

# Phenotypic characterization of TYLCD-resistance/tolerance, productivity, and fruit quality of tomato lines selected from commercial hybrids

Hassan A.A. Mohamed\*, Ahmed M.A. Mahmoud, Khaled E.A. Abdel-Ati and Ahmed A. Hassan

Department of Vegetable Crops, Faculty of Agriculture, Cairo University, P.O. Box 12613, Giza, Egypt.

\*E-mail: [hassan.a.mohamed@agr.cu.edu.eg](mailto:hassan.a.mohamed@agr.cu.edu.eg)

## Abstract

Tomato yellow leaf curl disease (TYLCD), caused by the *Tomato Yellow Leaf Curl Virus* (TYLCV), poses a significant threat to global tomato production, making resistance breeding a crucial endeavour. This study evaluated 17 tomato lines (TLs; F6 and F7) derived from F1 hybrids ‘65010’, ‘Nairouz’, ‘Tyrmes’ and ‘SVTD8320’ for 14 traits related to TYLCD resistance, yield, and fruit quality under natural whitefly-mediated inoculation during the 2022 and 2023 fall seasons. TLs demonstrated high TYLCD tolerance, characterised by low symptom severity and viral replication, and successfully detected symptomless plants using TEM scanning. TL1, TL5, TL6, and TL7 showed the lowest TYLCV-mean scores, ranging from 1.00 to 1.66 at 45 and 90 DAT. In contrast, susceptible ‘Castlerock’ had significantly higher TYLCD-mean scores (3.00 to 4.60). The highest total yield (g/plant) was for TL6 followed by TL5, TL1, TL7, and TL8, ranging from 3698.33 to 4413.33, while ‘Castlerock’ recorded the lowest value for yield ranging from 146.33 to 370.00. Climatic conditions may play a role in the differences in fruit quality traits of TLs, particularly the number of fruit locules, flesh thickness, and contents of vitamin C and lycopene, which varied between seasons, with higher temperatures and lower precipitation in the second season compared to the first.

**Key words:** *Begomovirus*, yield components, fruit quality, *Solanum lycopersicum*

## Introduction

Tomato yellow leaf curl disease (TYLCD), caused by the *Tomato Yellow Leaf Curl virus* (TYLCV), is a major threat to global tomato production. TYLCV is a member of the *Begomovirus* genus within the *Geminiviridae* family and is transmitted by the sweet potato whitefly (*Bemisia tabaci* Genn.; Homoptera, Aleyrodidae). TYLCD symptoms include yellowing and curling of leaves, stunted growth, flower absence, and fruit abortion. TYLCD can reduce tomato yield by up to 80-100%, particularly if infection occurs early in plant development. TYLCD also diminishes fruit quality, leading to smaller, deformed, and poorly colored fruits, which lower their market (Abhary *et al.*, 2007). TYLCD has spread globally due to the whitefly’s invasive spread, which has been found in over 175 countries (Ramos *et al.*, 2018). TYLCD pandemic outbreaks have occurred in about 70 countries (Mabvakure *et al.*, 2016), with climate factors like temperature and humidity worsening the spread by increasing whitefly populations and transmission rates, thereby increasing the disease risk, particularly with climate change (Koeda and Kitawaki, 2024).

The primary task of controlling TYLCD is to eliminate the whitefly vector, which is a challenging, costly, and labor-intensive task (Lapidot *et al.*, 2014). Effective management requires a combination of chemical, biological, and cultural strategies to control whiteflies and prevent virus transmission (Lapidot *et al.*, 2014). Insecticides, despite their effectiveness, are limited in use due to insect resistance development (Palumbo *et al.*, 2001), high cost, and harmful environmental and health effects (Czosnek, 2012). A key priority in reducing yield losses and virus spread

is through breeding TYLCD-resistant cultivars, which is a more sustainable, practical, and eco-friendly solution (Mahmoud and Osman, 2023).

The TYLCD resistance breeding program focuses on developing inoculation protocols, screening resistance sources, mapping resistance genes, transferring resistance genes to cultivated species, and evaluating introgressed lines. Whitefly-mediated inoculation is commonly used since whiteflies are the sole vector of TYLCV and are widely distributed (Mahmoud and Osman, 2023). Whiteflies can transmit TYLCV after 24 h of acquisition, with transmission efficiency reaching 100% when 5–15 whiteflies are present (Ghanim, 2014). Genotypes are evaluated during peak whitefly activity seasons in nurseries and fields without using insecticides (Hassan *et al.*, 2022; Mahmoud and Osman, 2023; Mahmoud *et al.*, 2023). Resistance is assessed based on symptom severity, with symptomless plants with low viral titer (confirmed by PCR) are considered resistant, and those with mild symptoms are considered tolerant (Mahmoud and Osman, 2023). Evaluation is usually performed 30 days after transplanting, allowing susceptible plants to develop symptoms in the presence of natural vector infestation (Ioannou, 1985).

TYLCD resistance was not found in tomato cultivars at first, but wild relatives like *S. chilense*, *S. habrochaites*, *S. peruvianum*, and *S. pennellii* have shown resistance (Yan *et al.*, 2018). The development of commercial TYLCD-resistant F<sub>1</sub> hybrids was a result of tomato breeding efforts that focused on transferring resistance genes from wild species into cultivated species (Dhaliwal *et al.*, 2020). TYLCD-resistant F<sub>1</sub> hybrids, with desirable traits like productivity and fruit quality, have become

important source of resistance in breeding programs (Mahmoud *et al.*, 2023). Segregating F<sub>1</sub> hybrid generations has resulted in significant progress in selecting TYLCD-resistant/tolerant lines in advanced selfed generations. Moustafa *et al.* (2005) selected TYLCD-resistant tomato lines with good fruit quality from the segregating generations of commercial hybrids ‘Fiona’ (F<sub>7</sub> line) and ‘Tyking’ (F<sub>7</sub> line). El-Morsy *et al.* (2021) selected 6 TYLCD-resistant F<sub>5</sub> tomato lines from 6 F<sub>2</sub> generations of hybrids ‘DANYA’, ‘6130’, ‘3017’, ‘783-N’, ‘TYG’, and ‘KIS-N’. Mahmoud and Osman (2023) selected 12 F<sub>9</sub> tomato lines from segregating generations of commercial hybrids ‘TH99802’ and ‘TH99806’, which exhibited TYLCD tolerance, strong growth, and high productivity. Nguyen *et al.* (2024) used a bulk selection program and selected ten F<sub>8</sub> elite tomato lines, which showed distinctive superior agronomical traits, including early maturing, highly efficient fruit set, higher yield, jointless pedicels, partial parthenocarp, and line ET3 consisting of *Ty-1/Ty-3* loci. However, Koeda *et al.* (2020) stated that homozygous resistance genes, particularly *Ty-1/Ty-3/Ty-3a*, in tomato cultivars/lines increase TYLCD resistance, but they may harm yield and quality traits because of linkage drag. Developing new TYLCD-resistant/tolerant lines is a key element in addressing the evolving nature of virus and resistance durability challenges while meeting commercial requirements for taste, appearance, and productivity.

To develop highly TYLCD-resistant and productive tomato lines, Mohamed (2021) selected several TYLCD-symptomless F<sub>3</sub> plants with desirable yield and fruit quality traits from the commercial tomato F<sub>1</sub> hybrids ‘SVTD8320’, ‘Tyrmes’, ‘Nirouz’, and ‘65010’. In continuation of this breeding effort, this study aimed to evaluate 17 improved tomato lines (TLs; F<sub>6</sub> and F<sub>7</sub> generations) for TYLCD resistance/tolerance, productivity, and

quality traits under natural whitefly-mediated inoculation during the 2022 and 2023 fall seasons.

## Materials and methods

**Tomato lines:** A bulk selection breeding program was initiated in 2017 to select TYLCD-resistant tomato lines from segregating generations of TYLCD-resistant commercial F<sub>1</sub> tomato hybrids: ‘Nairouz’, ‘65010’, ‘SV8320’, and ‘Tyrmes’ (Mohamed, 2021). Selections were performed during the fall seasons under a natural viruliferous whitefly field infestation at the Agricultural Experiment Station (AES) of the Faculty of Agriculture, Cairo University, Giza, Egypt (30°01’07’’N; 31°12’28’’E). Seventeen out of 40 symptomless F<sub>5</sub> plants, with average fruit weight >80 g, and high plant yield, fruit TSS content, and fruit firmness, were selected to be evaluated as new tomato lines (TLs).

**Planting:** Seventeen TLs along with susceptible ‘Castlerock’ were evaluated under natural viruliferous whitefly field infestation at the AES during the 2022 and 2023 fall seasons. Seeds were sown on the 1<sup>st</sup> of July of both seasons in seedling trays filled with a mixture of coconut peat and vermiculite (volume 1:1), and five-week-old seedlings were field-transplanted in a randomized complete block design (RCBD) with three replicates. Transplants were spaced 50 cm apart in two rows (1×3 m) per line for each experimental unit (EU). Plants were subjected to common agricultural practices without applying insecticides in the nursery and field.

**Whitefly-mediated natural inoculation:** Viruliferous whiteflies flourish in Egypt from April to November, with a peak in August to October (Abd-Rabou and Evans, 2020). The use of natural whitefly infestation for viral inoculation in this study was based on their spread in nurseries and fields due to the climate during

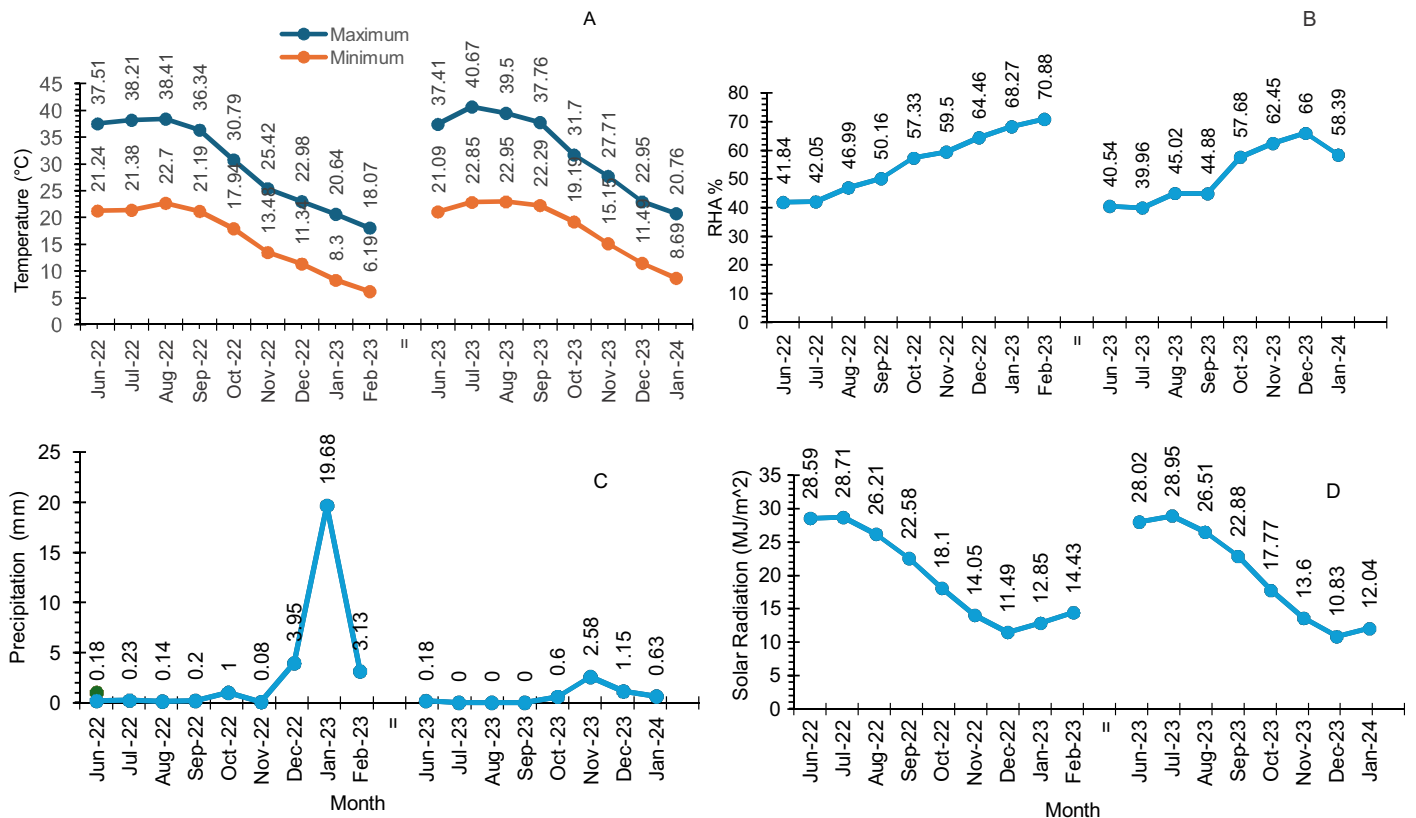


Fig. 1. Meteorological data of average monthly readings of minimum and maximum temperature (A), relative humidity (B), precipitation (C), and solar radiation (D) from June to February in the 2022 and 2023 seasons. Source: Central Laboratory for Agricultural Climate (CLAC), Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt.

both season, as shown in Fig. 1. Insecticides were not used to encourage the spread of whiteflies in these areas (Mahmoud and Osman, 2023).

**Evaluation of TYLCD resistance/tolerance:** TYLCD resistance/tolerance was evaluated based on TYLCD symptom severity in TLs, 45 and 90 days after transplanting (DAT) in both seasons. Symptoms were scored on a 1-5 scale for each plant, as described by Mahmoud *et al.* (2023), where 1: no symptoms appear on the plant, 2: slight symptoms on plant top, 3: moderate symptoms, 4: severe symptoms on the entire plant, and 5: severe symptoms and plant stunting. The severity (TYLCDS) of TYLCD symptoms for each line during each evaluation period were estimated based on the individual plant ratings using the following equations:

$$TYLCD-MS = \frac{\sum(\text{Number of plants of each grade} \times \text{disease grade})}{\text{Total number of plants}}$$

**Detection of TYLCV molecules by transmission electron microscopy leaf:** Samples from symptomless plants of TL6 and TL7 along with symptomatic plants of ‘Castlerock’, TL10, TL15, and TL16 at 90 DAT were examined using a transmission electron microscope (TEM) characterized viral molecules in cells. The fully extended plant leaves were collected from the experimental field, packed in plastic bags with moist tissue paper, and transported to a TEM unit. Plant tissue samples from petioles or veins were processed using standard procedures described by Cherif and Russo (1983).

**Plant yield and fruit quality traits:** The plant’s early (EY), total (TY), and marketable (MY) yields were estimated. The weight of all collected fruits from all harvests was used to estimate TY, while the weight of the first two harvests was used to estimate EY. To estimate MY, fruits with cracks, blossom-end rot, external watery transparent tissue, and fruits weighing less than 30 g were excluded from TY. The average fruit weight (AFW) was estimated as the mean weight of all the collected normal fruits of the plant. The number of plant fruits (NPF) was counted from all collected plant’s marketable fruits.

Fruit’s quality traits were evaluated by harvesting 20 fully red-ripe fruits from each EY during the peak harvesting period and washing them with distilled water. The fruit’s physical qualities were measured by their number of fruit locules (NFL), flesh thickness (FT), and fruit firmness (FF). Chemical quality included the content of total soluble solids (TSS), titratable acidity (TA), vitamin C (VC), lycopene (Lyc), and  $\beta$ -carotene ( $\beta$ -Car). TSS was measured using a digital refractometer (PR101, Palette Co. Ltd., Tokyo, Japan). TA was ascertained using 0.1 N NaOH solution and phenolphthalein as an indicator (AOAC, 1990). VC content was estimated according to Tareen *et al.* (2012). Five grams of fruit pulp was randomly collected, blended with 5 mL of 1.0% (w/v) hydrochloric acid, and centrifuged at 10,000x for 10 min. The absorbance of the supernatant (VC extract) was measured using a Jenway 6305 UV/visible spectrophotometer at wavelength 243 nm. The standard ascorbic acid solution was prepared using the same method to calibrate the spectrophotometer before VC estimation. The VC content was computed as mg 100 g<sup>-1</sup> of fresh weight. Lycopene and  $\beta$ -Carotene content of pericarp samples were determined according to Sánchez-González *et al.* (2016). One gram of fruit pericarp samples was used with 20 mL of acetone-hexane (4:6) solvent and homogenized in a test tube for 3 min. The optical density of the supernatant was measured

at 663, 645, 505, and 453 nm, using a Jenway 6305 UV/visible spectrophotometer. Lyc and  $\beta$ -Carot content (expressed in mg 100 mL<sup>-1</sup>FW) was calculated according to the following equations: Lyc =  $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$  and  $\beta$ -Carot =  $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$ .

**Statistical analysis:** The collected phenotypic data were checked for normality using the Shapiro—Wilk test. Data for TYLCD-MS, EY, TY, AFW, and TA were arcsine square root transformed (Wickens and Keppek, 2004). ANOVA was performed using RCBD for each season according to Wickens and Keppek (2004). Significant differences between the means were determined using Duncan’s multiple range test at a 5% probability level. The ANOVA and mean comparisons were conducted using MSTATc v.2.1 (Michigan State University, Michigan, USA).

## Results

Two F<sub>5</sub> symptomless plants of ‘Nairouz’, four of ‘65010’, ten of ‘STDV8320’, and one of ‘Tyrmes’ were selected as new tomato lines (TLs) from 47 symptomless F<sub>5</sub> plants. The selection traits were total yield/plant > 3000 g, average fruit weight >90 g, TSS content >3.6 °Brix, and fruit firmness > 4.5 kg cm<sup>-2</sup>. To determine genetic diversity and identify the elite lines, TYLCV resistance/tolerance, vegetative growth, and productivity of the selected TLs were evaluated throughout the F<sub>6</sub> and F<sub>7</sub> generations.

**TYLCV resistance/tolerance:** The phenotypic assessment of TYLCD resistance/tolerance in TLs was based on severity (TYLCD-S) of TYLCD symptoms at 45 and 90 DAT, as shown in Table 1. Highly significant differences ( $P < 0.001$ ) were identified among TLs for TYLCD-S at 45 and 90 DAT in both seasons (Table 1). TYLCD-S at 45DAT ranged between 1.00-3.57 in 2022 and 1.00-3.00 in 2023. TYLCD-S at 90DAT ranged between 1.00-4.60 in 2022 and 1.00-4.36 in 2023. TLs showed mild to moderate TYLCD symptoms at 45 (TYLCD-S ranging 1.00-2.46 in F<sub>6</sub> and 1.00-2.07 in F<sub>7</sub>) and 90 DAT (TYLCD-S ranging 1.00-2.56 in F<sub>6</sub> and 1.00-3.01 in F<sub>7</sub>). In both generations, TL1, and TL5-TL7 showed significantly lower ( $P < 0.05$ ) TYLCD-S values at 45 (ranging 1.00-1.32 in F<sub>6</sub> and 1.00-1.27 in F<sub>7</sub>) and 90 DAT (ranging 1.00-1.43 in F<sub>6</sub> and 1.00-1.66 in F<sub>7</sub>), with significant similarities ( $P < 0.05$ ) among them and TL2 and TL4 at 45 DAT in both generations, where TYLCD-S increased at 90DAT in TL2-F<sub>7</sub> (2.47) and TL4-F<sub>6</sub> (1.76) (Table 1). In both seasons, susceptible ‘Castlerock’ had significantly higher TYLCD-S at 45 (3.57 and 3.00, respectively) and 90DAT (4.60 and 4.36, respectively) (Table 1). Transmission electron microscope (TEM) was used to scan virus preparations from severe symptomatic ‘Castlerock’ plants, symptomless plants of TL6 and TL7, and mild symptomatic plants of TL10, TL15, and TL16. TEM images, as shown in Fig. 2, indicated the presence of scattered twinned icosahedral particles with diameters ranging from 16.6 to 47.9 nm.

**Plant yield components:** The evaluated lines showed highly significant ( $P < 0.001$ ) differences in plant yield components, *i.e.*, AFW, EY, TY, and MY among them during both seasons as in Table 2. NPF ranged from 5.10 with ‘Castlerock’ to 48.33 with TL4 in 2022 and 6.67 with ‘Castlerock’ to 46 with TL4 in 2023 (Table 2). In both seasons, the significantly ( $P < 0.05$ ) highest NPF was with TL4 (48.33 and 46.00, respectively) followed by TL6 (44.80 and 41.33, respectively) with significant differences ( $P < 0.05$ ) among them. Susceptible ‘Castlerock’ had the significantly

Table 1. TYLCD severity of 17 tomato lines and susceptible ‘Castlerock’ at 45 and 90 days after transplanting (DAT) under a natural TYLCDV-infection field during the 2022 and 2023 fall seasons

Line <sup>z</sup>	TYLCD severity <sup>y, x, w</sup>			
	45DAT		90DAT	
	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )
TL1	1.32de	1.00d	1.43ef	1.13f
TL2	1.08e	1.10cd	1.26f	2.47bc
TL3	1.66cd	1.30c	2.02cd	3.01b
TL4	1.33de	1.07cd	1.76de	1.19f
TL5	1.14e	1.27cd	1.17f	1.66d-f
TL6	1.00e	1.13cd	1.00f	1.58ef
TL7	1.02e	1.00d	1.02f	1.00f
TL8	2.00bc	2.00b	2.00cd	2.00c-e
TL9	2.00bc	2.00b	2.02cd	2.00c-e
TL10	2.46b	2.00b	2.46bc	2.23c-e
TL11	2.29b	2.00b	2.29b-d	2.51bc
TL12	2.02bc	2.00b	2.02cd	2.35b-d
TL13	2.12bc	2.00b	2.14b-d	2.41bc
TL14	2.02bc	2.07b	2.02cd	2.35b-d
TL15	2.05bc	2.00b	2.07b-d	2.40bc
TL16	2.00bc	2.00b	2.00cd	2.52bc
TL17	2.44b	2.00b	2.56b	2.45bc
‘Castlerock’	3.57a	3.00a	4.60a	4.36a
<i>F</i> <sub>(17, 34)</sub>	1.272***	35.580***	24.344***	12.188***

lowest NPF in both seasons (5.10 and 6.67, respectively), followed by TL17 (35 and 26.67, respectively) and TL3 (37.00 and 25.33, respectively) with significant differences ( $P < 0.05$ ) among them. AFW (g) ranged from 28.69 with ‘Castlerock’ to 122.34 with TL5 in 2022, and from 66.42 with ‘Castlerock’ to 111.11 with TL5 in 2023. In both seasons, the significantly ( $P < 0.05$ ) highest AFW was in TL5 (122.34 and 111.11, respectively), followed by TL7 (116.10 and 110.68, respectively) and TL9 (117.15 and 109.39, respectively) with significant ( $P < 0.05$ ) differences among them in 2022. Susceptible ‘Castlerock’ had the significantly lowest AFW in both seasons (28.69 and 66.42, respectively), followed by TL4 (81.74 and 82.05, respectively) and TL14 (80.96 and

90.12, respectively) with significant differences ( $P < 0.05$ ) among them (Table 2).

EY (g plant<sup>-1</sup>) ranged from 146.33 g with ‘Castlerock’ to 2233.33 g with TL5 in 2022, and from 310 g with ‘Castlerock’ to 1616.67 g with TL5 in 2023 (Table 2). The highest EY in both seasons was found with TL5 (2233.33 and 1616.67 g, respectively) and TL6 (2023.33 and 1548.33 g, respectively) with significant similarities ( $P < 0.05$ ) among them and TL2 in only 2022 (2033 g) and TL1 and TL7 in only 2023 (1536.7 and 1493.3, respectively). Susceptible ‘Castlerock’ plants had significantly the lowest EY in both seasons (146.33 and 310 g, respectively). The significantly lowest EY in both seasons among TLs was with TL4 (966.67 and 676.67 g, respectively), TL14 (900.00 and 560.00 g, respectively), TL16 (1033.33 and 705.00g, respectively), and TL17 (916.67 and 606.67g, respectively) with significant similarities ( $P < 0.05$ ) among them (Table 2).

TY (g plant<sup>-1</sup>) ranged from 146.33 with ‘Castlerock’ to 4413.33 with TL6 in 2022, and 370 g with ‘Castlerock’ to 4140 g with TL6 in 2023 (Table 2). In both seasons, the highest TY plant was showed with TL6 (4413.33 and 4140 g, respectively) followed by TL5 (4381.67 and 3663.33g, respectively) with a significant similarity ( $P < 0.05$ ) among them in 2022. Susceptible ‘Castlerock’ plants yielded the lowest TY in both seasons (146.33 and 370 g, respectively), followed by TL17 (3050 and 2383.33 g, respectively) with significant differences ( $P < 0.05$ ) among them (Table 2).

MY (g plant<sup>-1</sup>) ranged from 146.33 g with ‘Castlerock’ to 4388.33 g with TL6 in 2022, and from 310 g with ‘Castlerock’ to 3698.33 g with TL6 in 2023 (Table 2). The highest MY was with TL6 in both seasons (4388.33 and 3698.33 g, respectively), followed by lines TL5 (4197.50 and 3231.67 g, respectively), TL1 (3918.33 and 3206.67 g, respectively), TL2 (3866.67 and 3286.67 g, respectively), TL8 (3826.67 and 3140.00 g, respectively), and

Table 2. Plant yield components performance for 17 tomato lines and susceptible ‘Castlerock’ in a natural TYLCDV-infection field during the 2022 and 2023 fall seasons

Line <sup>z</sup>	Number of plant fruits <sup>y</sup>		Average fruit weight <sup>y, x</sup> (g)		Early yield <sup>y, x</sup> (g plant <sup>-1</sup> )		Total yield <sup>y, x</sup> (g plant <sup>-1</sup> )		Marketable yield <sup>y</sup> (g plant <sup>-1</sup> )	
	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )
	TL1	41.57cd	37.67c	96.25ef	95.03de	1975.00bc	1536.67ab	4038.33bc	3571.67cd	3918.33cd
TL2	41.40cd	34.33d	91.68fg	92.50ef	2033.33ab	1428.33b	4001.67bc	3190.00e	3866.67c-e	3286.67b
TL3	37.00g	25.33h	105.36c	97.41d	1116.67fg	735.00e-g	3983.33bc	2460.00i	3750.00de	2070.00j
TL4	48.33a	46.00 a	81.74h	82.05g	966.67fg	676.67f-h	4016.67bc	3723.33b	3716.67ef	3283.33b
TL5	37.00g	33.33de	122.34a	111.11a	2233.33a	1616.67a	4381.67a	3663.33bc	4197.50b	3231.67b
TL6	44.80b	41.33b	97.61 e	98.79cd	2023.33ab	1548.33ab	4413.33a	4140.00a	4388.33a	3698.33a
TL7	33.47h	31.67f	116.10b	110.68a	1865.00b-d	1493.33ab	3980.00bc	3496.67d	3720.00ef	3032.33b-d
TL8	37.80fg	34.33d	103.85cd	103.17bc	1848.33b-d	1283.33c	3985.00bc	3543.33d	3826.67 c-e	3140.00bc
TL9	35.00h	32.00ef	117.15b	109.39a	1633.33de	990.00d	4116.67b	3463.33d	3900.00c-e	3013.33b-e
TL10	40.00de	31.33ef	103.12cd	104.61b	1550.00e	1060.00d	4100.00bc	3276.67e	3983.33c	2914.00c-e
TL11	44.33b	32.33ef	88.24g	91.50ef	1766.67c-e	1000.00d	3950.00c	3066.67f	3766.67de	2774.00d-f
TL12	44.33b	33.33de	90.02g	91.30ef	1183.33f	850.00e	3983.33bc	3026.67f	3850.00c-e	2711.67e-g
TL13	38.00fg	31.33f	99.58de	99.31cd	1066.67fg	735.00e-g	3750.00d	2986.67f	3550.00f	2583.33f-h
TL14	42.33c	31.00f	80.96h	90.12f	900.00g	560.00h	3466.67e	2730.00h	3116.67g	2323.33hi
TL15	39.00ef	29.00g	89.49g	89.29f	1050.00fg	823.33ef	3516.67e	2653.33h	3183.33g	2238.33ji
TL16	39.00ef	31.67f	88.65g	92.11ef	1033.33fg	705.00e-h	3483.33e	2866.67g	3216.67g	2420.00g-i
TL17	35.00h	26.67h	86.56g	88.99f	916.67g	606.67gh	3050.00f	2383.33j	2750.00g	2036.67j
‘Castlerock’	5.10i	6.67i	28.69i	66.42h	146.33h	310.00i	146.33g	146.33i	146.33i	310.00k
<i>F</i> <sub>(17, 34)</sub>	247.347***	269.937***	158.209***	54.185***	48.962***	77.789***	411.790***	494.655***	266.681***	58.551***

<sup>z</sup>Lines were F<sub>6</sub> and F<sub>7</sub> generations from tomato commercial F<sub>1</sub> hybrids ‘Nairouz’ for TL-1andTL-2, ‘65010’ for TL-3andTL-6, ‘SVTD8320’ for TL-7-TL16, and ‘Tyrmes’ for TL-17.<sup>3</sup>Mean values followed by a letter in common are not significantly different according to Duncan’s multiple range test ( $P < 0.05$ ). <sup>x</sup>Data of AFW, EY, and TY were transformed by the arcsin equation for statistical analysis. \*\*\* Significant at 0.001 probability level.

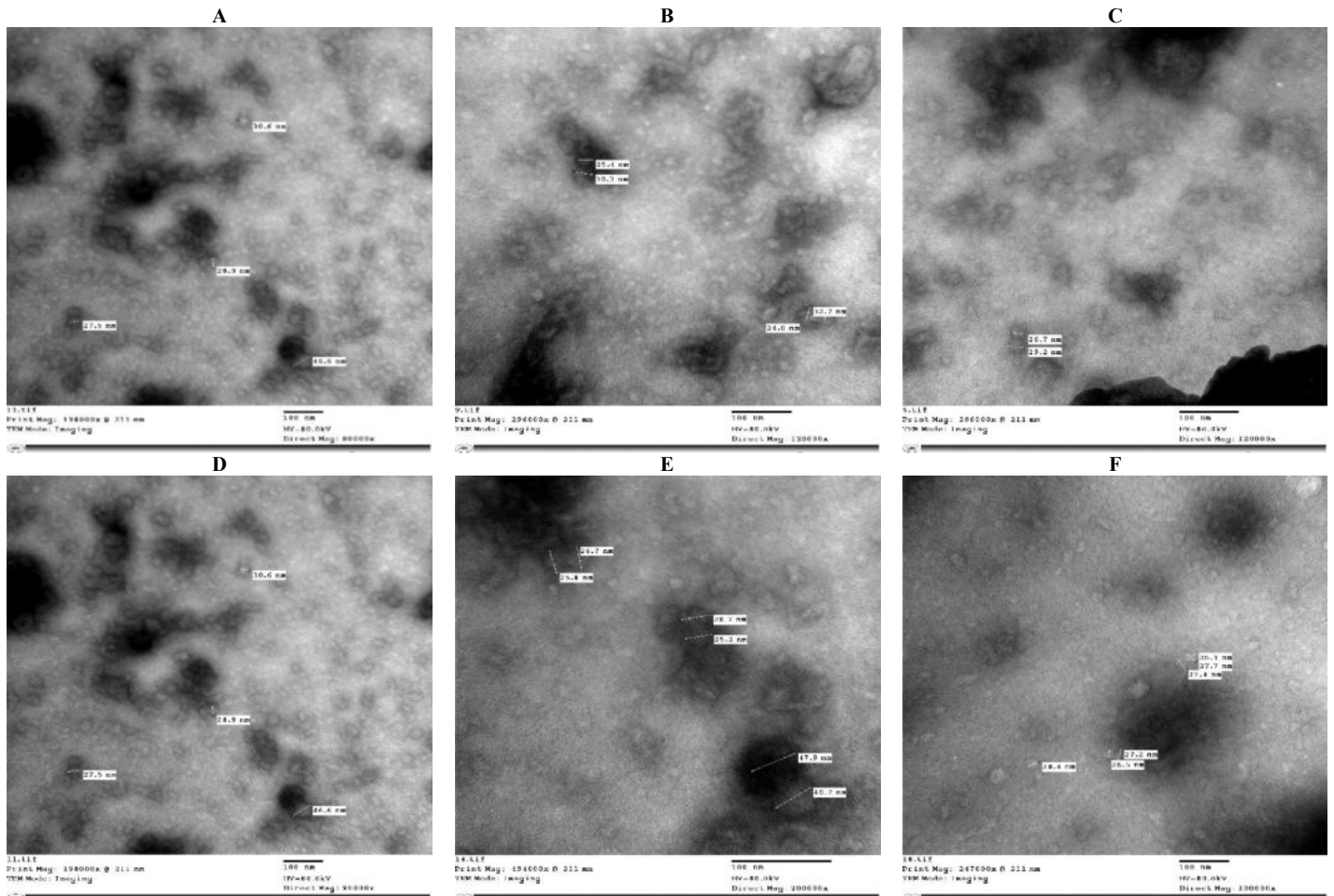


Fig. 2. Transmission electron microscopy image for viral particles in asymptomatic 'Castlerock' plants (A) and symptomless plants of TL6 (B), TL7 (C), TL10 (D), TL15 (E), and TL16 (F). Bar represents 100 nm. TL6: F<sub>7</sub> lines of '65010 F<sub>1</sub>'; TL7, TL10, TL15, and TL16: F<sub>7</sub> lines of 'SVTD8320 F<sub>1</sub>

TL9 (3900.00 and 3013.33 g, respectively), which had significant similarities ( $P < 0.05$ ) among them, especially in 2022. Susceptible 'Castlerock' plants had the significantly lowest MY in both seasons (146.33 and 310 g, respectively), followed by TL17 (2750.00 and

2036.67 g, respectively) with significant differences ( $P < 0.05$ ) among them (Table 2).

**Fruit quality traits:** The evaluated TLs showed highly significant differences ( $P < 0.001$ ) in fruit physical traits (FF, NFL, and FT) among them during both seasons as shown in Table 3. FF ( $\text{kg cm}^{-2}$ ) ranged from 3.25 with 'Castlerock' to 4.62 with TL5 in 2022, and from 3.73 with TL11 to 4.83 with TL5 in 2023. In both seasons, the highest FF was for TL5 (4.62 and 4.83, respectively) with significant similarities ( $P < 0.05$ ) with TL6 in 2022 (4.60) and TL14 in 2023 (4.70). The lowest FF was for 'Castlerock' (3.25 and 3.93, respectively), TL9 (3.74 and 3.83, respectively), and TL11 (3.72 and 3.73, respectively) with significant similarities ( $P < 0.05$ ) among them in 2023 and TL1 (3.83), TL6 (3.77), TL7 (3.93), TL9 (3.83), TL11 (3.73), TL12 (3.77), TL15 (3.77), TL16 (3.90) in only 2023.

NFL ranged from 2 with TL4 to 6 with TL5 and TL7 in 2022, and from 3 with TL4, TL15 TL16, and 'Castlerock' to 6 with TL2 and TL13 in 2023 (Table 3). NFL in TLs were inconsistent between seasons. The highest NFL was for TL5 and TL7 (6 for both) in 2022, and for TL2 and TL13 in 2023 (6 for both). The lowest NFL in both seasons was in TL4 (2 and 3, respectively) and TL15 (3 for both) with significant similarities ( $P < 0.05$ ) among them in 2023.

FT (cm) ranged from 0.30 with TL14 to 0.70 with TL10 in 2022, and from 0.50 with TL2, TL7, TL12, and TL14 to 0.73 with TL17 in 2023. FT results of TLs were inconsistent between seasons. The highest FT was for TL1, TL2, and TL10 in 2022

Table 3. Physical fruit performance for 17 tomato lines and susceptible 'Castlerock' in a natural TYLCD-infection field during the 2022 and 2023 fall seasons

Line <sup>z</sup>	Fruit firmness <sup>y</sup>   (kg cm <sup>-2</sup> )		Number of fruit locules <sup>y</sup>		Flesh thickness <sup>y</sup> (cm)	
	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )
TL1	3.88e	3.83d-f	4.00e	5.00b	0.68a	0.60c
TL2	3.99d	4.03b-d	4.00e	6.00a	0.69a	0.50e
TL3	3.74f	4.20b	3.00g	4.00c	0.40d	0.53d
TL4	3.68f	3.93c-e	2.00h	3.00d	0.50b	0.70b
TL5	4.62a	4.83a	6.00a	5.00b	0.50b	0.60c
TL6	4.60a	3.77ef	4.20d	5.00b	0.50b	0.70b
TL7	4.30b	3.93c-f	6.00a	5.00b	0.50b	0.50e
TL8	4.09c	4.00b-d	4.87c	5.00b	0.50b	0.60c
TL9	3.74f	3.83d-f	4.00e	4.00c	0.50b	0.60c
TL10	3.91de	4.10bc	4.00e	4.00c	0.70a	0.70b
TL11	3.72f	3.73f	4.00e	4.00c	0.43c	0.70b
TL12	4.37b	3.77ef	4.00e	4.00c	0.40d	0.50e
TL13	3.68f	4.10bc	5.00b	6.00a	0.50b	0.60c
TL14	3.65f	4.70a	5.00b	4.00c	0.30e	0.50e
TL15	3.91de	3.77ef	3.00g	3.00d	0.50b	0.60c
TL16	3.92de	3.90c-f	4.00e	3.00d	0.50b	0.70b
TL17	3.86e	4.13bc	4.00e	4.00c	0.50b	0.73a
'Castlerock'	3.25g	3.93c-f	3.27f	3.00d	0.33e	0.60c
F <sub>(17,34)</sub>	106.58***	21.16***	2013.34***	2.75***	167.21***	51.23***

with significant similarities ( $P < 0.05$ ) among them, while TL17 recorded the highest FT in 2023 (Table 3).

The evaluated TLs showed highly significant differences ( $P < 0.001$ ) in fruit chemical quality traits, *i.e.*, content of VC, Lyc,  $\beta$ -Car, TSS, and TA, along with TI and M, among them during both seasons as shown in Table 4. VC ( $\text{mg } 100\text{g}^{-1}$  FW) ranged from 6.61 with TL15 to 22.12 with TL5 in 2022 and 10.22 with TL10 to 20.10 with TL1 in 2023 (Table 4). VC content of TLs was inconsistent between seasons. In 2022, the highest VC was for TL5 (22.12), TL6 (20.69), and TL7 (19.99) with significant differences ( $P < 0.05$ ) among them. In 2023, TL1, TL4, and TL7 had the highest VC content with significant differences ( $P < 0.05$ ) among them. The lowest VC was for TL15 in 2022 (6.61) and for TL10 in 2023 (10.22). Fruit Lyc ( $\text{mg g}^{-1}$  FW) ranged from 0.27 with TL15 to 0.55 with TL10 in 2022, and from 0.32 with TL4 to 0.65  $\text{mg g}^{-1}$  FW with TL10 and TL14 in 2023. The highest Lyc was for TL10 in both seasons (0.55 and 0.65, respectively) with significant similarities ( $P < 0.05$ ) with TL6 (0.52), TL9 (0.64), and TL13 (0.56), TL14 (0.65), and 'Castlerock' (0.52) in only 2023. In 2022, the lowest Lyc was with 'Castlerock' (0.18), followed by TL13 (0.27), TL14 (0.28), TL15 (0.27), and TL16 (0.28). The lowest Lyc content in 2023 was identified with TL4 (0.32) with significant similarities ( $P < 0.05$ ) with TL1-TL3, TL5, TL7-TL8, TL11-TL12, TL15-TL17 (ranging 0.32-0.48).

Fruit  $\beta$ -Car ( $\text{mg g}^{-1}$  FW) ranged from 0.051 with 'Castlerock' to 0.185 with TL5 in 2022, and from 0.05 with 'Castlerock' to 0.20 with TL10 in 2023. The highest  $\beta$ -Car was showed in TL5 in 2022 (0.185), and TL9, TL10, and TL16 in 2023 (0.17, 0.20, and 0.15, respectively) with significant similarities ( $P < 0.05$ ) among them. In both seasons, the lowest  $\beta$ -Car was in 'Castlerock' (0.051 and 0.05, respectively) and TL12 (0.054 and 0.08, respectively) with significant similarities ( $P < 0.05$ ) among them.

TSS content ( $^{\circ}$ Brix) ranged from 4.65 with 'Castlerock' to 5.77 with TL4 in 2022, and from 4.40 with 'Castlerock' to 5.30 with TL11 in 2023. In both seasons, the highest TSS was

found with TL4 (5.77 and 5.22, respectively), TL8 (5.50 and 5.23, respectively), and TL11 (5.60 and 5.30, respectively) with significant similarities ( $P < 0.05$ ) among them, and TL2, TL3, TL10, TL13, TL14, TL16, and TL17 in 2023 (ranging 4.97 - 5.03). 'Castlerock' showed the lowest TSS in both seasons (4.65 and 4.40, respectively), followed by TL1 (4.93 and 4.80, respectively), TL7 (4.98 and 4.83, respectively), and TL6 (4.99 and 4.90, respectively) with significant differences ( $P < 0.05$ ) among them.

TA content ( $\text{mg citric acid } 100\text{g}^{-1}$  FW) ranged from 0.32 with TL4 to 0.69 with TL5 in 2022, and from 0.35 with TL4 and TL11 to 0.70 with TL5 in 2023. In both seasons, the highest TA showed with TL5 (0.69 and 0.70, respectively), TL7 (0.67 and 0.53, respectively), TL9 (0.63 and 0.52, respectively), TL10 (0.64 and 0.53, respectively), and TL14 (0.64 and 0.61, respectively) with significant similarities ( $P < 0.05$ ) among them in 2022. In both seasons, the lowest TA showed with TL4 (0.32 and 0.35, respectively) and TL1 (0.38 for both) with significant similarities ( $P < 0.05$ ) among them and TL2 in only 2022 (0.38), and TL3, TL11, TL12, TL15, TL17, and 'Castlerock' in only 2023 (ranging 0.35 - 0.44) (Table 4).

## Discussion

TYLCD symptoms, including yellowing and curling of leaves, typically appear 15–21 days after virus inoculation in susceptible plants (Ioannou, 1985). The plant's aging causes these symptoms to worsen, which can inhibit growth and fruit production, resulting in yield losses of 80-100%. Furthermore, the remaining fruits were frequently smaller, deformed, and less marketable (Abhary *et al.*, 2007; Ioannou, 1985). In this study, TYLCD symptoms were evaluated 45 DAT under natural whitefly-mediated inoculation, as they would be visible on susceptible 'Castlerock' plants. A reevaluation at 90 DAT evaluated the durability of resistance in TLs by checking for symptom development. Severe symptoms were observed in susceptible 'Castlerock' plants at 45 DAT, and they fully

Table 4. Chemical fruit performance and taste indices for 17 tomato lines and susceptible 'Castlerock' in a natural TYLCV-infection field during the 2022 and 2023 fall seasons

Line <sup>z</sup>	Vitamin C <sup>y</sup> ( $\text{mg } 100\text{g}^{-1}$ FW)		Lycopene <sup>y</sup> ( $\text{mg g}^{-1}$ FW)		$\beta$ -Carotene <sup>y</sup> ( $\text{mg g}^{-1}$ FW)		TSS <sup>y</sup> ( $^{\circ}$ Brix)		Titratable acidity <sup>y, x</sup> ( $\text{mg citric acid } 100\text{g}^{-1}$ FW)	
	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )	2022 (F <sub>6</sub> )	2023 (F <sub>7</sub> )
TL1	18.10de	20.10a	0.44c	0.37cd	0.156b	0.08d-g	4.93g	4.80c	0.38fg	0.38gh
TL2	17.51ef	16.05h	0.34de	0.38cd	0.124cd	0.07e-g	4.99fg	5.03a-c	0.38fg	0.47c-g
TL3	16.09g	16.60g	0.34de	0.36cd	0.130c	0.11c-g	5.27c-f	5.03a-c	0.47de	0.39f-h
TL4	17.09f	19.97b	0.38d	0.32d	0.105d-f	0.10c-g	5.77a	5.20ab	0.32g	0.35h
TL5	22.12a	13.48i	0.51b	0.43b-d	0.185a	0.09c-g	5.31b-e	4.90bc	0.69a	0.70a
TL6	20.69b	14.47i	0.33e	0.52a-c	0.128c	0.13b-e	4.99fg	4.90bc	0.58bc	0.47c-g
TL7	19.99c	19.66c	0.34de	0.40cd	0.128c	0.12b-e	4.98fg	4.83c	0.67a	0.53b-d
TL8	18.19d	11.70m	0.38de	0.43b-d	0.119c-d	0.14b-d	5.50a-c	5.23ab	0.58bc	0.49c-f
TL9	9.31m	10.97o	0.33e	0.64a	0.130c	0.17ab	4.97fg	4.77c	0.63ab	0.52b-e
TL10	14.74h	10.22q	0.55a	0.65a	0.164b	0.20a	4.97fg	4.97a-c	0.64ab	0.53b-d
TL11	7.29n	13.08k	0.34de	0.48b-d	0.128c	0.12b-f	5.60ab	5.30a	0.47de	0.35h
TL12	13.15i	11.71m	0.31f	0.46b-d	0.054h	0.08d-g	5.43b-d	4.90bc	0.44ef	0.42e-h
TL13	8.92m	17.02e	0.27g	0.56ab	0.116c-e	0.09d-g	5.10e-g	5.03a-c	0.54cd	0.49c-f
TL14	10.23l	11.43n	0.28g	0.65a	0.099e-g	0.06fg	5.07e-g	5.03a-c	0.64ab	0.61ab
TL15	6.61o	11.83l	0.27g	0.43b-d	0.083g	0.06fg	5.17 d-g	4.93bc	0.49de	0.42e-h
TL16	10.45l	16.69f	0.28g	0.37cd	0.087fg	0.15a-c	5.33b-e	4.97a-c	0.54cd	0.57bc
TL17	12.07i	10.27p	0.30f	0.37cd	0.090fg	0.11b-g	5.20c-g	5.07a-c	0.54cd	0.44d-h
'Castlerock'	11.12k	17.45d	0.18h	0.52a-c	0.051h	0.05g	4.65h	4.40d	0.46de	0.39f-h

<sup>z</sup>Lines were F<sub>6</sub> and F<sub>7</sub> generations from tomato commercial F<sub>1</sub> hybrids 'Nairouz' for TL-1 and TL-2, '65010' for TL-3 and TL-6, 'SVTD8320' for TL-7-TL16, and 'Tyrmes' for TL-17. <sup>y</sup>Mean values followed by a letter in common are not significantly different according to Duncan's multiple range test ( $P < 0.05$ ). <sup>x</sup>Data of TA was transformed by the arcsin equation for statistical analysis.

developed by 90 DAT (Table 1), indicating significant severity of the TYLCV infection. As a result, 'Castlerock' plants decreased fruit production and almost identical early and total plant yield (Table 2). Additionally, 'Castlerock' plants produced fewer fruits and smaller fruits, resulting in a lower total and marketable yield (Table 2). Most TLs were symptomless or displayed only mild symptoms under high inoculum pressure, with symptoms emerging later (Table 1). Early, total, and marketable yield of TLs were higher due to their higher fruit weights and numbers (Table 2) in comparison to those of the susceptible 'Castlerock' by 515.0 - 1426.2 % in 2022 and 80.6 - 421.5 % in 2023 for EY; 1984.3 - 2916.0 % in 2022 and 668.8 - 1235.5 % in 2023 for TY; and 1779.3 - 2898.9 % in 2022 and 557.0 - 1093.0 % in 2023 for MY. The fruit quality traits of TLs, particularly the number of fruit locules, flesh thickness, and content of vitamin C and lycopene, varied between seasons (Tables 4). These differences are likely attributed to climatic conditions, with higher temperatures and lower precipitation in the second season compared to the first (Fig. 1). According to Kascjan Maršić *et al.* (2005), tomato fruit quality is negatively impacted by high temperatures and low precipitation during growing seasons. Accumulation of vitamin C, lycopene, and  $\beta$ -carotene in tomato fruits is directly influenced by temperature, with higher temperatures leading to reduced levels (Leyva *et al.*, 2013; Tsaniklidis *et al.*, 2014).

Selection from segregating generations of tomato commercial F<sub>1</sub> hybrids has led to significant progress in developing lines with high TYLCD tolerance, yield, and good quality traits, as shown by lines TL2 and TL5 - TL8. These lines can serve as the foundation for developing tomato hybrids with improved yield and virus resistance, allowing further progress in breeding efforts.

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Received: March, 2025 ; Revised: May, 2025 ; Accepted: June, 2025