonista ou sinérgica dependendo da infecção mista. A melancia Charleston gray foi o mais suscetível à maioria das cepas virais. Assim, esta cultivar deve ser evitada nessas regiões.

PALAVRAS-CHAVE: *Citrullus lanatus*. *Cucurbita pepo*. Víruses

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