Doi: https://doi.org/10.17584/rcch.2021v15i1.10693

Zucchini lineages with levels of resistance to ZYMV and SqMV viruses

Linajes de calabacín con niveles de resistencia a los virus ZYMV y SqMV

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Zucchini viruses.
Photo: I.F. Beloti

ABSTRACT

Zucchini (*Cucurbita pepo* L.) is a horticultural plant species of great socioeconomic value in tropical countries such as Colombia and Brazil. The production of zucchini is qualitatively and quantitatively affected by many diseases, especially viruses belonging to the *Potyvirus* (*Zucchini yellow mosaic virus* - ZYMV) and *Comovirus* (*Squash mosaic virus* - SqMV) groups. The primary strategy to reduce the spread of potentially damaging plant viruses is the development of genotypes with genetic tolerance; however, there are not many zucchini genotypes with multiple tolerance. Therefore, this study evaluated 66 zucchini genotypes to find sources of tolerance to the ZYMV and SqMV viruses. This experiment was conducted in a completely randomized design using genotypes from the germplasm bank of the Federal University of Uberlândia, including the genotypes: Emanuela (common commercial genotype) 'Tronco Caserta' (susceptible genotype) and PX 13067051 (resistant genotype). Leaf extracts containing viral particles were used as inoculant, and the distribution of grades of tolerance was recorded at the seedling stage. The lineages UFU-C×UFU-A#18#3;1, UFU-C×UFU-F#19#11;3, UFU-F#4#9;1, and UFU-D×UFU-F#7#21;1 and the Emanuela cultivar are alternatives for the production of new zucchini genotypes or hybrids with tolerance to the viruses ZYMV and SqMV. More severe symptoms were observed, as well as a larger number of susceptible genotypes for the ZYMV virus, indicating that this virus has great potential for causing damage and losses to zucchini crops.

Additional keywords: *Cucurbita pepo*; marrow zucchini; courgetti; *Zucchini yellow mosaic virus*; *Squash mosaic virus*; genetic tolerance.

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RESUMEN

El calabacín (*Cucurbita pepo* L.) es una especie de planta hortícola de gran valor socioeconómico en países tropicales como Colombia y Brasil. Su producción se ve afectada tanto cualitativa como cuantitativamente por muchas enfermedades en especial por los virus pertenecientes a los grupos Potyvirus (Virus de mosaico amarillo de Zucchini - ZYMV) y Comovirus (Virus de mosaico de calabaza - SqMV). La estrategia principal para reducir la propagación de virus vegetales potencialmente dañinos es el desarrollo de genotipos con tolerancia genética; sin embargo, no hay muchos genotipos de calabacín con tolerancia múltiple. Por lo tanto, este estudio evaluó 66 genotipos de calabacín para encontrar fuentes de tolerancia a los virus ZYMV y SqMV. El experimento se llevó a cabo en un diseño completamente aleatorio utilizando genotipos del banco de germoplasma de la Universidad Federal de Uberlândia, incluyendo los genotipos: 'Emanuela' (genotipo comercial común), 'Caserta' (genotipo susceptible) y 'PX 13067051' (genotipo resistente). Los extractos de hojas que contienen partículas virales se utilizaron como inoculantes y la distribución de los grados de tolerancia se registró en la etapa de plántula. Los linajes UFU-C×UFU-A#18#3;1, UFU-C×UFU-F#19#11;3, UFU-F#4#9;1, UFU-D×UFU-F#7#21;1, y el cultivar Emanuela son alternativas para la producción de nuevos genotipos de calabacín o híbridos tolerancia a los virus ZYMV y SqMV. Se observaron síntomas más graves, así como un mayor número de genotipos susceptibles para el virus ZYMV, lo que indica que este virus tiene un gran potencial para causar daños y pérdidas al cultivo de calabacín.

Palabras clave adicionales: *Cucurbita pepo*; calabacín de médula; courgette; *Zucchini yellow mosaic virus*; *Squash mosaic virus*; tolerancia genética.

Received: 02-03-2020 Accepted: 31-10-2020 Published: 02-01-2021

INTRODUCTION

Among the pumpkin family, the zucchini (*Cucurbita pepo* L.), also commercially known as marrow zucchini, courgette, or Italian pumpkin, is widely used for raw consumption or in many cooking recipes. This horticultural fruit is an important source of B vitamins, niacin, calcium, phosphorus and iron. In Brazil, zucchini is among the top ten vegetables because of the great economic and social value, which can be found with a light green color, white or green with dark-colored stripes (Couto *et al*., 2009).

Several phytosanitary problems cause considerable damage to this crop, such as powdery mildew (*Podosphaera xanthii*), anthracnose (*Colletotrichum gloeosporioides* f. sp. *cucurbitae*), black rot (*Didymella bryoniae*), fusarium wilt (*Fusarium oxysporum*), bacterial blackleg (*Pectobacterium carotovorum* subsp. *carotovorum*) and virus (*Papaya ringspot virus*, *Zucchini yellow mosaic virus*, *Squash mosaic virus* and *Watermelon ringspot virus*) (Nogueira *et al*., 2011; Agrofit, 2017).

Among the diseases that occur in cucurbits, those caused by viruses seriously affect the quality and quantity of fruit production, representing one of the most important limiting factors for this crop production (Finetti-Sialer *et al*., 2012; Yesil, 2019). The phytoviroses have no curative control, and, thus, preventive measures are needed for crop management (Rodríguez *et al*., 2016; Cutler *et al*., 2018). Existing strategies to decrease dissemination include the development of cultivars or hybrids with genetic resistance to viral infections (Nogueira *et al*., 2011).

More than 20 species of viruses can naturally infect cucurbits, and those that belong to the genus *Potyvirus* (family *Potyviridae*) have proven to be the most important, especially ZYMV (*Zucchini yellow mosaic virus*). The symptoms include chlorosis of leaf veins, usually in the first leaves. The presence of severe mosaic and systemic necrosis presents a yellowish color in the leaves and sharp reductions in plant development (Finetti-Sialer *et al*., 2012). The fruits are stunted and deformed, resulting in reduced yield and making them non-marketable (Spadotti *et al*., 2015). ZYMV is markedly present in the producing regions of Brazil; Barbosa *et al*. (2016), in a survey in the Vale do Rio São Francisco, obtained 44% symptomatic plants with the presence of this virus.

Other viruses of importance include the *Squash mosaic virus* (SqMV), a member of the *Comovirus* genus of the subfamily *Comovirinae*, Family *Secoviridae*, and

order *Picornavirales* (Sanfacon *et al*., 2011). Alencar *et al*. (2012), in a survey of viruses that infect cucurbits in Tocantins State (Brazil), found that 56% of the samples were infected with the virus SqMV, a high incidence in cucurbits – contrary to that reported by other authors –. According to Alencar *et al*. (2016), symptoms depend on the virus isolate, plant species and environment; however, the majority of susceptible hosts present a severe systemic mosaic, with leaf and fruit deformation. The symptoms can also vary if the host plant is concomitantly infected by different viruses (Barbosa *et al*., 2016; Silva *et al*., 2016).

Historically, resistance to viruses was considered the most important goal in developing strains of *C. pepo*. Since pumpkins are harvested continuously, plants continue to grow and differentiate when exposed to infection by viruses (Whitaker and Robinson, 1986; Paris, 2016). The development of resistant cultivars to the cucurbits virus is usually a long and complex process. In the initial phase, it is necessary to select an appropriate source of resistance to certain species of viruses or to more than one species (Silveira *et al*., 2009; Nogueira *et al*., 2011).

There is little to no information about zucchini cultivars that are resistant to more than one virus; thus, this study aimed to evaluate zucchini genotypes to identify tolerance to the virus ZYMV and SqMV. This was a prospective study that intended to detect tolerant zucchini genotypes for future serological and molecular tests in thebreeding program.

MATERIALS AND METHODS

This experiment was conducted from January to August, 2018, at the Experimental Station of Vegetables of the Federal University of Uberlândia (UFU), Campus Monte Carmelo, Minas Gerais state, Brazil (18°42'43.19"S and 47°29'55.8", 873 m a.s.l.). The buffer solutions were prepared in the Laboratory of Seed Analysis and Genetic Resources (LAGEN) of the UFU.

This study was part of the Program of Genetic Improvement of Pumpkins of the UFU, Campus Monte Carmelo. The genotypes were obtained from the Vegetable Germplasm Bank of the same institution, from a free market in the region of Monte Carmelo City. The breeding method used to obtain the lineages was genealogic. The lineages were evaluated from crosses between: "UFU-A", "UFU-B", "UFU-C", "UFU-D", "UFU-E", "UFU-F", and "UFU-G". The visual criteria of desirable fruit numbers of male and female flowers, SPAD index and leaf temperature were used to advance the generations to F_4. The characteristics of parental genotypes are presented in table 1.

Table 1. Characterization of zucchini genotypes used as progenitors.

Genotypes	Fruit characteristics
UFU-A	Uniform fruits, light-green colored with dark-green shine stripes, presenting a discrete floral scar and superior quality
UFU-B	Uniform fruits and light-green colored with very dark-green stripes
UFU-C	Cylindrical green fruit with dark-green stripes
UFU-D	Fruits with clear, light coloring with dark-green stripes
UFU-E	Cylindrical fruits, slightly protuberant, and light green coloring
UFU-F	Fruits slightly protuberant and light green coloring
UFU-G	Cylindrical fruits and slightly protuberant

The genotypes evaluated in this study, except the controls, belonged to generation F_4 and were derived from selections made over 163 genotypes evaluated in this generation, selecting 66 genotypes (Tab. 2). The Emanuela cultivar, from the zucchini breeding program of the UFU, was included for the evaluation of virus resistance. A susceptible control was used, Tronco Caserta (ISLA®); a resistant control was used, hybrid PX 13067051 with resistance to ZYMV and PRSV-W (Seminis®).

The virus ZYMV and SqMV strains were obtained from the Central Viruses Indexing, located in the Department of Plant Pathology of the Federal University of Lavras in November, 2017. Serological tests were used to confirm virus presence in the plants. Maintenance was carried out weekly at the Experimental Station of Vegetables of the UFU, Campus Monte Carmelo, through the inoculation of zucchini seedlings.

Each 25 kg of commercial substrate had 0.5 kg of formulated 4-14-8 added, revolving til homogenization. This fertilization was required to obtain vigorous plants without "masking" the characteristic symptoms of the viruses.

Table 2. Zucchini genotypes evaluated.

Number	Genotype code	Number	Genotype code
1	UFU-A#6#6;3	34	UFU-D×UFU-C#8#12;1
2	UFU-B#1#7;2	35	UFU-D×UFU-C#8#14;2
3	UFU-B#9#4;3	36	UFU-D×UFU-C#8#17;3
4	UFU-B×UFU-A#17#3;2	37	UFU-D×UFU-C#8#6;3
5	UFU-B×UFU-A#17#4;1	38	UFU-D×UFU-C#8#9;2
6	UFU-B×UFU-A#17#5;1	39	UFU-D×UFU-F#7#1;1
7	UFU-B×UFU-A#17#5;25	40	UFU-D×UFU-F#7#11;1
8	UFU-B×UFU-F#9#1;1	41	UFU-D×UFU-F#7#12;3
9	UFU-B×UFU-F#9#12;2	42	UFU-D×UFU-F#7#14;1
10	UFU-B×UFU-F#9#13;3	43	UFU-D×UFU-F#7#16;1
11	UFU-B×UFU-F#9#14;1	44	UFU-D×UFU-F#7#18;1
12	UFU-B×UFU-F#9#16;2	45	UFU-D×UFU-F#7#19;2
13	UFU-B×UFU-F#9#2;3	46	UFU-D×UFU-F#7#2;2
14	UFU-B×UFU-F#9#9;2	47	UFU-D×UFU-F#7#2;3
15	UFU-C#3#4;1	48	UFU-D×UFU-F#7#20;3
16	UFU-C×UFU-A#18#3;1	49	UFU-D×UFU-F#7#21;1
17	UFU-C×UFU-F#19#10;2	50	UFU-D×UFU-F#7#23;2
18	UFU-C×UFU-F#19#11;3	51	UFU-D×UFU-F#7#6;1
19	UFU-C×UFU-F#19#9;1	52	UFU-D×UFU-F#7#9;1
20	UFU-F#4#9;1	53	UFU-D×UFU-E#11#10;2
21	UFU-F×UFU-A#12#10;1	54	UFU-D×UFU-E#11#11;2
22	UFU-F×UFU-A#12#9;2	55	UFU-DXUFU-E#11#12;2
23	UFU-D#5#1;1	56	UFU-DXUFU-E#11#13;1
24	UFU-D#5#2;1	57	UFU-DXUFU-E#11#7;2
25	UFU-D#5#3;2	58	UFU-E#12#8;1
26	UFU-D#5#4;1	59	cv. Tronco Caserta (susceptible)
27	UFU-D#5#4;3	60	UFU-E
28	UFU-D×UFU-A#16#12;1	61	UFU-A
29	UFU-D×UFU-A#16#2;1	62	UFU-B
30	UFU-D×UFU-A#16#2;2	63	UFU-C
31	UFU-D×UFU-A#16#3;2	64	PX 13067051 (resistant)
32	UFU-D×UFU-A#16#8;1	65	UFU-G
33	UFU-D×UFU-C#8#11;1	66	cv. Emanuela

The sowing occurred in polystyrene trays (128 cells), maintaining adequate moisture in the substrate before and after sowing. The susceptible cultivar used to maintain the virus was Tronco Caserta (ISLA®), which presents an upright growth habit, facilitating the cultural treatments in the greenhouse. The cotyledonary leaves of the seedlings were inoculated at 7 days after emergence (DAE) and at 14 DAE. The viruses were kept in protected cages with anti-aphid screens to avoid contamination by insect vectors, such as aphids and whiteflies.

The buffer solutions were prepared with the methodology (adapted) used by Maluf (1986): (1) 250 mL of

0.2 M KH_2PO_4 (monobasic potassium phosphate) + 0.2% Na_2SO_3 (sodium sulfite) solution – the final pH should be about 7.3 –; (2) 300 mL of 0.2 M K_2HPO_4 (potassium phosphate dibasic) + 0.2% Na_2SO_3 (sodium sulfite) solution – the final pH should be 9.0 –; (3) the pH of the K_2HPO_4 solution should be adjusted to 7.3 using the 0.2 M KH_2PO_4 solution. This solution was stored under refrigeration (2 to 5°C).

The virus inoculation method on the cotyledonary leaves, at 7 and 14 DAE, extract from infected pumpkin leaf with SqMV and ZYMV viruses (separately) was used to inoculate the zucchini plants. The infected leaves presented the mosaic symptom and leaf deformation. Each pumpkin leaf extract was prepared with the buffer solutions with leaf maceration in a mortar with a phosphate buffer. The proportion was 90 mL buffer and 10 g of fresh leaf tissue. Inoculations were performed by rubbing the extract with the fingertips on zucchini leaves. These leaves were first sprinkled with carborundum (400 mesh). Subsequently, the leaves were washed with tap water, and the plants were maintained in a greenhouse until the final evaluation of symptoms. The inoculations were done early in the morning, avoiding high temperatures.

The experiment design was completely randomized, with model (1):

$$Y_{ij} = \mu + t_i + e_{ij} \quad (1)$$

where: Y_{ij} is the observation made in the plot for the treatment of the repetition j; μ represents a constant inherent in the whole plot; t_i represents the effect of the treatment i; e_{ij} is the experiment error in plot i, j. The experiment unit was one seedling. Five replications were performed with four seedlings in each, totaling 20 seedlings per treatment (inoculation). The experiment was conducted in a greenhouse using cultural treatments recommended for zucchini cultures.

Evaluations

The characteristic symptoms were observed after the third week, i.e., a week after the second inoculation, and consisted of a single assessment. A diagrammatic scale of notes (1 to 5) was used to assess the susceptibility of the materials to the viruses according to the modified scale of Maluf *et al*. (1986):

1 = most of the leaves without symptoms, a new leaf showing mild symptoms and or mild whitening ribs; 2 = most of the leaves with mild symptoms, mild whitening ribs or sparse chlorotic spots; 3 = most of the leaves with mosaic, symptoms ranging from whitening ribs or chlorotic spots to sparse chloroses up to 50% of the leaf area; 4 = almost all the leaves with mosaic, coalescence of chlorotic areas, reaching up to 50% of leaf area; 5 = almost all the leaves with severe mosaic, leaves with more than 50% affected leaf area or with severe distortions.

The zero score scale was not used because of the absence of serological or molecular tests in the initial stage of the screening program. The genotypes with higher degrees of tolerance to these viruses will be selected for future characterizations of resistance. This strategy is intended to make breeding programs viable by saving resources in the initial stages of a breeding process and by generating other important characteristics of economic interest.

The symptoms observed at 21 d after the first inoculation were also described as follows: borders furrowed (Bf); blisters (Bl); leaf distortion (Ld); leaf curl (Lc); mosaic (M); parallel veins (Pv), and without symptoms (Ws).

The data were subjected to analysis of variance with an F test ($P<0.05$). The averages were compared in two distinct ways: the Scott-Knott test ($P<0.05$) and Dunnet's test ($P<0.05$), used to compare the performance of the genotypes and individually, with susceptible and resistant controls, respectively. All data were analyzed using the *R* software (R Core Team, 2014), testing the assumptions of normality of residues (Levene's test) and homogeneity of variances (Kolmogorov-Smirnov test), at the 0.05 significance level.

RESULTS

The evaluation of zucchini genotypes indicated significant differences between the evaluated materials for the tolerance to the SqMV and ZYMV viruses. The assessment identified a range of different symptoms among the studied genotypes (Tab. 3).

Table 3. Grades and symptoms attributed to the zucchini genotypes related to SqMV and ZYMV.

Genotypes	SQMV	Symptoms	ZYMV	Symptoms
UFU-A#6#6;3	3.00 b	pv/Bl/M/Ld	3,35 c-	BL/M/BF/Ld
UFU-B#1#7;2	2.78 b+	Ld/Bl/M/Pv/Ws	3,85 d-	M/BL/Pv
UFU-B#9#4;3	2.85 b	Pv/M/Bl/Ld/Ws	4,22 d-	M/BL/Pv/BF
UFU-B×UFU-A#17#3;2	3.20 b	M/Ld/Bl/Pv/Bf	2,95 b	M/BL//Ld/BF
UFU-B×UFU-A#17#4;1	2.80 b+	Ld/M/Bl/Pv/Ws	3,95 d-	M/BL/Ld
UFU-B×UFU-A#17#5;1	3.03 b	M/Bl/Ld/Pv/Ws	3,22 c-	Bl/M/Bf
UFU-B×UFU-A#17#5;25	2.43 a+	M/Bl/Pv/Ws/Ld	2,48 b+	Bl/M/Ld/BF
UFU-B×UFU-F#9#1;1	4.65 c-	Pv/Bl/M/Ld/Bf	4,00 d-	NP/M/BL/Ld/BF
UFU-B×UFU-F#9#12;2	2.82 b+	M/Bl/Ld/Bf	3,13 c-	M/BL/BF
UFU-B×UFU-F#9#13;3	3.05 b	Bl/Ld/M/Bf/Pv	3,27 c-	M/BL
UFU-B×UFU-F#9#14;1	2.55 b+	bl/Ld/Ws/Pv/Bf/M	3,30 c-	M/BL/BF/Ld
UFU-B×UFU-F#9#16;2	3.17 b	Bl/Pv/Ld	4,60 d-	M/BL/BF/Pv
UFU-B×UFU-F#9#2;3	4.07 c-	ld/Bf/Pv/M/Bl/Ws	4,52 d-	M/BL/Ld/BF
UFU-B×UFU-F#9#9;2	3.05 b	Ld/M/Bl/Pv	3,45 c-	M/BL/BF
UFU-C#3#4;1	2.28 a+	Bl/M/Ld/Ws/Pv	2,98 b	Bl/M/Ws
UFU-C×UFU-A#18#3;1	2.28 a+	M/Ld/Bl/Ws	2,20 a+	BL/M/Ws
UFU-C×UFU-F#19#10;2	2.00 a+	M/Ld/Bl/Ws/Pv	2,60 b+	M/Bl/BF
UFU-C×UFU-F#19#11;3	2.45 a+	M/Bl/Ld/Ws	2,10 a+	Bf/M/Bl/Ws
UFU-C×UFU-F#19#9;1	2.72 b+	M/Ld/Bl	3,00 b	M/Bl/Bf
UFU-F#4#9;1	2.20 a+	Bl/M/Ld	2,10 a+	M/Bl/BF
UFU-F×UFU-A#12#10;1	2.65 b+	Bl/Pv/Ld/Bf/M	3,62 c-	M/BL/Bf
UFU-F×UFU-A#12#9;2	2.90 b	M/Bl/Ld/Pv/Bf	3,68 c-	M/BL/Ld/BF
UFU-D#5#1;1	2.08 a+	M/Bl/Ld/Ws	3,57 c-	M/Bl/Ld/Bf
UFU-D#5#2;1	2.60 b+	pv/Ws/Bl/Ld/Bf	2,88 b	Bl/M/Ld/BF
UFU-D#5#3;2	2.22 a+	Bl/Ld/M/Ws	2,60 b+	M/Bl/Bf
UFU-D#5#4;1	2.22 a+	M/Ld/Bl/Ws	3,23 c-	M/Bl
UFU-D#5#4;3	2.55 b +	Ld/Bl/M/Ws	4,00 d-	M/BL/Bf/Ld
UFU-D×UFU-A#16#12;1	2.43 a+	M/Bl/Ws/Ld/Pv	3,07 c-	M/Bl/Bf
UFU-D×UFU-A#16#2;1	2.45 a+	Ws/Bl/Ld/M/Pv	3,20 c-	M/Bl/Ld/BF
UFU-D×UFU-A#16#2;2	2.48 a+	m/Ld/Bl/Pv/Bf/Ws	3,78 d-	M/Bl/Bf
UFU-D×UFU-A#16#3;2	2.68 b+	Bl/Ld/M/Bf/Pv	3,07 c-	M/Bl/Bf/Ld
UFU-D×UFU-A#16#8;1	2.60 b+	Bl/Ld/Bf/M/Pv	3,35 c-	Bl/Bf/M
UFU-D×UFU-C#8#11;1	2.40 a+	M/Bl/Bf/Ws/Ld	3,55 c-	M/Bl/Ld/Bf
UFU-D×UFU-C#8#12;1	4.45 c-	Ld/Bl/Bf/M/Pv	3,60 c-	Bl/M/Bf/Ld
UFU-D×UFU-C#8#14;2	2.53 b+	Pv/Bl/Ws/M/Ld	3,10 c-	Bl/M/Ld/BF
UFU-D×UFU-C#8#17;3	2.23 a+	M/Ld/Bl/Ws	2,72 b	Ld/Bl/M/Ws
UFU-D×UFU-C#8#6;3	2.88 b	Ws/M/Bl/Ld/Pv	2,80 b	M/Bl/Bf
UFU-D×UFU-C#8#9;2	2.30 a+	Ws/Bl/Ld/M	3,17 c-	M/Bl/Bf
UFU-D×UFU-F#7#1;1	2.60 b+	M/Bl/Ws/Pv/Ld	3,25 c-	Bl/M/Bf

Continued

Continuation Table 3. Grades and symptoms attributed to the zucchini genotypes related to SqMV and ZYMV.

Genotypes	SqMV	Symptoms	ZYMV	Symptoms
UFU-D×UFU-F#7#11;1	2.40 a+	Ws/Ld/Bl/M	2,98 b-	M/Bl/Bf/Ws
UFU-D×UFU-F#7#12;3	2.75 b+	M/Ld/Bl/Ws	3,08 c-	M/Bl/Bf/Ld
UFU-D×UFU-F#7#14;1	2.60 b+	Bl/Ws/M/Pv/Ld	2,80 b	M/Bl/Bf
UFU-D×UFU-F#7#16;1	2.45 a+	Bl/M/Ld/Ws	4,00 d-	M/Bl/Bf
UFU-D×UFU-F#7#18;1	2.58 b+	Bl/Ws/M/Ld	2,62 b+	M/Bl/Bf
UFU-D×UFU-F#7#19;2	2.65 b+	Bl/M/Ld	2,43 b+	M/Bl/Bf
UFU-D×UFU-F#7#2;2	2.80 b+	Bl/M/Ld/Bf	2,47 b+	M/Bl/Bf/Ld
UFU-D×UFU-F#7#2;3	3.28 b	Bl/M/Ld/Pv	3,55 c-	M/Bl/Bf
UFU-D×UFU-F#7#20;3	2.25 a+	Bl/Ld/Ws/M	2,90 b	M/Bl/Bf
UFU-D×UFU-F#7#21;1	2.37 a+	M/Ld/Bl/Ws	2,05 a	Bl/M/Ws
UFU-D×UFU-F#7#23;2	2.07 a+	Ws/Ld/Bl/M	3,55 c-	Bl/Bf/M/Ld
UFU-D×UFU-F#7#6;1	2.12 a+	ws/M/Bl/Pv/Bf/Ld	2,67 b+	Bf/Bl/M/Ld/Ws
UFU-D×UFU-F#7#9;1	2.25 a+	M/Bl/Ld	2,33 b+	M/Bl/Bf/Ws
UFU-D×UFU-E#11#10;2	2.60 b+	Bl/Ld/M/Bf/Ws	3,17 c-	Bl/M/Bf
UFU-D×UFU-E#11#11;2	3.08 b	M/Bl/Ld/Pv	3,40 c-	M/Ld/Bl/Bf
UFU-D×UFU-E#11#12;2	2.31 a+	M/Bl/Ws	3,31 c-	M/Bl/Bf
UFU-D×UFU-E#11#13;1	2.92 b	M/Ld/Bl/Pv	2,83 b	M/Bl/Bf/Ws
UFU-D×UFU-E#11#7;2	3.02 b	Ld/Bl/M/Pv/Bf	3,68 c-	M/Bl/Bf/Ld
UFU-E#12#8;1	2.60 b+	M/Bl/Ld/Ws	3,45 c-	M/Bl/Ws
'Tronco Caserta'	4.62 c	M/Pv/Ld/Bl	4,20 d	M/Bl/Pv/Bf
UFU-E	2.43 a+	Bl/Pv/M/Ws/Ld	3,45 c-	M/BF/Ld/BL/NP
UFU-A	2.57 b+	M/Bl/Ws/Ld	2,73 b+	Bl/Ld/M
UFU-B	3.07 b	M/Bl/Bf/Ld	2,70 b+	M/Bl/Ld
UFU-C	1.33 a+	M/Bl/Ws	3,73 d-	Ws/M/Bl
PX 13067051	1.67 a	Bl/Ws	1,77 a	Ws/Bl
UFU-G	3.33 b	M/Bl	1,53 a	M/Ws/Bl
'Emanuela'	1.73 a+	Bl/Bf/Ws/Bf/M	1,55 a+	Bl/Bf/M/Ws
CV (%)	21.61		18.43	
Kolmogorov-Smirnov	0.3118		0.1466	
Levene	0.6868		0.6868	

Means with different letters indicate statistically significant differences according to the Scott-Knott ($P<0.05$).
+: do not differ according to the Dunnett test ($P<0.05$) for the resistant genotype *PX 13067051*
-: do not differ according to the Dunnett test ($P<0.05$) for the resistant genotype *Tronco Caserta*
Bf: borders furrowed; Bl: blisters; Ld: leaf distortion; Lc: leaf curl; M: mosaic; Pv: parallel veins; Ws: without symptoms.

For SqMV, the genotypes UFU-B×UFU-A#17#5;25, UFU-C#3#4;1, UFU-C×UFU-A#18#3;1, UFU-C×UFU-F#19#10;2, UFU-C×UFU-F#19#11;3, UFU-F#4#9;1, UFU-D#5#1;1, UFU-D#5#3;2, UFU-D#5#4;1, UFU-D×UFU-A#16#12;1, UFU-D×UFU-A#16#2;1; UFU-D×UFU-A#16#2;2, UFU-D×UFU-C#8#11;1, UFU-D×UFU-C#8#17;3, UFU-D×UFU-C#8#9;2, UFU-D×UFU-F#7#11;1, UFU-D×UFU-F#7#16;1, UFU-D×UFU-F#7#20;3, UFU-D×UFU-F#7#21;1, UFU-D×UFU-F#7#23;2, UFU-D×UFU-F#7#6;1, UFU-D×UFU-F#7#9;1, UFU-D×UFU-F#7#9;1, UFU-DXUFU-E#11#12;2, UFU-E, and UFU-C and cv. Emanuela did not differ according to the Skott-knott test ($P>0.05$) from the PX 13067051 resistant genotype.

The ZYMV symptoms in the UFU-C×UFU-A#18#3;1, UFU-C×UFU-F#19#11;3, UFU-F#4#9;1, UFU-D×UFU-F#7#21;1, UFU-G and cv. Emanuela genotypes did not differ according to the Skott-Knott test ($P>0.05$) from PX 13067051 (resistant genotype to ZYMV). These genotypes, except UFU-G, presented good tolerance to both viruses.

The symptoms in the plants inoculated with SqMV evolved from simple mosaic, leaf blister, and distortion to more severe cases with the presence of parallel veins and furrowed edges. The symptoms in the zucchini genotypes inoculated with ZYMV evolved from simple to blister mosaic, soft leaf distortion to more severe cases of mosaic and leaf deformation with severe furrowed edges.

DISCUSSION

The phenotypic evaluations demonstrated that all zucchini plants of the 'Tronco Caserta' genotype showed symptoms after inoculation with the ZYMV and SqMV viruses. This result demonstrates the effectiveness of the inoculation procedure because 'Tronco Caserta' is susceptible to viruses and is commonly used in studies that evaluate the virulence of isolates (Oliveira *et al*., 2000; Tavares *et al*., 2014).

According to Finetti-Sialer *et al*. (2012), zucchini plants affected by the ZYMV virus may present mosaic, reduction of leaf blade area, leaf and fruit deformation, blisters and necrosis, and the symptoms vary depending on the infected host and the isolate used. Barbosa *et al*. (2017) evaluated the phenotypic reactions and behavior of 28 pumpkin accessions of the cucurbits Germplasm Bank of Embrapa Semiarid (Petrolina, PE, Brazil) to PRSV-W, ZYMV and WMV viruses, and none of the evaluated genotypes showed immunity to the viruses. More serious symptoms were observed in the accessions inoculated with ZYMV, where 50% of the accessions were highly susceptible. This fact was also observed by other authors: Moura *et al*. (2005), Oliveira *et al*. (2000), and Yakoubi *et al*. (2008).

Radwan *et al*. (2007) demonstrated that *C. pepo* cv. Eskandarani leaves infected with ZYMV presented varying degrees of symptoms, including severe mosaic, size reduction, dwarfism, and deformation. The viral infection also decreased the levels of pigments, proteins and carbohydrates. The peroxidase activity and the proline content were also induced.

Moura *et al*. (2005) analyzed the reaction of *Cucurbita* sp. accessions to ZYMV and verified that this virus causes strong disorganization in the arrangement and form of epidermal cells and palisade parenchyma, inducing hyperplasia (excessive multiplication of cells) and causing leaf distortion. In the present study, this symptom was only observed in genotypes with high susceptibility to the virus.

In the present study, 39 genotypes did not differ from the susceptible control cv. Tronco Caserta for ZYMV. A great number when compared to the SqMV, in which only three genotypes did not differ. Barbosa *et al*. (2017) evaluated the resistance of 28 different accessions of *Cucurbita* spp. to the PRSV-W, ZYMV and WMV viruses and came to the conclusion that more severe symptoms were observed in hosts inoculated with ZYMV with 50% being highly susceptible.

For SqMV, 46 genotypes did not differ from the resistant PX 13067051 genotype, while for ZYMV, only 14 genotypes did not differ. Moura *et al*. (2005) evaluated 100 accessions of *Cucurbita* sp. from the Active Germplasm Bank of the Federal University of Viçosa and found immunity to ZYMV in only three genotypes, while 26 were resistant and 48 were tolerant to ZYMV. These results demonstrate the higher aggressiveness of ZYMV than in the other virus.

Şevik and Toksöz (2008) reported that SqMV was detected with an incidence of 20.9% in *Cucurbita* sp. species after the analysis of symptomatic samples with DAS-ELISA (double antibody sandwich - Enzyme-Linked Immunosorbent Assay) in a survey conducted in Samsun (Turkey). The symptoms included severe or mild green mosaic, parallel veins, deformation or reduction in the shape and size of the leaves and fruits, similar to what was found in the present study.

This study presents important results of the evaluation of *Zucchini yellow mosaic virus* and the *Squash mosaic virus* for the selection of zucchini genotypes. No other study had assessed these viruses for this crop, indicating the importance of the selection of zucchini genotypes with some level of tolerance for progenitors in breeding programs.

CONCLUSION

The genotypes UFU-C×UFU-A#18#3;1, UFU-C×UFU-F#19#11;3, UFU-F#4#9;1, and UFU-D X UFU-F#7#21;1 and cv. Emanuela are alternatives for the production of new zucchini cultivars or hybrids tolerant to ZYMV and SqMV.

The five genotypes should be re-inoculated, evaluating again using an adapted scale of notes (including the note '0') and serological and molecular tests in further zucchini breeding studies.

ACKNOWLEDGMENTS

Coordination of Superior Level Staff Improvement (CAPES). Brazilian National Council for Scientific and Technological Development (CNPQ). Research Support Foundation of the State of Minas Gerais (FAPEMIG). Federal University of Uberlândia (UFU).

Conflict of interests: The manuscript was prepared and reviewed with the participation of the authors, who declare that there exists no conflict of interest that puts at risk the validity of the presented results.

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