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## **Characterization of F<sup>2</sup> generation tomato plants and marker assisted selection against tomato spotted wilt virus (tswv) and tomato yellow leaf curl virus (tylcv)**

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## **Abstract**

Identifying the morphological characteristics of genetic material such as leaf, flower, yield, and fruit shape is crucial to genetic diversity assessment. Agronomic and morphological traits of 47 tomato plants in  $F_2$  generation were assessed, as well as their resistance to Tomato Spotted Wilt Virus (TSWV) and Tomato Yellow Leaf Curl Virus (TYLCV). The highest average fruit weight of the tomato lines in  $F_2$  generation was measured in the plants of the line with pink beef fruit type (G300), while the lowest was measured in the plants of the lines with round (cocktail) (S15) and ovate (V30, V31 and V32) fruit types. The highest fruit flesh firmness was measured as  $2.74 \text{ kg/cm}^2$  in  $F_2$  plants of line S230 with single red fruit type. The highest SSC (soluble solids content) was measured in  $F_2$  plants of line V31 and S230 with 6.93% and 6.73%, respectively. The longest internode was determined in  $F_2$  plants of the line with single red (S230) fruit type, while the highest stem diameter was measured in plants of the line with pink (G300) fruit type. Despite the variation in leaf color, G300 and S230 plants have potato-shaped leaves, while the other lines have tomato-shaped leaves. There were 2 homozygote resistant plants and 8 heterozygote resistant plants among the  $F_2$  plants. Among the  $F_2$  plants, 2 plants were homozygote resistant and 8 plants were heterozygote resistant to TYLCV. Heterozygote resistance to TSWV was detected only in 6 plants of line V30 and no resistance to TSWV was detected in plants of other lines. The F<sup>3</sup> lines obtained by selfing because of the study can be the material of the breeding programmes in the coming years and testing studies against biotic and abiotic factors should be carried out. The results obtained here should be reinforced with further studies such as the determination of post-harvest preservation storage and shelf-life potentials.

**Keywords:** Fruit type, Selection, Susceptible, Resistant

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## **INTRODUCTION**

Tomato (*Solanum lycopersicum*) is an annual vegetable species belonging to the *Solanaceae* family, which is widely cultivated in the world. In the world, a total of 1.173.069.683.43 tonnes of vegetables were produced in 2022, with tomatoes (186.107.972 tonnes) accounting for approximately 16% of this vegetable production. While China ranks first in world tomato production, Türkiye ranks third after India (Fao, 2023). Due to the commercial importance of tomatoes, there is a great need for the development of new varieties with higher yield and disease resistance characteristics. In order to achieve this, plant breeders need to characterise well the properties of the genetic material they use. For a breeding programme to be established using a gene pool, a good understanding of genetic diversity is essential. Moreover, analysing the interrelationship between characters helps to select important traits that contribute to yield (Kouam et al., 2018; Grozeva et al., 2021). Therefore, the information in a collection of tomato genotypes can contribute to the formulation of the new breeding plan (Mitra et al., 2023). Tanksley & McCouch, (1997) reported that without genetic diversity, breeding efforts will result in failure and plants may lack important traits such as resistance.

Morphological and agronomic traits are widely used in the study of genetic diversity in plants (Tecirli et al., 2018; Athinodorou et al., 2021; Morilipınar et al., 2021; Yaman, 2022; Coşkun, 2023; Khan et al., 2024). Morphological characterization studies and determination of traits such as leaf, flower, yield and fruit shape of genetic material are critical in determining genetic diversity (Svetlana et al., 2012; Türkmen et al., 2022). In tomato breeding, factors during intensive selection and cultivation have led to a narrowing of genetic diversity. For these reasons, tomato is more prone to high disease incidence. From sowing to post-harvest, tomatoes worldwide can be affected by more than 200 diseases caused by different pathogens (Bai et al., 2007; Williams & St. Clair, 2011). The most important of these pathogens are tomato spotted wilt virus (TSWV) and Tomato Yellow Leaf Curl Virus (TYLCV). These viruses are extremely damaging in agricultural production and can cause serious economic losses and even devastating consequences in terms of yield in tomato production in some countries and regions (Qiao et al., 2023).

Various strategies can be applied to prevent economic damage caused by these viruses. These methods include cultural, physical, biological, plant-based, chemical and resistance mechanisms. However, the use of each method may depend on their suitability for the planting system and the scale of farmers' activities. Also, some methods may not be compatible when applied together to manage virus populations in the field. Therefore, to control pathogens, it is most practical to use resistant cultivars because of the reduced cost of production and because they are environmentally friendly and compatible with other control methods. Molecular techniques are very sensitive and versatile for the diagnosis of viruses and the selection of resistant plants (Noris & Miozzi, 2015). The aim of this study was to characterise tomato plants in  $F_2$  generation in terms of some agronomic and morphological characteristics and to determine their resistance status to TSWV and TYLCV.

#### **MATERIALS AND METHODS**

## **Plant Material**

In the experiment,  $F_2$  plants obtained by selfing of different types of tomato varieties widely used in greenhouse cultivation in Turkey in 2022-2023 were used as plant material. The nematode resistance status of 47  $F<sub>2</sub>$  generation tomato plants used in the study was determined by using MI-23 SCAR marker in the study conducted by Başak et. al., (2024) (Table 1).

## **Method**

Morphological and pomological characterization study was carried out in 2024 in the geothermally heated, venlo type, glass and fully automated R&D greenhouse of Kırşehir Ahi Evran University. Seed sowing was carried out in 128-well trays consisting of peat:perlite mixture at a ratio of 3:1. Plants were grown in the greenhouse by irrigation and fertilisation until the first true leaf stage. When the seedlings reached planting size (seedlings at the true 2-4 leaf stage), they were planted in cocopeat medium with a distance of 25 cm between rows and 100 cm between rows. In the experiment, the number of plants specified in Table 1 was planted from each  $F_2$  line. Irrigation, fertigation and acclimatization processes (the amount of water and fertilizer was adjusted depending on the plant growth stage and greenhouse temperature) were carried out with an automation system. Since the plants were in F<sub>2</sub> generation, the experiment was not set up with replicates. The averages of the measurements and observations were determined according to the number of  $F_2$  plants within the lines.

### **Examined Parameters**

In the experiment, morphological and pomological characterization was carried out according to UPOV criteria in terms of plant and fruit characteristics in the parameters specified in Table 2. Fruit measurements were completed when the first fruits ripened on the plants. Fruits were harvested 60 days after flowering. Observations, measurements and analyses were carried out on 3 fruits selected from each plant. The data obtained were averaged. During the observation and measurement period, the traits to be analysed were measured with a ruler for length and callipers for diameter. Fruit juice EC and pH values were measured with Extech device. Fruit flesh firmness was measured with PCEPTR 200 penetrometer. SSC (soluble solids content) was measured with Hanna HI96801 digital refractometer.

<b>DNA Code</b>	$F_2$ Code	Nematode Resistance $(Mi 1.2$ gene)	<b>DNA Code</b>	$F_2$ Code	Nematode Resistance $(Mi 1.2$ gene)
$\mathbf{1}$	$V30-1$	aa	25	$S15-1$	Aa
$\overline{c}$	$V30-2$	aa	26	$S15-2$	Aa
3	$V30-3$	aa	27	$S15-3$	Aa
$\overline{4}$	$V30-4$	aa	28	S15-4	Aa
5	$V30-5$	aa	29	$S15-5$	AA
6	$V30-6$	aa	30	$S15-6$	AA
7	V30-7	aa	31	S15-7	AA
$8\,$	$V30-8$	aa	32	$V31-1$	aa
9	G300-1	aa	33	$V31-2$	aa
10	G300-2	aa	34	$V31-3$	aa
11	G300-3	aa	35	$V31-4$	
12	G300-4	aa	36	$V31-5$	
13	G300-5	aa	37	$V31-6$	
14	G300-6	aa	38	$V31-7$	aa
15	G300-7	aa	39	$V31-8$	aa
16	G300-8	aa	40	$V-32-1$	aa
17	S230-1	Aa	41	$V-32-2$	aa
18	S230-2	AA	42	$V-32-3$	aa
19	S230-3	AA	43	$V-32-4$	aa
20	S230-4	Aa	44	$V-32-5$	aa
21	S230-5	$\qquad \qquad \blacksquare$	45	$V-32-6$	aa
22	S230-6	$\overline{a}$	46	$V-32-7$	$\overline{\phantom{a}}$
23	S230-7	AA	47	$V-32-8$	aa
24	S230-8	aa			

Table 1. Resistance of 47 tomato plants in F<sup>2</sup> generation against nematode races (*M*. *incognita*, *M*. *javanica*, *M*. *arenaria*)

(-) Band was unable to be obtained, aa: Susceptible, Aa: Heterozygote Resistant, AA: Homozygote Resistant





#### **DNA Isolation and PCR Analysis**

DNA isolation was performed by modification of the CTAB method developed according to Doyle and Doyle (1990). Fresh leaves were shredded in porcelain mortars using liquid nitrogen, then 100-200 mg aliquots were taken into a 1.5 ml eppendorf centrifuge tube and 250 µl of extraction solution [100 mM trisHC1, pH 8.0; 1.4 M NaC1; 20 mM EDTA; 2% hexadecyl-trimethyl-ammonium bromide (CTAB, Sigma Chemical Co, MO, USA); 0.4% β-mercaptoethanol] were added, mixed thoroughly and incubated in a water bath at 65 ºC for 30 min. 100 µl chloroform/isoamyl alcohol (24/1) was added, mixed well, centrifuged at full power for 3 min and the upper liquid phase was transferred to a new eppendorf tube. In order to precipitate DNA, 500 µl ethanol-acetate solution (96 mL EtOH, 4 mL 3 M NaAc, pH 5.2) was added and mixed gently and centrifuged at full power for 3 min. After the application, the liquid part was poured and left to dry at room temperature for 1 hour. At the end of the observations, 200 µl sterile distilled water was added and the DNA pellet was dissolved. Co-dominant SCAR (SW5-2F 5'- AATTAGGTTCTTGAAGCCCATCT -3' and SW5-2R 5'- TTCCGCATCAGCCAATAGTGT 3') markers developed in previous studies were used for selection of Sw-5 gene which provides resistance against TSWV in tomato (Dianese et al., 2010). P6-25 co-dominate SCAR (P6-25F 5'- GGTAGTGGAAATGATGCTGCTC -3' and P6-25R 5'- GCTCTGCCT ATTGTCCCATATATAACC 3') marker specific for Ty-3 gene was used against TYLCV (Jensen et al., 2007). PCR reactions were performed in a volume of 15 μl. Reactions were performed using 2.0 μl DNA (20 ng), 1.5 μl 10X PCR buffer, 1.0 μl dNTP (200 μM of each dNTP), 1.5 μl MgCl2 (25 mM), 0.2 μl Taq DNA polymerase (0.5 U/μl), 0.5 μl forward and reverse primers (0.3 μM of each primer) and 7.8 μl ddH2O. The PCR products were run on a 2% agarose gel at 115 V in 1x TBE buffer for 3 hours, photographed under UV light and scored to determine disease resistance.

#### **Statistical Analysis**

The data obtained in the studies were analysed by one-way analysis of variance (ANOVA) using SPSS 18.0 statistical software (IBM, Chicago, IL, USA) at 5% significance level and the difference between the means was determined by Duncan's multiple comparison test.

## **RESULTS**

When the plants in the  $F_2$  generation obtained by selfing 6 hybrid tomato varieties of different types, which are widely used in greenhouse cultivation, were classified according to fruit type, it was determined that line G300 had single-pink beef, line S15 had cluster-round (cocktail), line S230 had single-red, lines V30, V31 and V32 had single-ovate fruit type. Among 6 tomato lines in  $F_2$  generation, the highest average fruit weight was determined in line G300 with pink beef fruit type (219.81 g) and in line S230 with single red fruit type (159.47 g). Lines V30  $(32.19 \text{ g})$ , V32  $(20.35 \text{ g})$ , V31  $(18.10 \text{ g})$  with single ovate fruit type and line S15  $(16.64 \text{ g})$  with round (cocktail) fruit type had the lowest average fruit weight. Fruit length and diameter parameters were generally parallel with the average fruit weight. When the lines were evaluated in terms of fruit flesh firmness, the highest fruit flesh firmness was measured in line S230 (2.74 kg/cm<sup>2</sup>), which has single red fruit type, while there was no statistical difference between the other lines. The highest SSC was measured in line V31 (6.93%) and S230 (6.73%), while the lowest was measured in line V30 (5.53%). While there was no statistical difference in juice pH content of the lines, the highest fruit juice Ec was measured in line G300 (5.39 dS/m) and the lowest in line V31 (3.31 dS/m) (Table 3).



Table 3. Mean fruit weight, fruit length, fruit diameter, fruit flesh firmness, fruit juice SSC (soluble solids content),  $Ec$  and  $nH$  of tomato lines in  $F<sub>2</sub>$  generation with different fruit types

Different letters in the same column indicate that the difference between groups is significant  $p < 0.05$ . ns, nonsignificant. \*  $p < 0.05$ , \*\*  $p < 0.01$  and \*\*\*  $p < 0.001$ .

The fruit weights of  $8 F<sub>2</sub>$  plants of line G300 obtained by selfing of pink beef hybrid tomato cultivar varied between 216.50 g and 223 g, the fruit weights of plants of line S230 with single red fruit type varied between 103.50 g and 185.25 g, while the fruit weight of line S15 with cluster-round (cocktail) fruit type varied between 11.50 g and 20.33 g. The average internode length of line G300 was 7.05 cm, line S230 was 9.75 cm and line S15 was 7.00 cm. Among the lines, the thickest stem was measured in  $F_2$  plants of line G300 (11.58 mm), while the thinnest stem average was measured in  $F_2$  plants of line S15 (10.09 mm). Twenty-three  $F_2$  plants of lines G300, S230 and S15 had medium plant strength and potato leaf type. The  $F_2$  plants of lines G300 and S230 had semierect leaf attitude, while the  $F_2$  plants of line S15 had horizontal leaf attitude.  $F_2$  plants of line S15 had light green leaf color, while  $F_2$  plants of lines G300 and S230 had medium green leaf color (Table 4).

Table 4. Average fruit weight, internode length, stem diameter, plant strength, leaf attitude, leaf type and leaf intensity of green color of single pink beef (G300), single red (S230), and round cluster (cocktail) (S15) type  $F_2$ plants



Among the F<sub>2</sub> plants of lines V30, V31 and V32 obtained by selfing 3 hybrid tomato varieties with ovate fruit type, the highest fruit weight was measured in V30-1 plant with 48.28 g and the lowest was measured in  $F_2$  plant of V31-4 with 15.00 g. Regarding the internode length of the lines, the highest average internode length was obtained in the plants of line V32 (9.66 cm), while the lowest was measured in the plants of line V30 (7.58 cm). The average stem diameter of  $F_2$  plants of line V30 was 10.40 mm, of line V31 was 10.29 mm and of line V32 was 8.77 mm. The plant strength of the 3 lines with ovate type fruit type was found to be medium, leaf attitude was semi-erect, leaf type, tomato leaf type and leaf color were found to be dark green (Table 5).

Table 5. Average fruit weight, internode length, stem diameter, plant strength, leaf attitude, leaf type and leaf intensity of green color of  $F_2$  plants with ovate fruit type (V30, V31 and V32)  $r = c$ 



The resistance of plants to tomato yellow leaf curl virus (TYLCV) and tomato spotted wilt virus (TSWV) was evaluated. 2 plants were homozygote resistant and 8 plants were heterozygote resistant to TYLCV among tomato plants of different types. Only 6 plants of line V30 showed heterozygote resistance to TSWV, while no resistance was detected in plants of other lines (Table 6).

$\rm{F}_2$ Code	<b>TYLCV</b>	<b>TSWV</b>
$V30-1$	aa	Aa
$V30-2$	$\qquad \qquad -$	$\rm Aa$
$V30-3$	aa	$_{\rm aa}$
$V30-4$	Aa	Aa
$V30-5$	$_{\rm aa}$	Aa
$V30-6$	aa	Aa
$V30-7$	aa	$\overline{\phantom{a}}$
$V30-8$	$_{\rm aa}$	Aa
G300- $1$	Aa	$_{\rm aa}$
G300-2	${\bf A}{\bf A}$	aa
G300-3	$_{\rm aa}$	$\qquad \qquad -$
G300-4	$\rm Aa$	aa
G300-5	$\rm Aa$	aa
G300-6	Aa	$_{\rm aa}$
G300-7	$_{\rm aa}$	$_{\rm aa}$
G300-8	AA	aa
S230-1	$\rm Aa$	$_{\rm aa}$
S230-2	$_{\rm aa}$	aa
S230-3	aa	aa
S230-4	$\overline{\phantom{a}}$	aa
S230-5		$_{\rm aa}$
S230-6	$\overline{\phantom{a}}$	aa
S230-7	aa	aa
S230-8	$_{\rm aa}$	aa
$S15-1$	Aa	aa
$S15-2$	Aa	aa
$S15-3$	$_{\rm aa}$	$_{\rm aa}$
S15-4	aa	aa
$S15-5$	aa	$_{\rm aa}$
$S15-6$	aa	$\overline{\phantom{a}}$
S15-7	aa	aa
$V31-1$	aa	aa
$V31-2$	aa	$_{\rm aa}$
$V31-3$	aa	aa
$V31-4$	$_{\rm aa}$	aa
$V31-5$	$_{\rm aa}$	
$V31-6$	aa	$\overline{\phantom{a}}$
$V31-7$	aa	$_{\rm aa}$
$V31-8$	aa	$_{\rm aa}$
$V-32-1$	$_{\rm aa}$	$_{\rm aa}$
$V-32-2$	Aa	aa
$V-32-3$	aa	$\overline{\phantom{a}}$
$V-32-4$	aa	$_{\rm aa}$
$V-32-5$	aa	$_{\rm aa}$
$V-32-6$	aa	$_{\rm aa}$
$V-32-7$	$\overline{\phantom{a}}$	$_{\rm aa}$
$V-32-8$	Aa	$_{\rm aa}$

Table 6. Resistance to TYLCV (Tomato Yellow Leaf Curl Virus) and TSWV (Tomato Spotted Wilt Virus) of tomato plants in  $F_2$  generation with different fruit types

-: Band was unable to be obtained, aa: Susceptible, Aa: Heterozygote Resistant, AA: Homozygote Resistant

#### **DISCUSSION**

As a result of the characterization study carried out on the plants of the lines with different fruit types, the plants of the line with single pink beef fruit type had the highest fruit weight, while the lowest fruit weight was determined in the plants of the lines with ovate and cocktail fruit type. Fruit length and fruit diameter parameters were in parallel with fruit weight. Pradeepkumar et al. (2001) found that the average fruit weight of tomato fruits varied between 1.40-115.0 g. Turhan et al. (2022) reported that the fruit width of tomato genotypes varied between 33.0-93.0 mm and the average fruit weight varied between 18.18-332.45 g. Bernousi et al. (2011) determined the fruit heights of tomato genotypes in a study in which they determined tomato fruit height and found that fruit height of genotypes varied between 26.8-74.1 mm. The results obtained from the study are in parallel with the results of fruit heights, fruit diameter and average fruit weight in the studies conducted by previous researchers.

In the study, the highest fruit flesh firmness and SSC were determined in the plants of the line with single red fruit type, while the highest fruit juice Ec value was determined in the plants of the line with pink beef fruit type. Güngör et al. (2023) reported that the fruit flesh firmness of 14 different tomato genotypes of different types varied between 0.56 and 2.61 kg/cm<sup>2</sup>. Periago et al. (2002) reported that the average amount of SSC varied between 4.0-7.50%, Ziaf et al. (2016) reported the amount of SSC in tomato genotypes between 8.38-13.85%, Lázaro, (2018) reported an average of 6.73% in indigenous tomato genotypes in Madrid Region of Spain, Salim et al. (2020) between 2.97-5.51%, Bakir et al. (2020) between 5.5-9.42% and Athinodorou et al. (2021) between 3.20-5.07%. The reason for the difference in SSC in all studies is that the variety and climatic conditions have an effect on SSC. The longest internode was determined in the plants of the line with single red fruit type, while the highest stem diameter was measured in the plants of the line with pink fruit type. Leaf color varied between light green and dark green, and in terms of leaf type, plants belonging to line G300 and line S230 had potato leaf type, while plants belonging to other lines had tomato leaf type. Demir and Ünlü, (2023) reported that leaf color varied between light green and dark green in tomato genotypes.

The leaf attitude of the plants varies between horizontal and semi-pendent. Yana and Rahima, (2023) reported that stem diameter varied between 1.09 cm and 1.17 cm at the end of 60 days in three different tomato varieties Demir and Ünlü, (2023) reported that the leaf attitude of 24 tomato genotypes had horizontal, semi drooping or semi erect posture and the stem diameter varied between 13.06 mm and 20.99 mm.

An effective measure to manage TYLCV and TSWV does not exist, except for the cultivation of resistant crops. A small number of whiteflies can spread begomoviruses throughout a large area. (Horowitz et al., 2005; Schuster et al., 2010; Elbaz et al., 2016). For the management of TYLCV and TSWV, host plant resistance is the most effective, environmentally friendly and durable approach. Host plant resistance can be achieved by breeding or selection. Over the last few decades, breeding disease-resistant tomato cultivars has become increasingly important for introducing resistance genes. These resistant cultivars can reduce the need for chemical treatments and reduce environmental impact. Furthermore, they can help to reduce the amount of money spent on agricultural inputs. In regions and seasons prone to TYLCV and TSWV, resistant varieties/hybrids have significantly stabilized tomato production (Dhaliwal et al., 2020). The use of molecular markers in plant breeding has a wide range of applications. These markers can be used to select desirable traits, determine genetic diversity, and track genetic traits in plants. They can also be used to track the impact of agricultural activities such as pesticide use and climate change on plant populations. Several resistance genes discovered in wild tomato species have been transferred to cultivated tomatoes. This has resulted in varieties that are more resistant to diseases and pests.

Furthermore, the gene pyramid, which combines multiple resistance genes from various species through molecular markers, has been an important component of the modern tomato breeding program. Tomato is rich in the number of molecular markers available (Foolad, 2007). Marker-assisted selection (MAS) has been effectively used in tomato breeding program to transfer many disease resistance genes. Some of the resistance genes have been shown to be linked to other genes with epistatic traits (Consuegra et al., 2015; Gómez et al., 2004; Mejía et al., 2005; Rani et al., 2008). In our study, the co-dominant SCAR SW5-2 marker developed in previous studies was used for the selection of the Sw-5 gene that provides resistance to TSWV in tomato. P6-25 co-dominant SCAR P6-25 marker, which is specific to Ty-3 gene, was used against TYLCV.

In a previous study using 14 tomato cultivars with the SW5-2 marker, 4 cultivars were resistant with a 574 bp band, 2 cultivars were susceptible with a 510 bp DNA band and 8 cultivars were susceptible with a 464 bp band. It was reported that the SW5-2 primer set efficiently determines tospovirus resistance under greenhouse and field conditions and is a good marker for marker-assisted selection (Dianese et al., 2010). P6-25 marker was used for TYLCV resistance in different tomato varieties and it was reported that P6-25 marker was accurate and reliable in selection studies (Caro et al., 2015; Kim et al., 2020; Aktaş & Aydın, 2022). The P6-25 molecular DNA marker used was found to be helpful in determining the resistance responses of pink-fleshed tomato to TYLCV and the findings were rapid, accurate and reproducible. Due to the availability of this information and the fact that some tomatoes showed disease resistance, it was determined that the primers could be used in future breeding trials (Tekin et al., 2024).

## **CONCLUSION**

TYLCV and TSWV are a serious constraint in tomato cultivation worldwide. The identification of sources of resistance and their transfer to commercial varieties has stabilized tomato production in disease-prone regions and seasons. The late emergence of the disease does not threaten tomato production. Genetic diversity is necessary for the development of a new variety. The present study, the results of morphological and pomological traits obtained from F<sup>2</sup> generation plants and plants showing resistance to TSWV and TYLCV can be the material for future breeding programs and will help in the management, classification and conservation of germplasm.

## **Compliance with Ethical Standards**

**Peer-review**

Externally peer-reviewed.

**Conflict of interest**

The authors state there is no competing interest.

#### **Author contribution**

Authors' individual contributions to the article are equal.

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