Australian Journal of Crop Science AJCS

AJCS 13(09):1409-1415 (2019) ISSN:1835-2707
doi: 10.21475/ajcs.19.13.09.p1203

Selection of watermelon accessions for resistance to some important potyvirus species based on serological evaluation

Gerffeson Thiago Mota de Almeida Silva[1], José Albérsio de Araújo Lima[1], Graziela da Silva Barbosa[2], Ênio Gomes Flôr Souza[3], Giordanio Bruno Silva Oliveira[4], Manoel Abilio de Queiróz[2], Lindomar Maria da Silveira[4], Aline Kelly Queiróz do Nascimento[5], Aurélio Paes Barros Júnior*[4]

[1]Universidade Federal do Ceará (UFC)/Programa de Pós-Graduação em Fitotecnia, Fortaleza, CE, Brazil
[2]Universidade do Estado da Bahia (UNEB)/Departamento de Tecnologia e Ciências Sociais. Juazeiro, BA, Brazil
[3]Instituto Federal de Alagoas (IFAL)/Campus Piranhas. Piranhas, AL, Brazil
[4]Universidade Federal Rural do Semi-Árido (UFERSA)/Centro de Ciências Agrárias, Mossoró, RN, Brazil
[5]Syngenta Brasil. Aracati, CE, Brazil

***Corresponding author: aurelio.barros@ufersa.edu.br**

Abstract

We used 19 genotypes (plus controls) of watermelon from the collection of Cucurbitaceas germplasm of the Federal Rural University of the Semi-Arid to select watermelon plants resistant to Papaya ringspot virus "type Watermelon" (PRSV-W), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus (ZYMV). Twenty individual plants were tested for each genotype/accession. Three controls were also used (3 genotypes). Evaluations were performed under greenhouse in a completely randomized design with five replications. The first inoculation was performed on the seedlings before the appearance of the first definitive leaf. From each accession (genotype) 20 different individual plants were inoculated with the three viruses. The inoculum was prepared by infected leaf tissue. The viral suspension and gauze soaked in extract were wiped on the surface of the leaves. Ten days later, the symptom assessment was performed. Subsequently, the plants were individually tested against the specific viruses using indirect ELISA. ELISA negative plants were submitted to a second inoculation under greenhouse, as described for the first inoculation. Ten days after new inoculation, new symptomatological evaluations and serological tests were performed to confirm the resistance of the plants. Plants that were negative by ELISA were tested by RT-PCR for confirmation of resistance. Resistance to the three viruses was verified individually in several tested genotypes. We found resistance to the three viruses tested, but in different plant individuals, where 16 individual plants were WMV-resistant, 26 PRSV-W resistant and 30 ZYMV-resistant. These plants can be used to develop homozygous lines for resistance to the virus studied.

Keywords: *Citrullus lanatus;* Pre-breeding; Plant genetic resources.

Introduction

The watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai), originated from the tropical regions of equatorial Africa. It is a *Cucurbitaceae* cultivated in several countries around the world due to its easy handling and reduced production costs when compared to other vegetables. According to FAO data, in 2014 Brazil became the fourth largest watermelon producer among 119 countries listed, behind only China, Turkey, and Iran. Around the world, approximately 111 million tons were cultivated, distributed across 3.48 million hectares (FAO, 2017).

In Brazil, this crop is considered one of the most produced and commercialized, with production levels rivaling sweet potatoes, melon, and onion. In Brazil, only the production of tomatoes and potatoes is greater than watermelon production (IBGE, 2017). In 2014, the area of the country planted with watermelon was approximately 95 thousand ha, with a production volume of approximately 2,171 thousand tons (IBGE, 2017). Watermelon cultivation has been negatively affected by serious phytosanitary problems because of the genotypes cultivated in Brazil (with some exceptions) were developed for American and Japanese conditions, which are different from those in Brazil (Nascimento et al., 2011).

Viral diseases are responsible for the majority of phytosanitary problems, reducing their productivity, both quantitatively and qualitatively. In some cases, viral diseases can impair the cultivation in farms (Lima, 2015) due to the presence of a number of aphid vectors in the field and the large number of host aphid species, including those of the *Cucurbitaceae* family (Beserra Jr. et al. 2006).

In Brazil, at least ten viruses have been found to infect commercial *Cucurbitaceae* crops, such as the *Potyviridae* family, *Potyvirus* genus - *Papaya ringspot virus* type Watermelon (PRSV-W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV); *Bromoviridae* family, genus *Cucumovirus* - *Cucumber mosaic virus* (CMV);

Comoviridae family, genus *Comovirus* - *Squash mosaic virus* (SqMV) and *Bunyaviridae* family, genus *Tospovirus* - *Zucchini lethal chlorosis virus* (ZLCV) (Moura et al., 2001; Silveira et al., 2009; Rodrigues, 2011; Lima et al., 2016).

The *Potyviridae* family is considered to be one of the largest and most important families of virus that infect plants (FAUQUET et al., 2005), with reports of potyvirus occurring in all regions of the planet and infecting more than 2,000 plant species, 550 genera and 81 botanical families (BRUNT et al., 1997). Collectively, potyviruses are considered the most harmful to plants due to their high potential for causing losses compared to the other plant viruses (SHUKLA et al., 1994). In particular, for watermelon culture in the Northeast region of Brazil, three potyviruses (PRSV-W, WMV, and ZYMV) are considered of major importance.

Until a few decades ago, phytosanitary control was limited to methods such as chemical vector control, thermotherapy, meristematic cultures, cross-protection or eradication of infected plants (Fuchs and Gonsalves, 2007). In contrast, the use of resistant genotypes is indicated as the most effective strategy for the control of phytoviroses. However, the dynamism of these pathogens in the environment allows for constant evolution and emergence of isolates that can adapt to the new host. This adaptation can lead to the overcoming of genotype-specific resistance by one or more viruses and/or the emergence of a new species (Ge et al., 2007), which makes constant identification of new sources of resistance in the culture important.

The objective of this study was to evaluate and select watermelon plants resistant to PRSV-W, WMV, and ZYMV potyvirus in watermelon accessions belonging to the UFERSA collection of *Cucurbitaceae* germplasm and from traditional agriculture of Northeast Brazil to establish the basis for an improvement program.

Results and Discussion

Symptomatology

The symptoms of viral infection began to appear in plants from the fourth-day post-inoculation, differing according to the virus and its association with the various genotypes. The observed symptoms ranged from light mosaic to severe mosaic, leaf bloom, and deformation, with some necrosis plants at the end of the evaluation (Tables 2, 3 and 4). Leaf deformations (most severe in the plants inoculated with ZYMV) (Table 4) were able to affect the development of the plant, as they compromise the photosynthetic process due to the decreased surface area directly interfering with productivity (Basso et al., 2010). An increase in the aggressiveness of symptoms was observed for all the viruses throughout the symptomatic evaluation period.

The symptoms of viral infections are very similar and difficult to diagnose. In some cases, they may be easily confused with nutritional deficiency. In addition, mixed infections (which occur easily in the field) present varying symptoms, amplifying the problem of virus identification (Wilson, 2001). In mixed infections, a synergistic effect may occur, causing an increase or decrease in virus concentration in the plant, a change in symptoms, and even a change in the systemic movement of viruses (Oliveira et al., 2000). Viruses that infect *Cucurbitaceae* are difficult to distinguish because they all produce leaf mosaic symptoms when found in simple infections, indicating the need to use serological techniques to identify or diagnose these viruses in *Cucurbitaceae.*

Serology

The serological results confirmed the symptomatology (Table 5). Two accessions presented isolated resistance to PRSV-W (Accession 9 and Accession 11), one to WMV (Accession 5), and three to ZYMV (Accession 4, Accession 8, and Accession 17). The double resistance to the PRSV-W and WMV viruses could only be verified in Accession 16. In addition, three accessions (Accession 1, Accession 14, and Accession 21) with combined WMV and ZYMV resistance were found (Table 2). The Explorer hybrid showed resistance to the three viruses; however, the frequency of this resistance varied according to the inoculated virus (WMV - 60%, ZYMV - 50%, PRSV - 30%). It has been reported as being resistant to ZYMV and WMV (Agristar, 2013).

Some Explorer hybrid plants were infected with these viruses. This may have occurred due to inoculation with strains different to those of the region, where the hybrid was developed and/or by the difference in inoculation techniques used in the development of the hybrid and in this work (considering the high pressure that occurs in a mechanical inoculation). In addition to the hybrid, Accession 3, Accession 6, Accession 7, and Accession 12 showed resistance to the three viruses. No resistant plants were observed in Accession 4. Thus, 44 plants were classified as resistant. Ten plants were resistant to PRSV-W, fourteen plants were resistant to WMV, and twenty plants were resistant to ZYMV. It should be noted that the accessions presenting resistance to multiple potyviruses were from inoculations of separate plants. Multiple resistances were identified in accessions and not in the plants. Due to the relationship between the three virus species (Ramos et al., 2003b), the simultaneous inoculation of the three viruses in the same plant could lead to misinterpretation of the results, as there may be synergy between viruses in a mixed infection leading to possible changes in the symptoms of the disease (Ramos et al., 2003a).

Sources of resistance to potyvirus have been selected and reported in several *Cucurbitaceae* such as in accessions of *Cucurbita* spp. (Nascimento et al., 2012; Tavares et al., 2014); *Cucumis sativus* L. (Silva et al., 1978) and *Cucumis melo* L. (Rabelo Filho et al., 2010). In watermelon, Rabelo Filho et al. (2010) found genotypes resistant to PRSV-W and WMV but did not verify ZYMV resistant genotypes. Strange et al. (2002) found double resistance to PRSV-W and ZYMV in a watermelon genotype. Silveira et al. (2005) evaluated watermelon accessions and found resistance to PRSV-W, WMV, and ZYMV. When evaluating endogamic progenies obtained from these accessions, the authors found resistance to PRSV-W and WMV; however, no resistance was found for ZYMV, demonstrating segregation of this feature. Vieira (2005) found nineteen genotypes resistant to PRSV-W, nineteen to WMV, and thirteen to ZYMV.

Although there are reports of sources of resistance to potyvirus in some *Cucurbitaceae*, as well as in watermelon (Oliveira, 2002; Rabelo Filho et al., 2010; Vieira et al., 2010; Nascimento et al., 2012; Tavares et al., 2014), the identification of new sources is of fundamental importance due to the variability existing for these virus species. It is also worth mentioning that several sources of resistance

Table 1. Identification of watermelon accessions used for selection of sources of resistance to *Papaya ringspot virus* type Watermelon (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV).

Accession[1]	Code[2]	Species	Origin[3]
Accession 1	WGCUC001	*Citrullus lanatus*	Serra Talhada - PE
Accession 2	WGCUC002	*Citrullus lanatus*	Custódia - PE
Accession 3	WGCUC003	*Citrullus lanatus*	Apodi – RN
Accession 4	WGCUC004	*Citrullus lanatus*	Floresta – PE
Accession 5	WGCUC005	*Citrullus lanatus*	Custódia - PE
Accession 6	WGCUC006	*Citrullus lanatus*	Serra Talhada - PE
Accession 7	WGCUC007	*Citrullus lanatus*	Custódia - PE
Accession 8	WGCUC008	*Citrullus lanatus*	Apodi – RN
Accession 9	WGCUC009	*Citrullus lanatus*	Cerro Corá - RN
Accession 11	WGCUC011	*Citrullus lanatus*	Apodi – RN
Accession 12	WGCUC012	*Citrullus lanatus*	Apodi – RN
Accession 14	WGCUC014	*Citrullus lanatus*	Apodi – RN
Accession 16	WGCUC016	*Citrullus lanatus*	Apodi – RN
Accession 17	WGCUC017	*Citrullus lanatus*	Apodi – RN
Accession 21	WGCUC021	*Citrullus lanatus*	Apodi – RN
Accession 22	WGCUC022	*Citrullus lanatus*	Apodi – RN
Crimson Sweet[4]	-	*Citrullus lanatus*	Control
Explorer Hybrid[4]	-	*Citrullus lanatus*	Control
'Caserta'[4]	-	*Cucurbita pepo*	Control

[1]Accessions of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] collected in the states of Pernambuco and Rio Grande do Norte and belonging to the Collection of germplasm of cucurbitaceae of the Federal Rural University of the Semi-Arid (UFERSA).[2] Accession code in the UFERSA Cucurbit Germplasm Collection.[3] Location where the accessions were collected.
[4] Cultivars obtained commercially and used as control.

Fig 1. Municipalities in Northeastern Brazil, places of origin of watermelon accessions [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], used in a study to identify sources of resistance to viruses *Papaya ringspot virus* type Watermelon (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). Origin: Google Earth/2018.

Table 2. Symptomatology observed in watermelon plants [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] used in the study to identify sources of resistance to the *Watermelon mosaic virus* (WMV).

Accession[1]	Code[2]	Notes for symptoms[3]			
		1	2	3	4
Accession 1	WGCUC001	0.3	0.70	0.20	-
Accession 2	WGCUC002	-	1.00	-	-
Accession 3	WGCUC003	0.05	0.75	0.20	-
Accession 4	WGCUC004	-	1.00	-	-
Accession 5	WGCUC005	0.10	0.70	-	-
Accession 6	WGCUC006	0.15	0.85	0.10	-
Accession 7	WGCUC007	0.42	0.58	-	-
Accession 8	WGCUC008	0.15	0.75	-	-
Accession 9	WGCUC009	0.21	0.79	-	-
Accession 11	WGCUC011	0.10	0.90	-	-
Accession 12	WGCUC012	0.35	0.65	-	-
Accession 14	WGCUC014	0.32	0.68	-	-
Accession 16	WGCUC016	0.42	0.58	-	-
Accession 17	WGCUC017	0.30	0.70	-	-
Accession 21	WGCUC021	0.30	0.70	-	-
Accession 22	WGCUC022	0.37	0.47	0.16	-
Explorer Hybrid	-	0.60	0.40	-	-

[1]Accessions of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] collected in the states of Pernambuco and Rio Grande do Norte and belonging to the Collection of germplasm of cucurbitaceae of the Federal Rural University of the Semi-Arid (UFERSA). [2] Access code in the UFERSA Cucurbit Germplasm Collection.[3] Frequency of plants classified in each note. 1: No symptoms; 2 - Mosaic with or without whitening of the ribs and / or winding of the limbus; 3 - Mosaic, limb winding, shoot necrosis and / or bloom; 4 - Mosaic, limb winding, sprouting necrosis, blooming and / or severe deformation.

Table 3. Symptomatology observed in watermelon plants [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] used in the study to identify sources of resistance to the *Papaya ringspot virus* type Watermelon (PRSV-W).

Accession/genotypes[1]	Code[2]	Notes for symptoms[3]			
		1	2	3	1
Accession 1	WGCUC001	0.16	0.84	-	-
Accession 2	WGCUC002	-	0.80	0.20	-
Accession 3	WGCUC003	0.15	0.80	0.05	-
Accession 4	WGCUC004	0.15	0.85	-	-
Accession 5	WGCUC005	0.10	0.70	0.20	-
Accession 6	WGCUC006	0.05	0.75	0.20	-
Accession 7	WGCUC007	0.05	0.85	0.10	-
Accession 8	WGCUC008	0.05	0.75	0.20	-
Accession 9	WGCUC009	0.10	0.90	-	-
Accession 11	WGCUC011	0.05	0.65	0.30	-
Accession 12	WGCUC012	0.10	0.75	0.15	-
Accession 14	WGCUC014	-	1.00	-	-
Accession 16	WGCUC016	0.16-	0.84	-	-
Accession 17	WGCUC017	-	1.00	-	-
Accession 21	WGCUC021	-	1.00	-	-
Accession 22	WGCUC022	-	0.89	0.11	-
Explorer Hybrid	-	0.30	0.55	0.15	-

[1]Accessions of watermelon [Citrullus lanatus (Thunb.) Matsum. & Nakai] collected in the states of Pernambuco and Rio Grande do Norte and belonging to the Collection of germplasm of cucurbitaceae of the Federal Rural University of the Semi-Arid (UFERSA).[2] Access code in the UFERSA Cucurbit Germplasm Collection.[3] Frequency of plants classified in each note. 1: No symptoms; 2 - Mosaic with or without whitening of the ribs and / or winding of the limbus; 3 - Mosaic, limb winding, shoot necrosis and / or bloom; 4 - Mosaic, limb winding, sprouting necrosis, blooming and / or severe deformation.

Table 4. Symptomatology observed in watermelon plants [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] used in the study to identify sources of resistance to the *Zucchini yellow mosaic virus* (ZYMV).

Accession/genotypes[1]	Code[2]	Notes for symptoms[3]			
		1	2	3	1
Accession 1	WGCUC001	0.11	0.63	0.26	-
Accession 2	WGCUC002	0.15	0.65	0.20	-
Accession 3	WGCUC003	0.22	0.78	-	-
Accession 4	WGCUC004	0.11	0.37	0.53	-
Accession 5	WGCUC005	0.25	0.45	0.30	-
Accession 6	WGCUC006	0.20	0.50	0.30	-
Accession 7	WGCUC007	0.05	0.40	0.40	0.15
Accession 8	WGCUC008	0.11	0.72	0.17	-
Accession 9	WGCUC009	-	1.00	-	-
Accession 11	WGCUC011	0.10	0.60	0.30	-
Accession 12	WGCUC012	0.11	0.74	0.16	-
Accession 14	WGCUC014	0.11	0.89	-	-
Accession 16	WGCUC016	0.11	0.89	-	-
Accession 17	WGCUC017	0.15	0.40	0.45	-
Accession 21	WGCUC021	0.15	0.60	0.25	-
Accession 22	WGCUC022	0.16	0.32	0.32	0.21
Explorer Hybrid	-	0.50	0.50	-	-

[1]Accessions of watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] collected in the states of Pernambuco and Rio Grande do Norte and belonging to the Collection of germplasm of cucurbitaceae of the Federal Rural University of the Semi-Arid (UFERSA).[2] Access code in the UFERSA Cucurbit Germplasm Collection.[3] Frequency of plants classified in each note. 1: No symptoms; 2 - Mosaic with or without whitening of the ribs and / or winding of the limbus; 3 - Mosaic, limb winding, shoot necrosis and / or bloom; 4 - Mosaic, limb winding, sprouting necrosis, blooming and / or severe deformation.

Table 5. Serology of watermelon accessions evaluated for resistance to *Papaya ringspot virus* type Watermelon (PRSV-W), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV) under controlled greenhouse conditions.

Accession/genotypes[1]	Código[2]	Sorology (Number of individuals)[3]					
		PRSV-W		WMV		ZYMV	
		T	R	T	R	T	R
Accession 1	WGCUC001	20	0	20	1	20	1
Accession 2	WGCUC002	20	0	20	0	20	0
Accession 3	WGCUC003	20	1	20	1	20	1
Accession 4	WGCUC004	20	0	20	0	20	2
Accession 5	WGCUC005	20	0	20	2	20	0
Accession 6	WGCUC006	20	1	20	1	20	1
Accession 7	WGCUC007	20	1	20	2	20	1
Accession 8	WGCUC008	20	0	20	0	20	1
Accession 9	WGCUC009	20	2	20	0	20	0
Accession 11	WGCUC011	20	1	20	0	20	0
Accession 12	WGCUC012	20	2	20	1	20	2
Accession 14	WGCUC014	20	0	20	2	20	2
Accession 16	WGCUC016	20	2	20	2	20	0
Accession 17	WGCUC017	20	0	20	0	20	3
Accession 21	WGCUC021	20	0	20	1	20	3
Accession 22	WGCUC022	20	0	20	1	20	3
Crimson Sweet	-	20	0	20	0	20	0
Explorer Hybrid	-	20	6	20	12	20	10
TOTAL		340	16	340	26	340	30

[1]Accessions of watermelon Citrullus lanatus (Thunb.) Matsum. & Nakai collected in the states of Pernambuco and Rio Grande do Norte and belonging to the Collection of germplasm of cucurbitaceae of the Federal Rural University of the Semi-Arid (UFERSA).[2] Access code in the UFERSA Cucurbit Germplasm Collection.[3] T - Number of accession plants tested for each virus; R - number of resistant plants after being evaluated by indirect Elisa.

reported for *Cucurbitaceae* and watermelon present differentiated genetic control mechanisms (Silveira et al., 2015; 2014). It is also important to note that the presence of PRSV-W, WMV, and ZYMV potyviruses has been reported among viral infections in cucurbit production fields (Silveira et al., 2009; Barbosa et al., 2016; Silva et al., 2016; Soares et al., 2016). In addition, the use of plants resistant to only one of the viruses will not be efficient for the control of viruses. Thus, it is necessary to develop genotypes with resistance to multiple viruses occurring in a given region.

Molecular Assessment

The plants deemed negative by ELISA were also negative by PCR; thus, confirming the absence of virus in the selected plants. When cultured in natural conditions and retested by ELISA, the results of the controlled evaluation were repeated, except for four plants selected for WMV and two plants selected for ZYMV, which were susceptible to infection in the field. According to Acosta-Leal et al. (2010), the genetic variability is the primary factor in order for a virus to overcome resistance, adapt to a new host, and even change symptoms. This denotes the importance of associating selection under controlled conditions with field selection. Due to technical difficulties, most of the time identification only occurs at the species level, not differentiating the isolates. Thus, the occurrence of a different isolate in the field may render the selected resistance ineffective under controlled conditions.

Materials and Methods

Experimental area

The experiment was conducted in a greenhouse at the Federal University of Ceará (UFC), Fortaleza-CE, from July to October 2014.

Germplasm

Nineteen accessions of watermelon (20 individual plants each) [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] belonging to the cucurbit germplasm collection of the Federal Rural University of Semi-Arid (UFERSA) were tested. The accessions were collected from different cities of Northeast Brazil (Figure 1). The Crimson Sweet watermelon cultivar and the Caserta zucchini (*Cucurbita pepo* var. *melopepo*), described as susceptible, were used as a positive control for the susceptibility reaction to potyviruses. In addition, the commercial Explorer hybrid, described as resistant to ZYMV and WMV (ARISTAR, 2013), was evaluated. Thus, totally 22 genotypes were tested (including 20 individual plants for each accession) (Table 1).

Conducting the experiment

The genotypes were seeded in 5 L capacity polyethylene pots containing autoclave-sterilized (121 °C, 1 atm, for 40 minutes) substrate consisting of a mixture of soil and manure at a ratio of 2:1.

The viral inoculum used in the experimental trials was obtained from the Active Virus Bank of the Laboratory of Plant Virology of the Federal University of Ceará (UFC). They were isolated from naturally infected plant species in fields of melon and/or watermelon in the states of Ceará (*Papaya ringspot virus* type Watermelon PRSV-W), Rio Grande do Norte (*Zucchini yellow mosaic virus* ZYMV), Bahia, and Pernambuco (*Watermelon mosaic virus* WMV) (Oliveira et al., 2000). Inoculations were performed with PRSV-W, WMV, and ZYMV isolates. Each accession/genotype (20 plants) was inoculated with the three viruses but individually. Considering that viruses may have a synergistic interaction when they infect a plant at the same time (Ramos et al., 2003b) inoculation into separate plants allows avoiding possible errors in the interpretation of symptoms. Twenty plants of each accession were inoculated per virus. The inoculum was prepared by adding 0.05 M potassium phosphate buffer (K_2HPO_4), pH 7.5, to infected leaf tissue and macerating in a mortar at a ratio of 1 g of infected tissue to 2 mL of inoculation buffer. Subsequently, the suspension was filtered through gauze and a silicon carbide (carborundum) 400 mesh abrasive was added for friction. The viral suspension and the extract-soaked gauze were rubbed onto the surface of the sheets.

Inoculations

Two inoculations were performed. The first one when the cotyledon leaves were fully expanded and before the appearance of the first definitive leaf. The inoculated plants were kept in greenhouse for observation of symptomatic reactions. Ten days after the first inoculation, a symptomatological evaluation was performed based on a scale of scores varying from 1 to 4 and a serological evaluation by indirect ELISA (Enzyme-Linked Immunosorbent Assay) against specific antisera for PRSV-W, WMV and ZYMV potyviruses. Considering that some infected plants did not present symptoms, all plants were evaluated by indirect ELISA. The ELISA negative plants were submitted to a second inoculation as described for the first inoculation and were kept in the greenhouse for another ten days, when they were again evaluated by indirect ELISA. The second inoculation was performed to avoid escape and ensure that the selected plants are resistant to the inoculated virus.

Symptomatology

Symptom assessment was performed for each plant 10 days after inoculation and was determined according to the severity classification system used by Silveira (2008), which establishes four notes according to the degree of infection: **1** - No symptoms; **2** - Mosaic with or without whitening of the ribs and/or winding of the limbs; **3** - Mosaic, limb winding, shoot necrosis and/or bloom; **4** - Mosaic, limb winding, sprouting necrosis, blooming and/or severe deformation.

Serology

For the serological evaluations, the indirect ELISA (Mowat and Davidson, 1987) was used. For this, we worked with specific antisera for PRSV-W, WMV, and ZYMV. Samples with readings twice the absorbance values of healthy plant extracts (negative control) were considered positive (Ramos et al., 2003a). The plants that remained negative in both ELISA were evaluated by molecular techniques to verify the resistance.

Molecular assessment

The ELISA is not sensitive enough to determinate cases of low virus concentration. In order to confirm the resistance to the virus, each plant was tested by RT-PCR (Almeida and Lima, 2001).

The confirmation of the absence of virus in the plants selected as resistant was determined by PCR (Polymerase Chain Reaction). For PCRs, indirect ELISA and a sample of infected and healthy plants extracted total RNA from the leaves of the plants negative. The leaf samples were macerated (mechanically homogenized) in sterile microtubes in the presence of liquid nitrogen. After maceration, 1 mL of Brazol was added. Then the tubes were vortexed for two minutes, 250 µL of ice-cold chloroform added and shaken again. After this step, the samples were centrifuged at 12,000 × *g* at 4 °C for 20 minutes. After centrifugation, the supernatant was transferred to a new tube containing 500 µL of ice-cold isopropanol. The tubes were mixed by inversion for two minutes, followed by centrifugation at 12,000 × *g* at 4 °C for 15 minutes. After centrifugation, the supernatant was carefully removed by aspiration. The precipitate was then washed with 500 µL of 70% ethanol, mixed by inversion, centrifuged at 12,000 × *g* at 4 °C for 10 minutes and the supernatant discarded. The precipitate was resuspended in 50 µL of deionized sterile water. The PCR was prepared in a 25 µL reaction volume using a PROMEGA® kit. Each reaction contained 5 µL of 5x buffer (100 mM Tris-HCl, 50 mM KCl, pH 8.3), 2.5 µL of $MgCl_2$ (25 mM), 1 µL of dNTPs (10 mM), 0.5 µL of each oligonucleotide (25 pmol), 0.3 µL (1.25 units) of Taq DNA polymerase (PROMEGA) and 2 µL of the extracted RNA. The volume was adjusted with deionized sterile water. RNA fragments were amplified in a thermocycler (Mastercycler Gradient - Eppendorf) using the RNA cycle: 95 °C for 2 min for initial heating, followed by 31 cycles of denaturation at 95 °C for 1 min, annealing of the oligonucleotides at 55.4 °C for PRSV-W and ZYMV and 57.5 °C for WMV and finally, extension at 72°C for 2 min. The sequences were obtained using oligonucleotides specific to the CP (protein coat) regions of PRSV-W, ZYMV, and WMV: PRSV-W *cp:*

(F) 5'-TGAACGTGAGAGGGGAGACT-3'; for PRSV-W-CP

(R) 5'-CAGCAAACACACAAGCGCGA-3'; ZYMV *cp:*

(F) 5'- CTACCTACAAGCCCTCCATC-3', CP

(R) 5'- CAGCGAATCGATAACCTAGG-3' and WMV *cp*:

(F) 5'-AACTCGCTGCATCCGGAAAA-3',

(R) 5'-CGCAAATGCTAACTGTGACC-3'

PCR products were visualized on 0.8% agarose gels prepared with 0.5 M 0.5x TBE buffer. PCR samples (7 µL) containing 4 µL of loading dye (0.25% bromophenol blue, 0.25% xylene cyanol, and 30% glycerol) were separated by electrophoresis at 90 volts in a horizontal system for 50 minutes. After the run, the gel was stained for 15 min with ethidium bromide (0.1 µg/mL) and the electrophoretic profile was visualized and photographed under ultraviolet light using a Pro Gel Logic Photodocumentator 212.

Conclusions

Sixteen WMV resistant plants, 26 PRSV-W resistant plants and 30 ZYMV resistant plants were identified and selected. These plants can be used to develop homozygous watermelon lines for resistance to the viruses studied.

Acknowedgments

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Instituto Nacional do Semiárido (INSA).

References

Acosta-Leal R, Bryan BK, Rush CM (2010) Host effect on the genetic diversification of *Beet necrotic yellow vein virus* single-plant populations. Phytopathology. 100:1204-1212.

Agristar (2013) Melancia Explore F_1 in Folha Verde - Informativo do Grupo Agristar do Brasil, 3.

Almeida AMR, Lima, JAA (2001) Princípios e técnicas de diagnose em fitovirologia. Brasília-DF/Fortaleza-CE, Brasil: Publicação SBF. 186p.

Barbosa GS, Lima JAA, Queiroz MA, Dias RCS, Lima CS (2016) Identification and effects of mixed infection of potyvirus isolates with *Cucumber mosaic virus* in cucurbits. Rev Caatinga. 29(4):1028-1035.

Basso MF, Fajardo TVM, Santos HP, Guerra CC, Ayub RA (2010) Fisiologia foliar e qualidade enológica da uva em videiras infectadas por vírus. Tropical Plant Pathology. 35:351-359.

Beserra Jr JEA, Figueira AR, Maluf WR (2006) Seleção de genótipos de melancia resistentes ao *Watermelon mosaic virus* e ao *Papaya ringspot virus*. Ciência e agrotecnologia. 31:1563-1568.

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (1997) Plant Viruses Online.

FAO (2017) Agricultural production: 2016 Primary crops. Rome, Disponível em:<

http://www.fao.org>. Acesso em: 10 de janeiro. 2016

Fauquet CM, Mayo M A, Maniloff J, Desselberger U, Ball L A (2005) Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses. London: Academic Press.1,162p.

Fuchs M, Gonsalves D (2007) Safety of virus-resistant transgenic plants two decades after their introduction: lessons from realistic field risk assessment studies. Annual Review of Phytopathology. 45:173-202.

Ge L, Zhang J, Zhou X, Li H (2007) Genetic structure and population variability of Tomato yellow leaf curl China virus. Journal of Virology. 81:5902-5907.

IBGE (2017) Indicadores conjunturais; produção agrícola; agricultura.Disponível em:<http://biblioteca.ibge.gov.br/visualizacao/periodicos/66/pam_2014_v41_br.pdf>. Acesso em: 10 de janeiro. 2017.

Lima JAA (2015) Virologia essencial & viroses em culturas tropicais. 1. ed.: Edições UFC. Fortaleza, CE, Brasil. 605p.

Lima RN, Oliveira AS, Leastro MO, Blawid R, Nagata T, Resende RO, Melo FL (2016) The complete genome of the tospovirus *Zucchini lethal chlorosis virus*. Virology Journal. 3-7.

Moura MCCL, Albersio JAA, Lima A, Oliveira VB, Gonçalves MFB (2001) Identificação sorológica de espécies de vírus que infetam cucurbitáceas em áreas produtoras do Maranhão. Fitopatologia brasileira. 26:90-92.

Mowat WP, Dawson S (1987) Detection of plant viruses by ELISA using crude sap extracts and unfractionated antisera. Journal of Virology Methods. 15:233-247.

Nascimento IR, Santos GR, Maluf WR (2011) Melhoramento genético e cultivares de melancia. In: Santos GR, Zambolin L. Tecnologias para produção sustentável da melancia no Brasil. 1 ed. Editora UFV, Viçosa-MG, Brasil. 268p.

Nascimento IR, Santos LB, Sarmento RA, FigueiraAR, Oliveira GIS, Aguiar RWS (2012) Reação fenotípica de genótipos de abóboras ao virus da mancha anelar do mamoeiro , estirpe Melancia (*Papaya ringspot virus*, strain Watermelon – PRSV-W). Bioscience Journal. 28:191-197.

Oliveira VB, Lima JAA, Vale CC, Paiva WO (2000) Caracterização biológica e sorológica de isolados de potyvirus obtidos de cucurbitáceas no Nordeste brasileiro. Fitopatologia Brasileira. 25:628-636.

Oliveira VB, Queiroz MA, Lima AAJ (2002) Fontes de resistência em melancia aos principais potyvírus isolados de cucurbitáceas no Nordeste brasileiro. Horticultura Brasileira. 20:589-592.

Rabelo Filho FAC, Carvalho KF, Lima JAA, Queiroz MA, Paiva WO, Nascimento AKQ (2010) Fontes de resistência em melancia e meloeiro a vírus do gênero *Potyvirus*. Revista Brasileira de Ciências Agrárias - Brazilian Journal of Agricultural Sciences. 5:187-191.

Ramos NF, Lima JAA, Santos AA, Gonçalves MFB (2003a) Plantas de meloeiro com infecção mista de espécies de vírus em campos experimentais. Fitopatologia Brasileira. 27:211 supl.

Ramos NF, Lima JAA, Gonçalves MFB (2003b) Efeitos da interação de *Potyvirus* em híbridos de meloeiro, variedades de melancia e abobrinha. Fitopatologia Brasileira. 28:199-203.

Rodrigues AM (2011) Ocorrência, Distribuição e Diagnose de Viroses Associadas a Cultura da Melancia no Estado do Tocantins. 97 f. (Dissertação) Universidade Federal do Tocantins,Gurupi-TO,Brasil.

Silveira LM, Queiróz MA, Lima JAA, Nunes GHS, Nascimento AKQ, Lima Neto IS (2015) Herança da resistência ao Papaya ringspot virus em melancia. Caatinga (Online). 28:128-136.

Silveira LM, Queiróz MA, Lima JAA, Nunes GHS, Nascimento AKQ, Lima Neto IS (2014) Herança da resistência a Watermelon mosaic virus em melancia. Ceres. 61:530-537.

Shukla DD, Lauricella R, Ward CW, Brunt AA (1994) The Potyviridae. Wallingford: CAB International. Wallingford, UK, 516p.

Silva GTMA, Ribeiro RMP, Barros Junior AP, Silveira LM (2016) Characterization of cucurbit production systems and disease prevalence in municipalities in pernambuco. Rev Caatinga (Online). 29: 774–779.

Silva N, Costa CP (1978) Triagem de cultivares e híbridos de pepino (*Cucumis sativus* L.) para resistência a WMV-1 (*Watermelon mosaic virus*-1). Summa Phytopathologica. 4: 71-75.

Silveira LM (2008) Levantamento sorológico de vírus em cucurbitáceas na região do Submédio São Francisco e determinação de fontes e herança de resistência em melancia a espécies de Potyvirus. 129f. (Tese). Universidade Federal Rural do Semi-Árido, Mossoró-RN, Brasil.

Silveira LM, Queiróz MA, Lima JAA, Negreiros MZ (2005) Seleção de acessos e progênies de *Citrullus* spp . para resistência a três . Fitopatologia Brasileira.30:394-399.

Silveira LM, Queiroz MA, Lima JAA, Nascimento AKQ, Lima Neto IS (2009) Levantamento sorológico de vírus em espécies de cucurbitáceas na região do submédio São Francisco. Tropical Plant Pathology. 34:123-126.

Soares MGO, Soares JDA, Cezar MA, Cardoso TADL, Lima JA A (2016) Ocorrência de patógenos em cultivos de melancia e abóbora no sertão da Paraíba. Revista Verde de Agroecologia e Desenvolvimento Sustentável. 11: 07-13.

Strange EB, Guner N, Pesic-Vanesbroeck Z, Wehner TC (2002) Plant genetic resources screening the watermelon germplasm collection for resistance to *Papaya ringspot virus* type-W. Crop Science. 42:1324-1330.

Tavares AT, Chaves PPN, Aguiar RWS, Santos MF, Sarmento RA, Nascimento IR (2014) Phenotypic response of pumpkin and melon plants to infection by simple isolates of ZYMV and mixed ZYMV + SQMV. Journal of Biotechnology and Biodiversity. 5:79-87.

Vieira J (2005) Avaliação da coleção de germoplasma de melancia da Embrapa Hortaliças para tolerância a viroses. Embrapa Hortaliças. Brasília-DF, Brasil. 13p.

Vieira JV, Ávila AC, Olegário G (2010) Avaliação de genótipos de melancia para resistência ao *Papaya ringspot virus*, estirpe melancia. Horticultura Brasileira. 28:7-11.

Wilson CR (2001) Resistance to infection and translocation of *Tomato spotted wilt virus* in potatoes. Plant Pathology. 50:402-410.