Zucchini lineages with levels of resistance to ZYMV and SqMV viruses

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

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 andSqMV 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, UFU-C×UFU-F#19#11, 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.
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 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 (Rodriguez et al., 2016; Cutler et al., 2018). Existing strategies to decrease dissemination include the development of cultivars or hybrids with genetic resistance (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 (Sanfalcon 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 the breeding 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 F₄. The characteristics of parental genotypes are presented in table 1.

| 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 F₄ 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 till homogenization. This fertilization was required to obtain vigorous plants without “masking” the characteristic symptoms of the viruses.
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

### 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-F#7#11;2    |
| 21     | UFU-F×UFU-A#12#10;1   | 54     | UFU-D×UFU-F#7#11;2    |
| 22     | UFU-F×UFU-A#12#9;2    | 55     | UFU-D×UFU-F#7#12;2    |
| 23     | UFU-D#5#1;1           | 56     | UFU-D×UFU-F#7#13;1    |
| 24     | UFU-D#5#2;1           | 57     | UFU-D×UFU-F#7#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          |
0.2 M KH$_2$PO$_4$ (monobasic potassium phosphate) + 0.2% Na$_2$SO$_3$ (sodium sulfite) solution – the final pH should be about 7.3 –; (2) 300 mL of 0.2 M K$_2$HPO$_4$ (potassium phosphate dibasic) + 0.2% Na$_2$SO$_3$ (sodium sulfite) solution – the final pH should be 9.0 –; (3) the pH of the K$_2$HPO$_4$ solution should be adjusted to 7.3 using the 0.2 M KH$_2$PO$_4$ 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):

\[
Y_{ij} = \mu + t_i + e_{ij}
\]

where: \(Y_{ij}\) is the observation made in the plot for the treatment of the repetition \(j\); \(\mu\) 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        | 3.35 c-       |
| UFU-B#1#7:2        | 2.78 b+       | 3.85 d-       |
| UFU-B#9#4:3        | 2.85 b        | 4.22 d-       |
| UFU-B×UFU-A#17#3:2 | 3.20 b        | 2.95 b        |
| UFU-B×UFU-A#17#4:1 | 3.80 b+       | M/BL/Ld       |
| UFU-B×UFU-A#17#5:1 | 3.03 b        | M/BL/Ld/Pv    |
| UFU-B×UFU-A#17#5:25| 2.43 a+       | 2.48 b+       |
| UFU-B×UFU-F#9#1:1  | 4.65 c-       | 4.00 d-       |
| UFU-B×UFU-F#9#12:2 | 2.82 b+       | 3.13 c-       |
| UFU-B×UFU-F#9#13:3 | 3.05 b        | M/BL/Ld       |
| UFU-B×UFU-F#9#14:1 | 2.55 b+       | 3.30 c-       |
| UFU-B×UFU-F#9#16:2 | 3.17 b        | 4.60 d-       |
| UFU-B×UFU-F#9#2:3  | 4.07 c-       | 4.52 d-       |
| UFU-B×UFU-F#9#9:2  | 3.05 b        | 3.45 c-       |
| UFU-C#3#4:1        | 2.28 a+       | 2.98 b        |
| UFU-C×UFU-A#18#3:1 | 2.28 a+       | 2.20 a+       |
| UFU-C×UFU-F#19#10:2| 2.00 a+       | 2.60 b+       |
| UFU-C×UFU-F#19#11:3| 2.45 a+       | 2.10 a+       |
| UFU-C×UFU-F#19#9:1 | 2.72 b+       | 3.00 b        |
| UFU-F#4#9:1        | 2.20 a+       | 2.10 a+       |
| UFU-F×UFU-A#12#10:1| 2.65 b+       | 3.62 c-       |
| UFU-F×UFU-A#12#9:2 | 2.90 b        | 3.68 c-       |
| UFU-D#5#1:1        | 2.08 a+       | 3.57 c-       |
| UFU-D#5#2#2:1      | 2.60 b+       | 2.88 b        |
| UFU-D#5#3:2        | 2.22 a+       | 2.60 b+       |
| UFU-D#5#4:1        | 2.22 a+       | 3.23 c-       |
| UFU-D#5#4:3        | 2.55 b+       | 4.00 d-       |
| UFU-D×UFU-A#16#12:1| 2.43 a+       | 3.07 c-       |
| UFU-D×UFU-A#16#2:1 | 2.45 a+       | 3.20 c-       |
| UFU-D×UFU-A#16#2:2 | 2.48 a+       | 3.78 d-       |
| UFU-D×UFU-A#16#3:2 | 2.68 b+       | 3.07 c-       |
| UFU-D×UFU-A#16#8:1 | 2.60 b+       | 3.35 c-       |
| UFU-D×UFU-C#8#11:1 | 2.40 a+       | 3.55 c-       |
| UFU-D×UFU-C#8#12:1 | 4.45 c-       | 3.60 c-       |
| UFU-D×UFU-C#8#14:2 | 2.53 b+       | 3.10 c-       |
| UFU-D×UFU-C#8#17:3 | 2.23 a+       | 2.72 b        |
| UFU-D×UFU-C#8#6:3  | 2.88 b        | 2.80 b        |
| UFU-D×UFU-C#9#2    | 2.30 a+       | 3.17 c-       |
| UFU-D×UFU-F#7#1:1  | 2.60 b+       | 3.25 c-       |

Continued
For SqMV, the genotypes UFU-B×UFU-A#17#5;25, 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×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#6;1, UFU-D×UFU-F#7#9;1, UFU-D×UFU-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.

### 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+ | M/Bl/Ws |
| UFU-D×UFU-F#7#12;3 | 2.75 b+ | M/Ld/Bl/Ws |
| UFU-D×UFU-F#7#14;1 | 2.60 b+ | B/Ld/Ws/M/Pv/Ld |
| UFU-D×UFU-F#7#16;1 | 2.45 a+ | B/M/Ld/Ws |
| UFU-D×UFU-F#7#18;1 | 2.58 b+ | B/Bl/M/Ld |
| UFU-D×UFU-F#7#19;2 | 2.65 b+ | M/Ld |
| UFU-D×UFU-F#7#2;2 | 2.80 b+ | M/Ld/Bf |
| UFU-D×UFU-F#7#3;3 | 3.28 b | M/Ld/Pv |
| UFU-D×UFU-F#7#20;3 | 2.25 a+ | M/Ld/Ws/M |
| UFU-D×UFU-F#7#21;1 | 2.37 a+ | M/Ld/Ws |
| UFU-D×UFU-F#7#23;2 | 2.07 a+ | Ws/Ld/M |
| UFU-D×UFU-F#7#6;1 | 2.12 a+ | ws/M/Pv/Bf/Ld |
| UFU-D×UFU-E#11#10;2 | 2.60 b+ | M/Ld/M/Bf |
| UFU-D×UFU-E#11#11;2 | 3.08 b | M/Ld/Pv |
| UFU-D×UFU-E#11#12;2 | 2.31 a+ | M/Ld/M |
| UFU-D×UFU-E#11#13;1 | 2.92 b | M/Ld/Pv |
| UFU-D×UFU-E#11#7;2 | 3.02 b | Ld/M/Pv/Bf |
| UFU-E#12#8;1 | 2.60 b+ | M/Ld/Ws |
| ‘Tronco Caserta’ | 4.62 c | M/Pv/Ld/Bf |
| UFU-E | 2.43 a+ | M/Pv/M/Ws/Ld |
| UFU-A | 2.57 b+ | M/Ld/Ws/Ld |
| UFU-B | 3.07 b | M/Ld/Ld |
| UFU-C | 1.33 a+ | M/Ld/Ws |
| PX 13067051 | 1.67 a | B/M/Ws |
| UFU-G | 3.33 b | M/Ld/Ws |
| ‘Emanuela’ | 1.73 a+ | B/M/Ws |

| CV(%) | Kolmogorov-Smirnov | Levene |
|-------|---------------------|--------|
| 21.61 | 0.3118 | 0.6868 |
| 18.43 | 0.1466 | 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.
The ZYMV symptoms in the UFU-CxUFU-A#18#3;1, UFU-CxUFU-F#19#11;3,
UFU-F#4#9;1, UFU-DxUFU-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 distor-
tion to more severe cases with the presence of parallel
veins and furrowed edges. The symptoms in the zuc-
chini genotypes inoculated with ZYMV evolved from
simple to blister mosaic, soft leaf distortion to more
severe cases of mosaic and leaf deformation with se-
vere 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 ef-
ficiveness of the inoculation procedure because
‘Tronco Caserta’ is susceptible to viruses and is com-
monly 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 mo-
saic, reduction of leaf blade area, leaf and fruit de-
formation, 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 cucurbit 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 au-
thors: 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 mo-
saic, 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, in-
ducing 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 ac-
cessions 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 resis-
tant PX 13067051 genotype, while for ZYMV, only
14 genotypes did not differ. Moura et al. (2005) evalu-
ated 100 accessions of Cucurbita sp. from the Active
Germplasm Bank of the Federal University of Viçosa
and found immunity to ZYMV in only three geno-
types, while 26 were resistant and 48 were tolerant to
ZYMV. These results demonstrate the higher aggres-
siveness 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 - En-
zyme-Linked Immunosorbent Assay) in a survey con-
ducted in Samsun (Turkey). The symptoms included
severe or mild green mosaic, parallel veins, deforma-
tion 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 evalua-
tion of Zucchini yellow mosaic virus and the Squash mo-
saic 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 progeni-
tors 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.

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