Bulgarian Journal of Agricultural Science, 32 (No 1) 2026, 137–141

Characterisation of F_3 tomato breeding generations for resistance to *Tomato spotted wilt virus* and *Tomato mosaic virus*

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Abstract

Pasev, G., Radeva-Ivanova, V., Pashkoulova, V., Grozeva, S. & Ganeva, D. (2026). Characterisation of F3 tomato breeding generations for resistance to *Tomato spotted wilt virus* and *Tomato mosaic virus*. *Bulg. J. Agric. Sci., 32*(1), 137–141

Tomato spotted wilt virus (TSWV) and *Tomato mosaic virus* (ToMV) are some of the most dangerous viruses affecting tomatoes, because they cause serious damage to tomato production, especially on susceptible local cultivars in Bulgaria. The current study characterises the resistance to TSWV and ToMV of ten F_3 tomato crosses by detection of molecular markers for resistance genes *Sw5* and $Tm2^2$, respectively. Pautaliya and 975 were used as donors of *Tm2*^{2} in crosses with five susceptible cultivars and 1627 as a donor of *Sw5* in crosses with five other susceptible cultivars. Genotyping of 107 plants in total resulted in identifying 20 homozygous for *Sw5*, 15 homozygous for *Tm2*^{2} and one homozygous for both genes. Cluster analysis of characteristics such as growth habit, inflorescence type, presence of joint in the pedicel, colour and shape of the fruits, and resistance to TSWV and/or ToMV segregated the tested plants into a variety of combinations of valuable traits for future tomato breeding programs.

Keywords: breeding; resistance; tomato; *Tomato mosaic virus*; *Tomato spotted wilt virus*

Introduction

Tomato spotted wilt virus (TSWV, *Orthotospovirus*, *Bunyaviridae*) and *Tomato mosaic virus* (ToMV, *Tobamovirus*, *Virgaviridae*) are two of the most dangerous viruses affecting vegetable crops in the world, especially tomatoes (Pappu et al., 2009, Davide, 2023). *Tomato spotted wilt virus* is transmitted by several thrips species (*Thysanoptera, Thripidae*), while ToMV is transmitted mechanically. Both viruses are of considerable economic importance because they lead to poor quality production and low yields. Currently, the most successful way to prevent TSWV and ToMV infection is to use resistant tomato varieties.

Resistance to TSWV is controlled by several dominant and recessive genes – *Sw1a*, *Sw1b*, *sw2*, *sw3*, *sw4*, *Sw5*, *Sw6* and *Sw7*. Only the *Sw5b* gene from the *Sw5* cluster leads to broad spectrum resistance to orthotospoviruses, while resistance conferred by *Sw1a*, *Sw1b*, *sw2*, *sw3* and *sw4* is rapidly overcome, and that conferred by *Sw6* has narrow specificity to certain virus isolates (Qi et al., 2021). Effective protection against the different ToMV pathotypes is provided by the dominant *Tm2*^{2} gene (Pfitzner, 2006).

The aim of the present study is to assess the resistance of F_3 tomato generations by means of molecular markers for *Sw5* and *Tm2*^{2}, and to characterise individual resistant plants for key morphological traits.

Materials and Methods

Plant material

Nineteen F_3 tomato populations, originating from ten crosses, were used in the study. Pautaliya and 975 were used as donors of *Tm2*^{2} in crosses with five susceptible to ToMV cultivars (Ideal, Rozovo sartse, Karobeta, Aleno sartse, 1627), whilst line 1627 was used as a donor of *Sw5* in crosses with five susceptible to TSWV cultivars (Pautaliya, Karobeta, Neven, Opalka, Kamen bryag) (Table 1). Plants were grown in a growth chamber at 22–25°C and 14h light. Each population was represented with up to eight plants.

Table 1. List of F_3 populations

№	Crosses	Population ID	Analysis
1	975 (pink) × Ideal (red)	1799	*Tm2*^{2}
2	975 × Rozovo sartse (pink)	1802	*Tm2*^{2}
		1806	*Tm2*^{2}
3	975 × Karobeta (orange)	1808	*Tm2*^{2}
		1810	*Tm2*^{2}
4	Pautaliya (red) × Aleno sartse (red)	1815	*Tm2*^{2}
		1819	*Tm2*^{2}
5	Pautaliya × 1627 (red)	1820	*Sw5, Tm2*^{2}
		1821	*Sw5, Tm2*^{2}
		1822	*Sw5, Tm2*^{2}
		1824	*Sw5, Tm2*^{2}
6	Karobeta × 1627	1838	*Sw5*
		1839	*Sw5*
7	Neven (orange) × 1627	1870	*Sw5*
8	Opalka (red) × 1627	1900	*Sw5*
9	1627 × Kamen bryag (red)	1929	*Sw5*
		1931	*Sw5*
10	Kamen bryag × 1627	1786	*Sw5*
		1788	*Sw5*

Source: Authors' own elaboration

Homozygous plants for *Sw5* and/or *Tm2*^{2} were transferred to five-litre pots and grown in a greenhouse until seed set.

Molecular analysis

DNA was isolated according to standard CTAB protocol (Edwards et al., 1991) from young lyophilised apical leaves and resuspended in 100 µl ddH_2O. The concentration was measured spectrophotometrically and normalised to 50 ng/µl. Genotyping of the *Sw5* allele was performed according to Dianese et al. (2010), and for *Tm2*^{2} according to Panthee et al. (2013).

Phenotyping

Phenotyping of five morphological traits was performed according to UPOV (https://www.upov.int/edocs/tgdocs/en/tg044.pdf) with some modifications. Multiple categories were assigned to each of the five descriptors:

- Plant growth habit: 1. Determinate, 2. Semi-determinate, 3. Indeterminate;
- Joint in the pedicel: 0. Absent, 1. Present;
- Inflorescence type: 1. Uniparous, 2. Fishbone, 3. Forked, 4. Irregular, 5. Compound;
- Predominant fruit shape: 1. Flat, 2. Rectangular, 3. Ellipsoid, 4. Obovoid, 5. Round,
- 6. Oxheart, 7. Long, 8. Heart, 9. Other;
- External mature fruit colour: 1. Yellow, 2. Orange, 3. Pink, 4. Red, 5. Purple, 6. Brown, 7. Other

All traits were scored manually and recorded by Field book app v. 5.5 (Rife and Poland, 2014).

Cluster analysis

Collected morphological data were processed by the *snow Cluster*: *Multivariate Analysis* (v. 7.1.7; https://github.com/hyunsooseol/snowCluster) module of Jamovi software (v. 2.3.28), using Euclidean distance and Ward.D2 clustering method to construct a dendrogram.

Results

Genotyping

The current study was engaged in the genotyping of 107 plants in total from 19 F_3 populations, originating from ten crosses, by markers for *Sw5* and *Tm2*^{2} genes for resistance to TSWV and ToMV, respectively. Resistance was identified in 20 plants homozygous for *Sw5*, and 15 plants homozygous for *Tm2*^{2}. Homozygous resistance to both viruses was found in one plant in a population of Pautaliya × 1627. Only in the cross Neven x 1627 no plants resistant to either virus were found. Genotyping results are shown in Table 2.

Phenotyping

Plants possessing at least one homozygous resistance gene were phenotyped for five morphological characteristics. The majority of the plants were with indeterminate growth habit, except one from the cross 975 × Karobeta exhibiting determinate growth. Approximately 48% and 42% of the plants inherited forked and uniparous inflorescence, respectively. About 6% and 3% possessed irregular and compound inflorescence, respectively. Regarding the mature fruit colour more than 82% were with red fruits. Interestingly, in crosses 975 × Karobeta and Karobeta × 1627 plants with orange-coloured fruits, resembling those of Karobeta appeared.

Plants with jointless pedicels were found in Pautaliya × 1627, 975 × Karobeta and Karobeta × 1627 crosses. This trait is valuable in germplasm, intended for breeding of processing

Table 2. Results of the genotyping of ten crosses for *Sw5* and *Tm2*[2] genes

№	Crosses	№ of plants	Sw5			Tm2[2]		
			S	H	R	S	H	R
1	975 × Ideal	6				2	2	2
2	975 × Rozovo sartse	11				3	6	2
3	975 × Karobeta	13				2	10	1
4	Pautaliya × Aleno sartse	13				3	6	4
5	Pautaliya × 1627	18	5	7	6*	7	4	7*
6	Karobeta × 1627	9	4	2	3			
7	Neven × 1627	7	3	4	0			
8	Opalka x 1627	4	0	3	1			
9	1627 × Kamen bryag	15	3	7	5			
10	Kamen bryag × 1627	11	2	3	6			

Legend: S – susceptible homozygous (*sw5*/*sw5*, *tm2*/*tm2*); H – resistant heterozygous (*Sw5*/*sw5*, *Tm2*[2]/*tm2*);
R – resistant homozygous (*Sw5*/*Sw5*, *Tm2*[2]/*Tm2*[2]); * – including one plant homozygous for both *Sw5* and *Tm2*[2].
Source: Authors' own elaboration

Table 3. Phenotypic characteristics of individuals, resistant to TSWV and/or ToMV from nine crosses

№	Crosses	Plant ID	*Sw5*	*Tm2*[2]	Growth habit	Joint in the pedicel	Inflorescence type	Fruit shape	Mature fruit colour
1	975 × Ideal	1799-3	S	R	3 Indeterminate	1 Present	1 Uniparous	3 Ellipsoid	3 Pink
		1799-6	S	R	3 Indeterminate	1 Present	4 Irregular	5 Round	3 Pink
2	975 × Rozovo sartse	1806-3	S	R	3 Indeterminate	1 Present	3 Forked	5 Round	3 Pink
3	975 × Karobeta	1808-3	S	R	1 Determinate	0 Absent	5 Compound	5 Round	2 Orange
4	Pautaliya × Aleno sartse	1815-5	S	R	3 Indeterminate	1 Present	1 Uniparous	8 Heart	4 Red
		1819-1	S	R	3 Indeterminate	1 Present	3 Forked	2 Rectangular	4 Red
		1819-4	S	R	3 Indeterminate	1 Present	3 Forked	7 Long	4 Red
		1819-5	S	R	3 Indeterminate	1 Present	1 Uniparous	2 Rectangular	4 Red
5	Pautaliya × 1627	1820-1	R	R	3 Indeterminate	1 Present	1 Uniparous	5 Round	4 Red
		1820-2	H	R	3 Indeterminate	1 Present	3 Forked	5 Round	4 Red
		1820-3	S	R	3 Indeterminate	1 Present	1 Uniparous	5 Round	4 Red
		1820-4	H	R	3 Indeterminate	1 Present	1 Uniparous	8 Heart	4 Red
		1820-5	S	R	3 Indeterminate	0 Absent	3 Forked	8 Heart	4 Red
		1820-6	H	R	3 Indeterminate	1 Present	1 Uniparous	5 Round	4 Red
		1822-1	R	H	3 Indeterminate	1 Present	1 Uniparous	5 Round	4 Red
		1822-5	S	R	3 Indeterminate	0 Absent	3 Forked	5 Round	4 Red
		1824-2	R	S	3 Indeterminate	0 Absent	3 Forked	5 Round	4 Red
		1824-3	R	S	3 Indeterminate	0 Absent	3 Forked	5 Round	4 Red
		1824-5	R	S	3 Indeterminate	0 Absent	3 Forked	5 Round	4 Red
6	Karobeta × 1627	1838-1	R	S	3 Indeterminate	0 Absent	1 Uniparous	5 Round	4 Red
		1839-3	R	S	3 Indeterminate	1 Present	3 Forked	1 Flat	4 Red
		1839-6	R	S	3 Indeterminate	1 Present	1 Uniparous	5 Round	2 Orange
8	Opalka × 1627	1900-4	R	S	3 Indeterminate	1 Present	1 Uniparous	6 Oxheart	4 Red
9	1627 × Kamen bryag	1929-2	R	S	3 Indeterminate	1 Present	1 Uniparous	8 Heart	4 Red
		1929-5	R	S	3 Indeterminate	1 Present	1 Uniparous	6 Oxheart	4 Red
		1929-6	R	S	3 Indeterminate	1 Present	3 Forked	5 Round	4 Red
		1931-2	R	S	3 Indeterminate	1 Present	3 Forked	5 Round	4 Red

Table 3. Phenotypic characteristics of individuals, resistant to TSWV and/or ToMV from nine crosses

№	Crosses	Plant ID	*Sw5*	*Tm2*[2]	Growth habit	Joint in the pedicel	Inflorescence type	Fruit shape	Mature fruit colour
10	Kamen bryag × 1627	1786-1	R	S	3 Indeterminate	1 Present	1 Uniparous	5 Round	4 Red
		1786-2	R	S	3 Indeterminate	1 Present	3 Forked	1 Flat	4 Red
		1786-3	R	S	3 Indeterminate	1 Present	3 Forked	5 Round	4 Red
		1786-4	R	S	3 Indeterminate	1 Present	3 Forked	5 Round	4 Red
		1786-6	R	S	3 Indeterminate	1 Present	3 Forked	6 Oxheart	4 Red
		1788-6	R	S	3 Indeterminate	1 Present	4 Irregular	1 Flat	4 Red

Note: Resistant plants from only nine crosses have been morphologically characterised because no resistant individuals were found in cross №7 Neven x 1627.
Source: Authors' own elaboration

tomatoes, where stem-free harvest of fruit is recommended. Complete morphological data are presented in Table 3.

Cluster analysis

Phenotyping data were put to cluster analysis in order to visualise the differences between plants from investigated crosses (Figure 1).

The algorithm divided individuals into two main clusters on the basis of plant growth habit. The first group included one cluster, cluster I, with one determinate plant from the cross 975 x Karobeta, resistant to ToMV, with round orange fruits and compound inflorescence, lacking a joint in the pedicel. The second group consisted of indeterminate plants in 17 unique subclusters. Clusters IV-VII, X and XIII included multiple plants from different crosses, sharing morphological characteristics. Clusters IV and XIII consisted of plants with round red fruits on forked inflorescence. The two clusters differed only in the absence or presence of a joint in the pedicel, respectively. Clusters V, VI and VII all included plants with red-coloured fruits on uniparous inflorescence with jointed pedicel. However, the three groups differed in fruit shape – plants in cluster V had heart-shaped fruits. In cluster VI fruits were round, and in cluster VII oxheart-shaped fruits were observed.

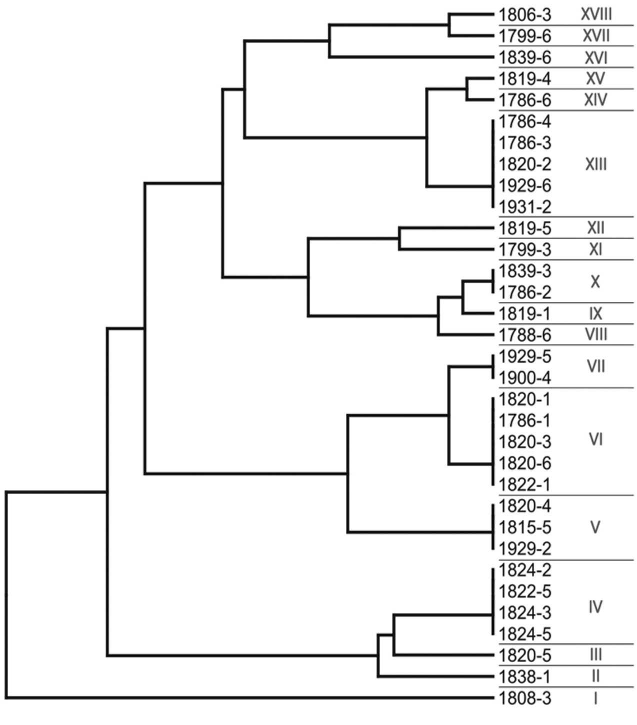

Fig. 1. Cluster dendrogram of plants resistant to TSWV and/or ToMV, based on five morphological traits
Source: Authors' own elaboration

The rest of the clusters (II, III, VIII, IX, XI, XII and XIV–XVIII) each included only one individual plant. These plants all possessed unique combinations of morphological characteristics, thus they were segregated into separate clusters.

Discussion

Development of tomato germplasm resistant to major viral diseases caused by ToMV and TSWV, is of great importance for Bulgaria, where these viruses are widespread and cause severe pressure on tomato production. The current paper engaged in the screening of 19 populations of ten crosses, for identifying homozygous individuals, resistant to these viruses, in order to start development of stable lines with specific morphological traits. Combination of both resistance genes in one genotype was expected in four populations, originating from the cross Pautaliya x 1627, since the parent plants were donors of *Tm2*[2] and *Sw5*, respectively. Only one plant possessing the desired gene combination was identified. This plant was indeterminate with round red fruits on uniparous inflorescence with a joint in the pedicel.

Pyramiding resistance genes are explored intensively in many tomato breeding programs. For example, García-Martínez et al. (2016) have introduced genes for resistance to ToMV and TSWV in 'De la Pera' tomato type for releasing lines UMH 1353 and UMH 1354. Similarly, Parrella and Troiano (2022) have used a backcrossing scheme to introduce both resistance genes in a Sorrento GP line originating from Italian local landrace "Pomodoro di Sorrento".

The tomatoes intended for fresh consumption and canning have different key characteristics. Those for fresh consumption are indeterminate or semi-determinate, and have a joint in the pedicel, allowing the whole truss to be cut and harvested at once. In contrast, canning tomatoes are indeterminate and jointless, making machine harvesting easy. In our crosses Pautaliya × 1627 and Karobeta × 1627, we have obtained individuals sharing characteristics of both types (Table 3).

The colour and shape of tomatoes are characteristics that greatly influence consumer preferences. In recent years pink tomatoes are strongly preferred by Bulgarian consumers (Casals et al., 2024). The representatives of populations 1799 and 1806 can be used for developing pink tomato lines and can also be included in other crossing schemes for germplasm improvement.

Conclusions

The current study identified F_3 plants, originating from ten tomato crosses, possessing homozygous resistance to TSWV and/or ToMV. Furthermore, these representatives were evaluated by plant growth habit, inflorescence type, presence of a joint in the pedicel, fruit shape and colour. A wide variety of combinations of valuable morphological traits were observed, providing an excellent starting material for tomato breeding programs to meet the requirements of growers and consumers.

Acknowledgements

The authors express their acknowledgement to the financial support of the European Union's Horizon 2020 research and innovation program project PlantaSYST (No. 664620), and to Agricultural Academy.

Conflict of interest

The authors declare that they have no conflict of interest.

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Received: **December, 05, 2024**; *Approved*: **January, 10, 2025**; *Published*: **February, 202**