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Screening Local Sicilian Tomato Ecotypes to Evaluate the Response of Tomato Brown Rugose Fruit Virus Infection

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Abstract: Tomato is one of the most important crops worldwide, with a production of ≈190 million tons, but it is constantly threatened by several viral diseases. Tomato brown rugose fruit virus (ToBRFV), identified in 2014 on tomato plants and subsequently reported in many countries, represents one of the major threats to tomato crops, due to production losses, different transmission modes and its rapid spread. This work aimed to evaluate 37 local Sicilian tomato ecotypes against ToBRFV infection. After a preliminary screening by molecular analyses for tomato mosaic virus (ToMV) and pepino mosaic virus (PepMV), and ToBRFV detection, tomato plants were grown in a greenhouse for their morphological characterization and for evaluating resistance and tolerance to ToBRFV. Resistance and tolerance levels were estimated by mechanical inoculation with ToB SIC01/19 ToBRFV isolate in ten plants per ecotype and evaluating virus accumulation by RT-qPCR and visual observation of symptoms. All ecotypes were infected with ToBRFV, showing several symptoms with different disease severity. No tomato ecotype showed a high level of resistance, but two ecotypes, Pop27 and Pop35, showed very moderate symptoms and therefore a high tolerance. These Sicilian tomato ecotypes could be used in genetic breeding programs as parental ones to obtain cultivars tolerant to ToBRFV.

Keywords: tomato; tobamovirus; ToBRFV; ecotype evaluation; virus tolerance

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1. Introduction

Tomato (*Solanum lycopersicum* L., family *Solanaceae*) is one of the most important, widespread and cultivated horticultural species worldwide. A total world tomato production of ≈190 million tons was recorded, according to the latest data reported by the Food and Agriculture Organization of the United Nations [1]. China is the first producer of tomato, followed by India and Turkey. Italy is the fifth largest producer country worldwide, with a predominant position among European countries in terms of cultivated area (102,060 ha) and production (6,644,790 t) [1], with the Sicily region playing a leading role at the national level for tomato greenhouse cultivation. In the last decade, there has been a considerable increase in cultivated area and total production, thanks to the progress of protected environment cultivation, which allows constant production cycles in periods during the year otherwise unfavorable to the crop. However, the emergence of new pathogens and the recrudescence of others already present in tomato production areas have severely undermined the quantity and quality of tomato production [2,3].

Several viruses were identified on tomato plants in the European and Mediterranean regions, such as tomato mosaic virus (ToMV) [4], tomato spotted wilt virus (TSWV) [5], parietaria mottle virus (PMoV) [6,7], tomato yellow leaf curl disease (TYLCD) (including tomato yellow leaf curl virus—TYLCV, tomato yellow leaf curl Sardinia virus—TYLCSV and their recombinants) [8,9], pepino mosaic virus (PepMV) [10], tomato leaf curl New Delhi virus (ToLCNDV) [11] and tomato brown rugose fruit virus (ToBRFV) [12].

Recently, tobamoviruses have played and still play a fundamental role in tomato phytosanitary management. Among them, ToBRFV (*Tobamovirus* genus, *Virgaviridae* family) is an emerging, stable and highly infectious virus, able to infect tomato plants harboring the *Tm* resistance genes [13]. This virus has a typical tobamovirus genomic organization, with a positive-sense single-stranded RNA (ssRNA+) of ≈6400 nucleotides (nt). The genome contains four open reading frames (ORFs), encoding two replication-related protein complexes of 126 kDa (ORF1a) and 183 kDa (ORF1b, expressed by the partial suppression of the stop codon), the movement protein (MP) of ≈30 kDa (ORF2) and the coat protein (CP) of ≈17.5 kDa (ORF3) [14,15]. The main hosts are tomato and pepper (*Capsicum annuum*) [15], while different grasses and weeds are also included in the host range, such as several species mainly belonging to *Nicotiana*, *Chenopodium*, *Datura* and *Physalis* genera [13,15,16].

ToBRFV induces different symptoms in tomato plants, including mild to severe leaf chlorotic mosaic [15], interveinal yellowing, deformation and narrowing. Leaflets can show apical brown necrosis [12]. Moreover, necrotic lesions on peduncles, calyxes and petioles have also been reported, along with longitudinal necrosis on stem/sepals [17]. In severe cases, the leaves may wither, with pronounced yellowing, and the whole plant can collapse and die. Yellow spots [14], deformations and irregular ripening can be observed in tomato berries [18]. In young and ripe berries, symptoms degenerate into marbling, frequently associated with the appearance of wrinkled necrotic areas [19].

Dispersal of ToBRFV is mainly mechanical (infected propagation material, direct plant-to-plant contact, infected sap from different surfaces, irrigation or drainage water, etc.), and it can be carried for long distances via contaminated seeds (classified as a seedborne virus) and berries [12]. Also, the bumblebee *Bombus terrestris* L. (*Hymenoptera*: *Apidae*) can spread the virus during pollination activity via mechanical transmission without virus ingestion and incubation [20]. The different and efficient transmission modes make the management and containment of this pathogen extremely difficult.

In this context, agricultural biodiversity plays a key role in ensuring the long-term ecosystems' resilience facing climate change [21], in producing food diversity to support human health [22], and in reducing the risk of yield losses [23]. Ecotypes and autochthonous varieties, which represent the genetic resources of a plant species, are adapted to local environmental conditions, which can also result in increased resistance and/or tolerance to biotic and abiotic stresses [24]. In recent decades, however, selective pressure towards specific phenotypes has caused a reduction in allelic diversity, with the consequent replacement of traditional and local species and cultivars with highly selected and specialized ones, leading to an overall biodiversity loss [25]. In particular, the genetic diversity of horticultural crops in Sicily has been even more threatened due to additional factors, such as the abandonment of rural areas, the aging of the agricultural population and the lack of information transmission [26]. However, the landscape's heterogeneity and geomorphological and pedogenetic characteristics are reflected in the presence of numerous ecotypes, historically adapted to the territories where they have been cultivated. In recent years, the appreciation of autochthonous tomato cultivars has gained more attention for their possible use as a source of adaptive and quality traits in breeding programs [27]. Limited information are available on ancient tomato ecotypes and autochthonous varieties to date. In the last few decades, efforts have been made in tomato breeding programs to obtain resistant cultivars to tobamoviruses. Tomato mosaic virus (ToMV), which spread rapidly throughout the world, has been primarily responsible for the forced evolution of this horticultural crop, leading breeders to select tomato cultivars with resistance genes to mitigate the production losses caused by this pathogen [28]. The impact of ToMV has

reduced the use of local cultivars, contributing to a reduction in biodiversity. On the other hand, the resistant commercial hybrids became inefficient because of the emergence of new resistance-breaking viruses. Thus, the emergence of ToBRFV has forced the search for new resistance sources to be implemented in tomato cultivars by plant breeding. However, no resistance source based on the absence of systemic infection of ToBRFV has been found so far. Thus, it would be interesting to evaluate tomato cultivars for relative resistance (reduction of virus accumulation) or tolerance (reduction of symptom severity while supporting virus infection).

It is necessary to identify and re-evaluate local ecotypes with potential sources of resistance or tolerance to ToBRFV to be used both in direct cultivation and as starting material for tomato breeding programs. The main aim of the present study was to evaluate the response to ToBRFV infection and morphological characteristics of different Sicilian tomato ecotypes.

2. Materials and Methods

2.1. Preliminary Screening of the Selected Sicilian Tomato Ecotypes by Molecular Analyses

A total of 37 ancient local ecotypes of *S. lycopersicum* L. retrieved from the Sicilian territory (Italy) and kept in collection at the Agricultural, Food and Forestry Sciences Department (SAAF) (University of Palermo, Italy) were evaluated. Specifically, the seed samples were identified by alphanumeric codes (Pop1–Pop37). Preliminary tests were conducted to confirm the absence of the main seedborne viruses present in the Sicilian territory (ToMV, PepMV and ToBRFV). In detail, total RNA was extracted starting from 10 tomato seeds of each ecotype homogenized in an extraction plastic bag (BIOREBA AG, Reinach, Switzerland) containing 1 mL of extraction buffer (0.5 M Tris-HCl, 0.14 M NaCl, 2% (*w*/*v*) PVP MW 24,000, 1% (*w*/*v*) PEG MW 6000, and 0.05% (*v*/*v*) Tween 20 in 1 L of distilled water, pH 8.2) using HOMEX 6 homogenizer (BIOREBA AG, Reinach, Switzerland). Subsequently, the "Plant/Fungi Total RNA Purification Kit" (Norgen Biotek Corp., Thorold, ON, Canada) was used, following the manufacturer's instructions. The yield and quality of purified total RNA was evaluated by a UV–Vis NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); samples were adjusted to ≈50 ng/µL and stored at −80 °C.

Two-step end-point reverse-transcription polymerase chain reaction (RT-PCR) assays were performed for ToMV, PepMV and ToBRFV. In detail, the ToMV1/ToMV2 and PepMV1/PepMV2-PepMV3 primer pairs were used according to the protocol developed by Panno and co-authors [29], while the ToBRFV-F/ToBRFV-R primer pairs [30] were used for ToBRFV detection. RNAs from ToMV-, PepMV- and ToBRFV-infected tomato plants were used as positive controls, while molecular-grade water and total RNA extracted from healthy tomato plants were used as negative controls. The RT-PCR products were electrophoresed on 2% (*w*/*v*) agarose gel at 100 V, stained with SYBR Safe (Thermo Fisher Scientific, Waltham, MA, USA), and visualized under UV light. Each sample was analyzed twice in three independent assays.

2.2. Tomato Plant Cultivation

After the verification of the absence of ToMV, PepMV and ToBRFV, a total of 12 seeds for each ecotype were sown in previously sterilized alveolate containers (one seed per alveolate) with an appropriate fertilized soil. The containers were then placed in a climate chamber under optimal germination conditions (25 ± 1 °C temperature and 80% relative humidity). At the 3rd to 4th true leaf stage, the 12 seedlings per ecotype were transplanted into larger pots and grown in an insect-proofed glasshouse at 28/20 °C day/night temperature, 14/10 h photoperiod, and 50–70% relative humidity.

2.3. Plant Inoculation with ToBRFV

The Sicilian ToBRFV isolate (ToB-SIC01/19) (GenBank acc.no. MN167466), previously characterized and stored at the "Bruno Rosciglione" plant virology laboratory of the

Agricultural, Food and Forestry Sciences Department (SAAF) (University of Palermo), was used to inoculate ten plants per each tomato ecotype, and two plants of each ecotype were left non-inoculated as negative controls. The inoculum was prepared starting from a ToBRFV-infected tomato leaf (≈200 mg) and manually homogenized in a mortar with 6 mL of phosphate buffer pH 7 (0.2 M NaH_2PO_4, 0.2 M $Na_2HPO_4 \times 7H_2O$). The plant material used for inoculum preparation was collected from a ToBRFV-infected tomato plant (cv Marmande). To quantify and standardize the inoculum and its viral concentration, an RT-qPCR analysis was carried out before mechanical transmission, following the protocol described by Panno and co-authors [18]. Based on obtained RT-qPCR results, each µL of infected sap extract contained 9.01×10^7 ToBRFV RNA copies.

For mechanical transmission, 30 µL of virus inoculum was rubbed onto three carborundum-dusted (320 mesh) leaves of each young tomato plant (10 µL/leaf) to cause micro-lesions and facilitate the passage of virions [18]. Inoculated plants were grown in an insect-proof glasshouse under the conditions reported above.

2.4. Evaluation of Symptoms and Accumulation of ToBRFV by RT-qPCR on the Selected Sicilian Ecotypes

Visual inspection of the symptoms on leaves and stems was performed at 7, 14, 21 and 28 days post-inoculation (dpi). Four symptomatic characters were evaluated: leaf mosaic, leaf bubbling, leaf deformation and stunted growth. Intensity of each symptom had four values: 0, asymptomatic; 1, mild; 2, medium or moderate; and 3, severe. A symptom severity index was estimated by adding the intensity values of the four symptoms, having a range from 0 to 12, which can be considered as an inverse of the tolerance level.

To estimate viral accumulation, ≈200 mg of young apex leaves was collected from the ToBRFV-inoculated plants (10 plants per ecotype) and the non-inoculated plants used as negative control at 28 dpi. Total RNA was extracted, as described in Section 2.1, and analyzed by RT-qPCR. To standardize the samples and minimize errors for different efficiency of RNA extraction, the concentration of total RNA was adjusted to 10 ng/µL. Each sample was analyzed in duplicate in two independent assays, and considered positive when Ct value (cycle threshold, defined as the number of cycles required for the fluorescent signal to cross the threshold) was <40. Moreover, to quantify the viral concentration in each sample, an external standard curve was generated, using 10-fold serial dilutions of in vitro-synthesized RNA transcripts from the ToB-SIC01/19 isolate; RT-qPCR curves were generated by linear regression analysis, plotting the Ct value vs. the logarithm of the starting RNA dilutions [18]. The Ct value is inversely correlated to viral titer; specifically, a low Ct value corresponds to a high viral titer, and vice versa, while a high RNA copies number corresponds to a high viral titer. Thus, based on the definition of resistance as the inverse of viral accumulation, Ct values were used as a proxy for resistance levels.

The tomato ecotypes were classified in different groups based on statistically significant differences in symptoms or viral accumulation analyzed with ANOVA and the relevance of these differences [31]. Symptom index and viral accumulation obtained from the experiment were processed with Statgraphics® Centurion XVIII (v. 18.1.16) software (StatPoint Technologies Inc., Warrenton, VA, USA). A one-way ANOVA was performed for each trait at the end of the experiment. The means were compared using Fisher's least significant difference (LSD) test ($p < 0.05$). Pearson correlation between the symptoms and between symptoms and viral accumulation was estimated.

2.5. Morphological Characterization of the Selected Sicilian Tomato Ecotypes

In order to carry out the morphological characterization of the 37 Sicilian tomato ecotypes and to search for useful characters for plant breeding, 20 seeds of each ecotype were supplied to the company "Tecnoplant Società Semplice", located in Agrigento Province (Sicily, Italy), and the ecotypes were cultivated in a greenhouse.

After sowing, each ecotype was immediately placed in the germination chamber and then transferred to the acclimatization greenhouse. After approximately four weeks

from sowing, the fully acclimatized seedlings were transplanted into the greenhouse and cultivated under standard conditions.

After full vegetative development and fruit maturity, observations of different plant and fruit characters were recorded from each ecotype. Each accession was characterized by measuring some of the traits from the phenotypic scale developed by the International Plant Genetic Resources Institute (IPGRI) [32] with some modifications (Supplementary Table S1). In particular, the 17 traits considered were plant growth type, leaf attitude, leaf type, predominant fruit shape and size, fruit size homogeneity within a plant, exterior and flesh color of mature fruit and their intensity, color intensity of the core, fruit shoulder shape, shape of pistil scar, fruit blossom end, fruit cross-sectional shape, ribbing at calyx end, easiness of fruit to detach from the pedicel. Each parameter was recorded from ten different plants or from the average of 10 fruits from different plants. All the fruit traits were recorded on the 3rd fruit of the 2nd and/or 3rd fruit branch at the full maturity stage.

3. Results

3.1. ToBRFV Symptoms and Evaluation of Tolerance Levels

All ToBRFV-inoculated plants of the Sicilian ecotypes showed infection, indicating that none of these ecotypes showed resistance to ToBRFV. Symptoms occurred gradually over the four weeks following the inoculation of the ToB-SIC01/19 isolate (Supplementary Table S2). At 28 dpi, the most severe symptoms were observed on the leaves: mosaic, bubbling and deformation, whereas only 13 out of 37 ecotypes showed stunted growth, which was mild or moderated.

Leaf mosaic was observed in all evaluated ecotypes at 28 dpi, although it was mild for ecotypes Pop24, Pop25, Pop27, Pop30 and Pop32. Leaf bubbling was only absent in ecotype Pop27, mild in Pop25 and Pop35 and moderate to severe in the rest of ecotypes. Variability of this symptom was low among plants from the same ecotype, except for Pop32, with half of the plants showing mild bubbling and the other plants showing a medium intensity. Leaf deformation was observed in all ecotypes, except Pop11, Pop15, Pop27 and Pop35, ranging from mild to severe. Pop22 showed the highest variability of this symptom, with mild deformation for four plants and moderate for the other six.

Since the correlation between symptoms was low, a symptom severity index was created by the addition of the values observed for the four symptoms analyzed: leaf mosaic, bubbling, deformation and stunted growth. These four symptoms can take four values in each plant: 0, asymptomatic; 1, mild; 2, medium or moderate; and 3, severe, whereas the symptom severity index ranks from 0 to 12. The mean value for ten plants per symptom and ecotype was calculated following this scale, so the ecotypes with lower symptom values are considered more tolerant to ToBRFV. The tomato ecotypes were classified into four groups, from more sensitive to more tolerant, based on the mean symptom severity index at 28 dpi (Figure 1).

Group A (index > 8.6): ecotypes Pop4, Pop7, Pop8, Pop13, Pop14, Pop17, Pop18, Pop19, Pop20, Pop23, Pop26, Pop28 and Pop36; group B (index 5.6–8.5): ecotypes Pop1, Pop2, Pop3, Pop5, Pop6, Pop9, Pop10, Pop12, Pop16, Pop21, Pop22, Pop24, Pop25, Pop29, Pop30, Pop31, Pop33, Pop34 and Pop37; group C (index 2.6–5.5): ecotypes Pop11, Pop15, Pop32 and Pop35; and group D (index < 2.5): ecotype Pop27.

Ecotype Pop27 was the most tolerant to ToBRFV, with a symptom severity index of one, showing a mild mosaic and absence of bubbling, deformation and stunted growth. Other ecotypes with high levels of tolerance were Pop35, with moderate leaf mosaic and mild bubbling and absence of leaf deformation and stunted growth, and Pop32, with mild mosaic and deformation, moderate bubbling and no stunted growth (Figure 2).

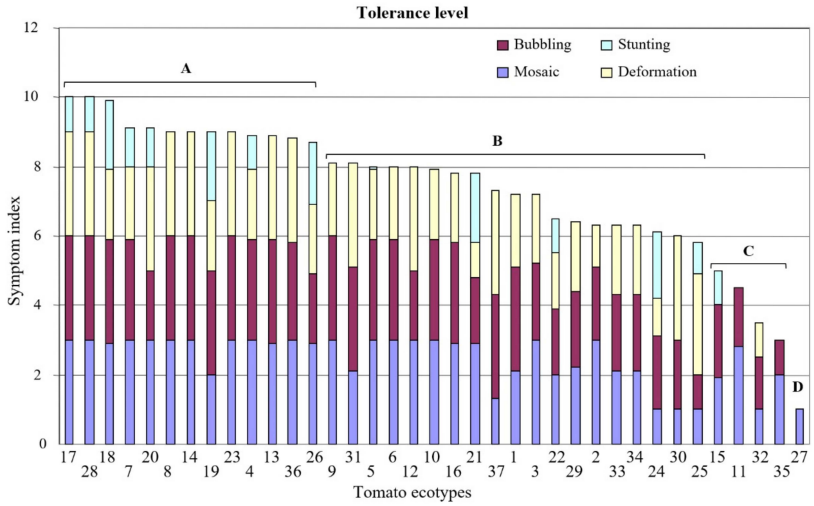

Figure 1. Tolerance levels to ToBRFV of 37 tomato ecotypes, estimated as the inverse of symptom severity index, measured as the addition of the values of four symptoms: leaf mosaic, bubbling, deformation and stunted growth. Average values for ten plants per symptom and ecotype were calculated based on this scale: 0, asymptomatic; 1, mild; 2, medium; and 3, severe for each symptom. The ecotypes were classified into four groups: A, B, C and D, based on statistical analysis and the relevance of differences.

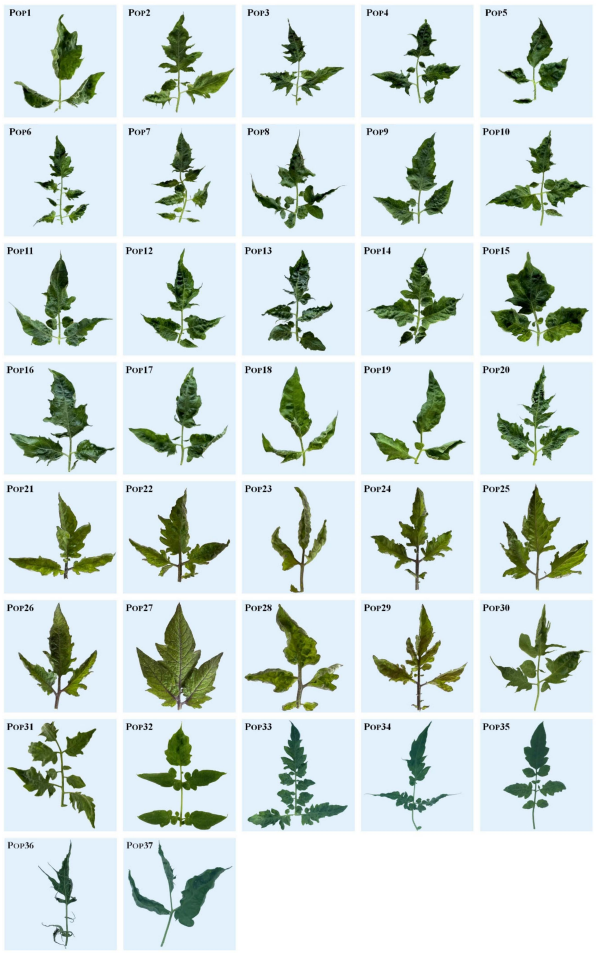

Figure 2. Different symptoms induced by ToBRFV in the 37 ecotypes evaluated.

The proportion of asymptomatic or mild-symptom ecotypes decreased with time (Figure 3).

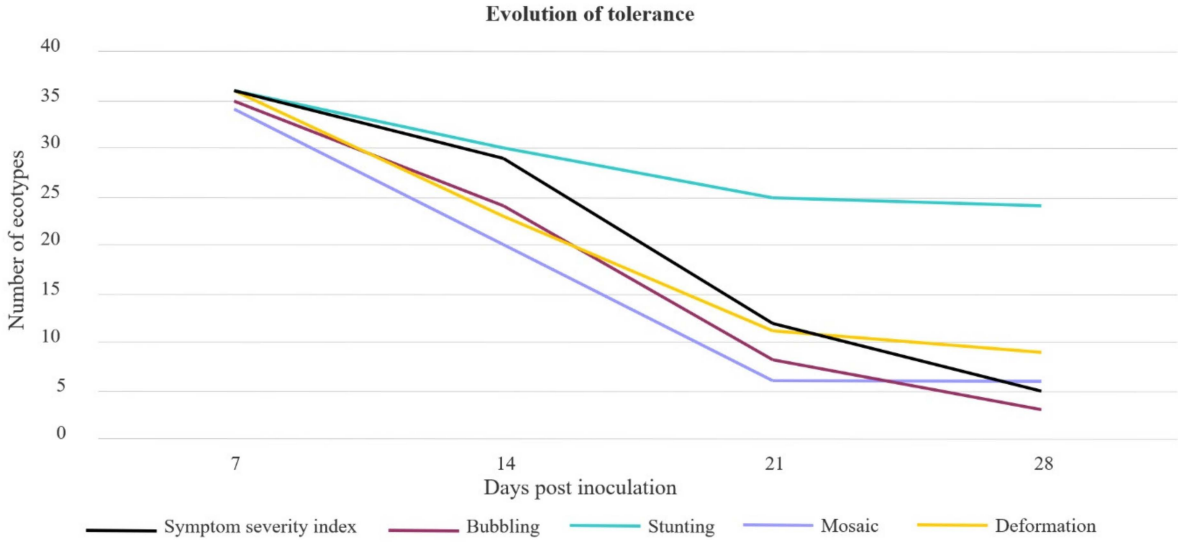

Figure 3. Number of ecotypes showing a mild symptom severity index, mild symptoms for leaf bubbling, mosaic and deformation, and no stunting growth.

However, some ecotypes differed in the evolution of symptoms (Supplementary Table S2). For example, the leaf bubbling in ecotype Pop4 was absent at 7 dpi, mild at 14 dpi, medium at 21 dpi and severe at 28 dpi, whereas ecotype Pop25 showed mild bubbling at 7, 14, 21 and 28 dpi. That means that symptoms should be evaluated at least at 28 dpi to estimate the tolerance level.

3.2. ToBRFV Accumulation and Evaluation of Resistance Levels

Molecular analyses at 28 days post-inoculation showed that all 37 Sicilian tomato ecotypes mechanically inoculated with the Sicilian isolate of ToBRFV (ToB-SIC01/19) were positive by RT-qPCR, in which the virus replicated significantly, showing a Ct values range from 15.1 (Pop31) to 24.7 (Pop14), corresponding to an RNA copies number range from 4.5×10^6 (Pop31) to 1.5×10^4 (Pop14); therefore, none of the tested ecotypes showed absolute resistance. However, statistically significant differences in the Ct values and RNA copies number between ecotypes suggest a partial resistance for some of them. The ecotypes were classified into five groups from more susceptible to more resistant, based on Ct values at 28 dpi (Figure 4).

Group A (Ct < 15.4): ecotype Pop31; group B (Ct 15.5–17.4): ecotypes Pop2, Pop33, Pop34 and Pop36; group C (Ct 17.5–19.4): ecotypes Pop26, Pop27, Pop30, Pop32, Pop35 and Pop37; group D (Ct 19.5–21.6): ecotypes Pop1, Pop4, Pop8, Pop10, Pop19, Pop21, Pop22, Pop23, Pop24, Pop25 and Pop28; Group E (Ct 21.6–23.5): ecotypes Pop3, Pop6, Pop7, Pop9, Pop11, Pop12, Pop13, Pop15, Pop16, Pop18, Pop20 and Pop29; and group F (Ct 23.6–25.5): ecotypes Pop5, Pop14 and Pop17.

Ct values showed a low correlation with symptoms, suggesting that differences in symptom intensity are related to tolerance rather than resistance. Thus, ecotypes Pop27 and Pop35 showed low and mild symptoms (the lowest symptom severity index, 1.0 and 3.0, respectively) and high virus accumulation (Ct values 19.0 and 18.2, respectively). Also, ecotypes Pop14 and Pop17, with severe symptoms (symptom severity index 10.0 and 9.0, respectively) showed the lowest viral concentration (Ct values 24.7 and 24.4, respectively) (Supplementary Table S2). Regarding the viral concentration, an inversely proportional trend of the RNA copy number to the Ct value in each ecotype was observed. In detail, starting from 30 µL of virus inoculum for each plant (9.01×10^7 ToBRFV RNA copies/µL), the Pop31 ecotype (group A) showed the highest virus accumulation

(4.5×10^6), followed by group B (3.6×10^6–1.1×10^6), group C (1.09×10^6–3.5×10^5), group D (3.3×10^5–9.6×10^4), group E (9.1×10^4–3.1×10^4) and group F (2.9×10^4–9.5×10^3). Therefore, the evaluation of viral accumulation in plants is not a good predictor of the damage caused by ToBRFV.

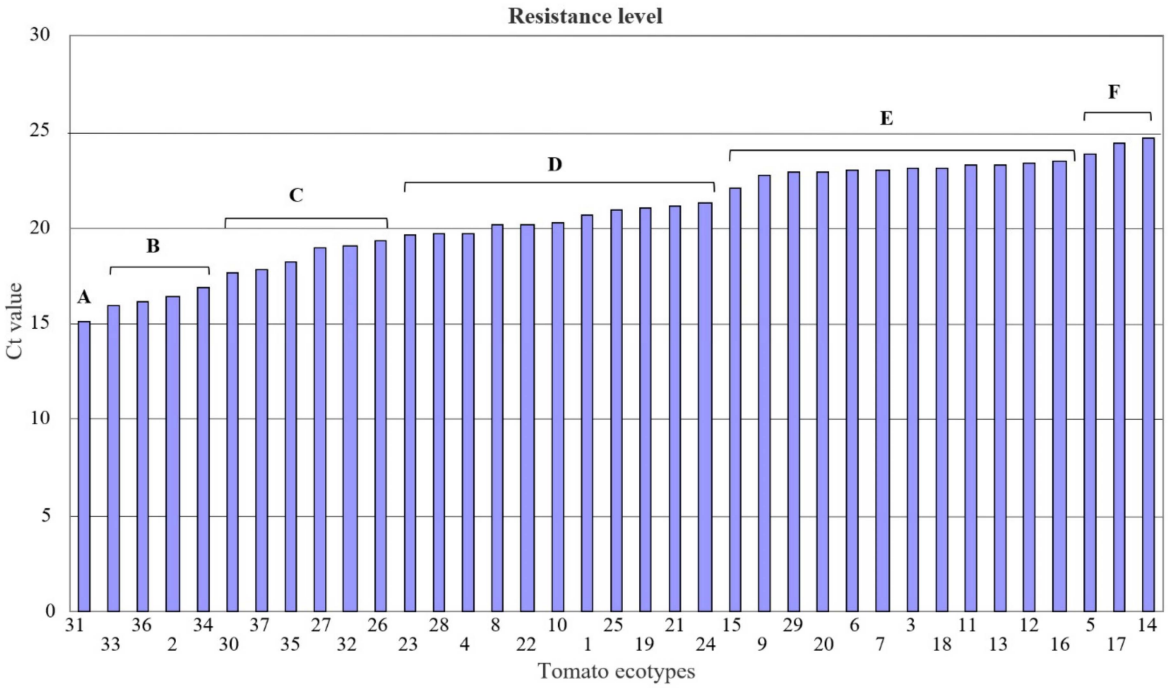

Figure 4. Resistance levels of 37 tomato ecotypes to ToBRFV estimated using Ct values obtained by RT-qPCR, which is inversely proportional to ToBRFV accumulation. The graphic represents the average Ct value calculated from ten plants per ecotype. The ecotypes were classified into six groups: A, B, C, D, E and F, based on statistical analysis and the relevance of differences.

3.3. Morphological Characterization of the Selected Sicilian Tomato Ecotypes

Most of the ecotypes showed indeterminate or semi-determinate plant growth, with a percentage of 45.9% (17 ecotypes) and 40.5% (15 ecotypes), respectively (Supplementary Table S1). Four ecotypes showed determinate plant growth (10.8%), whereas only one showed dwarf plant growth (Pop6) (Supplementary Table S3). Regarding the leaf attitude, three types were observed: horizontal leaf attitude (48.6%), drooping leaves (35.1%) and semi-erect leaves (16.3%). The leaf type did not show a wide range of variation and was mainly of the standard type (22 ecotypes—59.5%), followed by potato leaf type (11 ecotypes—29.7%) and peruvianum type (four ecotypes—10.8%).

All the predominant fruit shapes of tomato descriptors were observed; the fruits were rounded in 16 ecotypes (43.2%), flattened in 10 ecotypes (27.0%), highly rounded in five ecotypes (13.5%), slightly flattened in four ecotypes (10.8%), and only one ecotype had heart-shaped (Pop9) or ellipsoid fruit (Pop6) (Supplementary Table S3). Most of the 37 tomato ecotypes (70.3%) produced small fruits, while the other ecotypes had large (16.2%) (Pop12, Pop13, Pop14, Pop26, Pop27, Pop28) or intermediate (13.5%) (Pop1, Pop2, Pop3, Pop30, Pop33) fruit sizes. The small fruit size is usually associated with a high fruit homogeneity within the branch and the plant. Only nine ecotypes showed a low fruit-size homogeneity, whereas the other ecotypes recorded intermediate (10) or high (18) fruit-size homogeneity. At the ripening stage, the exterior color of the fruits was red in about 92% of the studied ecotypes, while only three ecotypes showed orange fruits (Pop5, Pop15, Pop35). The intensity of the exterior color was mainly dark (51.4%) or intermediate (43.2%). Even the flesh color of the pericarp was mainly red (86.5%), with various flesh color intensities: dark (40.5%), intermediate (35.1%) and light (24.3%). Pop15 was the

only ecotype with a yellow flesh color. No green or white color of the core was observed. Twenty-one ecotypes (56.8%) showed an intermediate core color, while eleven ecotypes had a core with light color, and only the ecotypes Pop19, Pop25, Pop30, Pop32 and Pop34 had a dark core. All the types of fruit shoulder were recorded in the tested ecotypes. Fifteen ecotypes (40.5%) showed a flat fruit shoulder, whereas eight (21.6%) showed a slightly depressed or moderately depressed fruit shoulder shape. Only six ecotypes (16.2%) had a strongly depressed fruit shoulder. The most frequent shape of the pistil scar was dot (64.9%), followed by irregular (27.0%) and stellate (8.1%). The flat blossom-end shape was predominant (78.4%), whereas the other ecotypes had fruits with pointed blossom ends (21.6%). Fruit cross-sectional shape of the tested ecotypes was classified into round (54.1%), irregular (27.0%) and angular (18.9%). Eleven ecotypes (29.7%) did not show ribbing at the calyx end of the fruits. The other ecotypes widely ranged for this trait: very weak (27.0%), weak (8.1%), intermediate (13.5%) and strong (21.6%). The easiness of fruit to detach from the pedicel was distributed among all the classes of this trait: 12 ecotypes (32.4%) had fruits easy to detach, 15 ecotypes (40.5%) showed an intermediate easiness, and 10 ecotypes were classified as difficult to detach from the pedicel.

Particularly, as regards the morphological characterization of Pop27 and Pop35 ecotypes, they showed semi-determinate (Pop27) and indeterminate (Pop35) plant growth, with a horizontal and drooping leaf attitude, respectively. The fruits produced by the Pop27 ecotype showed a flattened shape, large size with a low homogeneity and a dark red exterior color, while the Pop35 fruits showed a rounded shape, small size with a high homogeneity and an intermediate orange exterior color. Furthermore, the first fruit branch inserted on average above the third (Pop27) and fourth (Pop35) nodes, respectively; branches with a low number (3–5 fruits with an average weight of 46.9 g) and a higher number of fruits (7–12 fruits with an average weight of 14.7 g) were recorded in Pop27 and Pop35, respectively.

4. Discussion

Cultivation techniques have been differentiated and implemented in different environmental conditions and with changing cultivars to improve yield, production schedule, cultivation practices and fruit quality. The broad diversification that has evolved in tomato cultivation in recent decades, crop intensification and worldwide trading have helped to change the phytosanitary situation in the open field and greenhouse, especially in cases of new diseases' introduction [3].

Regarding viral diseases, tobamoviruses are the first described viruses [33] that cause the most devastating diseases in solanaceous crops, in particular tomato, and are the most persistent due to their ability to survive in dead plant tissues, outside plant cells and on different surfaces; tobacco mosaic virus (TMV) and tomato mosaic virus (ToMV) are the two best-known viruses of this genus [4,34].

Preventive agrotechnological techniques, such as tool disinfections, crop rotation, use of virus-free propagation material and infected plant removal were the main ways to contain tobamoviruses [4]. The introgression of two genes (*Tm-1* and *Tm-2*) into cultivated tomatoes conferred genetic resistance to ToMV during the last decades. Moreover, the *Tm-2* maps to the tomato chromosome 9, harboring two resistant alleles (*Tm-2* and *Tm-2*^{2}) [35]. It was demonstrated that *Tm-2*^{2} is more durable than *Tm-2,* and it consequently became extremely valuable due to its exploitation as a resistance source in tomato breeding programs to ToMV, being stable and effective in containing the disease [36]. This resistance was therefore effective in the control of tobamoviruses on tomato plants for several decades, until the spread of ToBRFV in tomato crops [12]. However, *Tm-1*, *Tm-2* and *Tm-2*^{2} genes are not effective in controlling ToBRFV infection in tomato plants [37].

The ability of ToBRFV to overcome the resistance genes found in commercial tomato cultivars highlights the challenge of developing efficient and durable tolerance/resistance traits [38].

Our results showed little differences in the resistance level between the Sicilian tomato ecotypes, which were unrelated to symptom severity and useless in reducing the damage caused by ToBRFV. However, some ecotypes remained with mild symptoms until 28 dpi, indicating a high level of tolerance to ToBRFV. Tolerance is a valid and stable strategy to reduce crop damage and yield loss caused by pathogens, referred to as limited symptom development or reduction in plant vigor or yield in a cultivar despite a normal virus accumulation that would be expected in a susceptible cultivar [39,40]. Tolerance has been evaluated for a few viruses and crops, for example, blackeye cowpea mosaic virus in cowpea [41], TYLCV in tomato [42], TSWV in pepper [43].

A wide variation of the morphological characteristics of plants and fruit was observed in the Sicilian ecotypes. Some of these characteristics along with the high level of tolerance to ToBRFV could be of interest for tomato breeding programs.

5. Conclusions

Just as ToMV prompted breeders to search for cultivars with ToMV resistance genes, ToBRFV will direct breeders to force new evolution to obtain plants resistant to it. Based on this consideration, there is a need to identify and valorize specific local ecotypes to be included in a breeding program and, therefore, develop new, more efficient, and durable resistance genes that could be able to provide adequate control against the pathogen, alone or in combination with other already identified resistances.

The present study, aimed at evaluating local tomato ecotypes, is a valuable contribution to tomato breeding programs. ToBRFV infects the 37 Sicilian tomato ecotypes; however, the tolerance of the Pop27 and Pop35 ecotypes to ToBRFV provides new perspectives to control this viral disease and could be used in tomato breeding programs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14030412/s1, Table S1: Distribution of Sicilian tomato ecotypes based on plant and fruit traits measured using the phenotypic scale developed by IPGRI (1996), Table S2: Average symptom and Ct values of Sicilian tomato ecotypes inoculated with ToBRFV, Table S3: Morphological characterization of Sicilian tomato ecotypes (*Solanum lycopersicum* L.) according to the phenotypic scale developed by IPGRI (1996).

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References

1. FAO—Food and Agriculture Organization of the United Nations. 2021. Available online: http://www.fao.org/faostat/en/#home (accessed on 10 December 2023).
2. Hanssen, I.M.; Lapidot, M.; Thomma, B.P. Emerging viral diseases of tomato crops. *Mol. Plant Microbe Interact.* **2010**, *23*, 539–548. [CrossRef]
3. Blancard, D. *Tomato Diseases: Identification, Biology and Control: A Colour Handbook*; CRC Press: Boca Raton, FL, USA, 2012.
4. Broadbent, L. Epidemiology and control of tomato mosaic virus. *Annu. Rev. Phytopathol.* **1976**, *14*, 75–96. [CrossRef]
5. Bellardi, M.G.; Vicchi, V. TSWV: Nuova insidia per la produzione agricola italiana. *Inf. Fitopatol.* **1990**, *40*, 17–24.
6. Roggero, P.; Ciuffo, M.; Katis, N.; Alioto, D.; Crescenzi, A.; Parrella, G.; Gallitelli, D. Necrotic disease in tomatoes in Greece and southern Italy caused by the tomato strain of Parietaria mottle virus. *J. Plant Pathol.* **2000**, *82*, 159.
7. Panno, S.; Caruso, A.G.; Bertacca, S.; Matić, S.; Davino, S.; Parrella, G. Detection of parietaria mottle virus by RT-qPCR: An emerging virus native of Mediterranean area that undermine tomato and pepper production in Southern Italy. *Front. Plant Sci.* **2021**, *12*, 698573. [CrossRef]
8. Moriones, E.; Navas-Castillo, J. Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Res.* **2000**, *71*, 123–134. [CrossRef] [PubMed]
9. Panno, S.; Caruso, A.G.; Davino, S. The nucleotide sequence of a recombinant tomato yellow leaf curl virus strain frequently detected in Sicily isolated from tomato plants carrying the *Ty-1* resistance gene. *Arch. Virol.* **2018**, *163*, 795–797. [CrossRef] [PubMed]
10. Davino, S.; Panno, S.; Iacono, G.; Sabatino, L.; D'Anna, F.; Iapichino, G.; Olmos, A.; Scuderi, G.; Rubio, L.; Tomassoli, L.; et al. Genetic variation and evolutionary analysis of Pepino mosaic virus in Sicily: Insights into the dispersion and epidemiology. *Plant Pathol.* **2017**, *66*, 368–375. [CrossRef]
11. Ruiz, M.L.; Simón, A.; Velasco, L.; García, M.C.; Janssen, D. First report of Tomato leaf curl New Delhi virus infecting tomato in Spain. *Plant Dis.* **2015**, *99*, 894. [CrossRef]
12. Caruso, A.G.; Bertacca, S.; Parrella, G.; Rizzo, R.; Davino, S.; Panno, S. Tomato brown rugose fruit virus: A pathogen that is changing the tomato production worldwide. *Ann. Appl. Biol.* **2022**, *181*, 258–274. [CrossRef]
13. Salem, N.M.; Jewehan, A.; Aranda, M.A.; Fox, A. Tomato Brown Rugose Fruit Virus Pandemic. *Annu. Rev. Phytopathol.* **2023**, *61*, 137–164. [CrossRef]
14. Salem, N.; Mansour, A.; Ciuffo, M.; Falk, B.W.; Turina, M. A new tobamovirus infecting tomato crops in Jordan. *Arch. Virol.* **2016**, *161*, 503–506. [CrossRef]
15. Luria, N.; Smith, E.; Reingold, V.; Bekelman, I.; Lapidot, M.; Levin, I.; Elad, N.; Tam, Y.; Sela, N.; Abu-Ras, A.; et al. A new Israeli Tobamovirus isolate infects tomato plants harboring *Tm-2*^{2} resistance genes. *PLoS ONE* **2017**, *12*, e0170429. [CrossRef]
16. Chanda, B.; Gilliard, A.; Jaiswal, N.; Ling, K.S. Comparative analysis of host range, ability to infect Tomato cultivars with *Tm-2*^{2} gene, and real-time reverse transcription PCR detection of Tomato Brown rugose fruit virus. *Plant Dis.* **2021**, *105*, 3643–3652. [CrossRef]
17. Oladokun, J.O.; Halabi, M.H.; Barua, P.; Nath, P.D. Tomato brown rugose fruit disease: Current distribution, knowledge and future prospects. *Plant Pathol.* **2019**, *68*, 1579–1586. [CrossRef]
18. Panno, S.; Ruiz-Ruiz, S.; Caruso, A.G.; Alfaro-Fernandez, A.; San Ambrosio, M.I.F.; Davino, S. Real-time reverse transcription polymerase chain reaction development for rapid detection of Tomato brown rugose fruit virus and comparison with other techniques. *PeerJ* **2019**, *7*, e7928. [CrossRef]
19. Menzel, W.; Winter, S. Identification of novel and known tobamoviruses in tomato and other solanaceous crops using a new pair of generic primers and development of a specific RT-qPCR for ToBRFV. In Proceedings of the VI International Symposium on Tomato Diseases: Managing Tomato Diseases in the Face of Globalization and Climate Change, Taipei, Taiwan, 6–9 May 2019; Volume 1316, pp. 143–148. [CrossRef]
20. Levitzky, N.; Smith, E.; Lachman, O.; Luria, N.; Mizrahi, Y.; Bakelman, H.; Sela, N.; Laskar, O.; Milrot, E.; Dombrovsky, A. The bumblebee Bombus terrestris carries a primary inoculum of Tomato brown rugose fruit virus contributing to disease spread in tomatoes. *PLoS ONE* **2019**, *14*, e0210871. [CrossRef] [PubMed]
21. Loreau, M.; De Mazancourt, C. Biodiversity and ecosystem stability: A synthesis of underlying mechanisms. *Ecol. Lett.* **2013**, *16*, 106–115. [CrossRef] [PubMed]
22. Singh, R.K.; Chang, H.W.; Yan, D.I.; Lee, K.M.; Ucmak, D.; Wong, K.; Abrouk, M.; Faeahnik, B.; Nakamura, M.; Zhu, T.H.; et al. Influence of diet on the gut microbiome and implications for human health. *J. Transl. Med.* **2017**, *15*, 1–17. [CrossRef]
23. Renard, D.; Tilman, D. National food production stabilized by crop diversity. *Nature* **2019**, *571*, 257–260. [CrossRef] [PubMed]
24. Campbell, A.K. Save those molecules! Molecular biodiversity and life. *J. Appl. Ecol.* **2003**, *40*, 193–203. [CrossRef]
25. Elia, A.; Santamaria, P. Biodiversity in vegetable crops, a heritage to save: The case of Puglia region. *Ital. J. Agron.* **2013**, *8*, e4. [CrossRef]
26. Hammer, K.; Laghetti, G. Genetic Erosion–Examples from Italy 1, 2. *Genet. Resour. Crop Evol.* **2005**, *52*, 629–634. [CrossRef]
27. Dwivedi, S.L.; Ceccarelli, S.; Blair, M.W.; Upadhyaya, H.D.; Are, A.K.; Ortiz, R. Landrace germplasm for improving yield and abiotic stress adaptation. *Trends Plant Sci.* **2016**, *21*, 31–42. [CrossRef]
28. Ishibashi, K.; Kubota, K.; Kano, A.; Ishikawa, M. Tobamoviruses: Old and new threats to tomato cultivation. *J. Gen. Plant Pathol.* **2023**, *89*, 305–321. [CrossRef]

29. Panno, S.; Davino, S.; Rubio, L.; Rangel, E.; Davino, M.; García-Hernández, J.; Olmos, A. Simultaneous detection of the seven main tomato-infecting RNA viruses by two multiplex reverse transcription polymerase chain reactions. *J. Virol. Methods* **2012**, *186*, 152–156. [CrossRef]
30. Alkowni, R.; Alabdallah, O.; Fadda, Z. Molecular identification of tomato brown rugose fruit virus in tomato in Palestine. *J. Plant Pathol.* **2019**, *101*, 719–723. [CrossRef]
31. Peiró, R.; Merchán, M.; Santacreu, M.A.; Argente, M.J.; García, M.L.; Folch, J.M.; Blasco, A. Identification of single-nucleotide polymorphism in the progesterone receptor gene and its association with reproductive traits in rabbits. *Genetics* **2008**, *180*, 1699–1705. [CrossRef] [PubMed]
32. IPGRI (International Plant Genetic Resources Institute). *Descriptors for Tomato (Lycopersicon spp.)*; IPGRI: Rome, Italy, 1996.
33. Van Damme, M.; Zois, R.; Verbeek, M.; Bai, Y.; Wolters, A.M.A. Directions from Nature: How to Halt the Tomato Brown Rugose Fruit Virus. *Agronomy* **2023**, *13*, 1300. [CrossRef]
34. Lewandowski, D.J. *Tobamovirus*; Elsevier: Amsterdam, The Netherlands, 2008.
35. Young, N.D.; Tanksley, S.D. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. *Theor. Appl. Genet.* **1989**, *77*, 353–359. [CrossRef] [PubMed]
36. Lapidot, M.; Levin, I. Genetic resistance to viruses in tomato. In *Achieving Sustainable Cultivation of Tomatoes*; Mattoo, A., Handa, A., Eds.; Burleigh Dodds Science Publishing: Cambridge, UK, 2017.
37. Zinger, A.; Lapidot, M.; Harel, A.; Doron-Faigenboim, A.; Gelbart, D.; Levin, I. Identification and mapping of tomato genome loci controlling tolerance and resistance to tomato brown rugose fruit virus. *Plants* **2021**, *10*, 179. [CrossRef]
38. Nicaise, V. Crop immunity against viruses: Outcomes and future challenges. *Front. Plant Sci.* **2014**, *5*, 660. [CrossRef] [PubMed]
39. Pagán, I.; García-Arenal, F. Tolerance of plants to pathogens: A unifying view. *Annu. Rev. Phytopathol.* **2020**, *58*, 77–96. [CrossRef] [PubMed]
40. Jeger, M.J. Tolerance of plant virus disease: Its genetic, physiological, and epidemiological significance. *Food Energy Secur.* **2022**, *14*, e440. [CrossRef]
41. Anderson, E.J.; Kline, A.S.; Morelock, T.E.; McNew, R.W. Tolerance to Blackeye cowpea mosaic potyvirus not correlated with decreased virus accumulation or protection from cowpea stunt disease. *Plant Dis.* **1996**, *80*, 847–852. [CrossRef]
42. Rubio, L.; Herrero, J.R.; Sarrió, J.; Moreno, P.; Guerri, J. A new approach to evaluate relative resistance and tolerance of tomato cultivars to begomoviruses causing the tomato yellow leaf curl virus in Spain. *Plant Pathol.* **2003**, *52*, 763–769. [CrossRef]
43. Soler, S.; Debreczeni, D.E.; Vidal, E.; Aramburu, J.; López, C.; Galipienso, L.; Rubio, L. A new *Capsicum baccatum* accession shows tolerance to wild-type and resistance-breaking isolates of tomato spotted wilt virus. *Ann. Appl. Biol.* **2015**, *167*, 343–353. [CrossRef]