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Tomato lines from interspecific hybridization – donors of resistance to leaf bacterial infections

Daniela Ganeva[1,*], Nevena Bogatzevska[2]

[1] *Agricultural Academy, Maritsa Vegetable Crops Research Institute, 4003 Plovdiv, Bulgaria*
[2] *Agricultural Academy, Institute of Soil Science, Agrotechnologies and Plant Protection "N. Pushkarov", 1331 Sofia, Bulgaria*
**Corresponding author:* dganeva@abv.bg

Abstract

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Leaf bacterial diseases (bacterial spot and speck) infect leaves, stems and fruit of tomatoes and cause both poor yield and fruit grade losses due to defoliation and fruit lesions. Natural populations of causative agents differ in species and race composition. *Pseudomonas syringae* pv. *tomato*, races R0 and R1 (bacterial speck) and *Xanthomonas vesicatoria* (pepper-and-tomato (PT), tomato (T) pathotype, races T1,T2,T3), *X. gardneri* and *X. euvesicatoria* (PT,T1,T2,T3) are widely distributed across tomato crops in Bulgaria. Fifteen interspecific hybrid tomato lines derived from crosses with the following wild species from genus *Solanum*: *S. cheesmaniae*, *S. pimpinellifolium*, *S. racemigerum*, *S. chilense* and *S. hirsutum* were studied in this experiment in order to establish their response to diseases.

Development of intraspecific hybrid tomato lines (crosses between *S. lycopersicum* and *S. chilense*, *S. pimpinellifolium* or *S. racemigerum*) is a prerequisite for improving the resistance to *X. vesicatoria* and *X. gardneri*. Two lines – L1052 (*S. lycopersicum* x *S. pimpinellifolium*) and L1791 (*S. lycopersicum* x *S. racemigerum*) were found to be resistant to the races of *X. vesicatoria*. Performed sequentially individual plant selection in L1787 (*S. racemigerum*), L1927 and L1921 (*S. chilense*) reduced the mean disease score and stabilized resistance to the races of *X. vesicatoria*. The lines L1787 and L1791 were found to be resistant to *X. gardneri* are *S. racemigerum*. The wild species *S. racemigerum* (L1787) was found to have multiple resistance to *X. gardneri*, the races of *X. vesicatoria* and *P. syringae* pv. *tomato*. This is the first study reporting on the influence of wild species *S. racemigerum* on the resistance of interspecific hybrid tomato lines towards *X. gardneri* races of *X. vesicatoria* and *P. syringae* pv. *tomato*.

Keywords: resistance; wild species; *Pseudomonas syringae; tomato* races 0, 1; *Xanthomonas vesicatoria; Xanthomonas gardneri*

Introduction

Leaf bacterial diseases (bacterial spot and speck) infect leaves, stems and fruit of tomatoes and cause both poor yield and fruit grade losses due to defoliation and fruit lesions. Natural populations of causative agents differ in species and race composition. *Pseudomonas syringae* pv. *tomato*, races R0 and R1 (bacterial speck) and *Xanthomonas vesicatoria* (pepper-and-tomato (PT), tomato (T) pathotype, races T1,T2,T3), *X. gardneri* and *X. euvesicatoria* (PT,T1,T2,T3) are widely distributed across tomato crops in Bulgaria (Bogatzevska, 2002; Bogatzevska & Sotirova, 2002; Kizheva et al., 2013, 2016; Stoyanova et al., 2014, 2015).

Both Bulgarian and introduced tomato varieties grown in the country are sensitive to the above listed races of bacterial speck and spot diseases (Aleksandrova et al., 2013; Alek-

sandrova et al., 2014a, b). Development of tomato cultivars with resistance to bacterial diseases is a desirable goal within the breeding programmers and an essential component of the integrated pest and disease control strategies within tomato producers worldwide. However, breeding for disease resistance has been difficult due to the necessity of selecting against multiple pathogens and the emergence of new species and races. When multiple races exist as this is the case for both studied pathogens, probability of the loss of effective resistance becomes an important consideration. An existence of sources of resistance is not only an important condition to perform genetic studies within the host-pathogen system but they also must endure long enough to ensure that overall benefits are greater than the costs incurred in the breeding efforts.

Domesticated tomato species *S. lycopersicum* possesses insufficient level of resistance to the agents of bacterial speck and spot (Danailov, 2012). The wild species from genus *Solanum* are certainly deficient in many characteristics required for the current cultivated systems (Zhang et al., 2002). However, they are used as a gene pool for resistant genes (R genes) that can be incorporated easily into commercially acceptable varieties (Caicedo & Schaal, 2004). When selecting donors of resistance, it is important to know the genetic distance between wild and domesticated species, as well as the existence of any incompatibility barriers and ways to overcome them (Danailov, 2012). Sources of resistance towards 16 pests and 32 diseases have been identified in wild tomato species (Rick, 1986).

The main source for genetic resistance to *X. vesicatoria*, *X. gardneri*, *P. syringae* pv. tomato are several wild tomato species including *S. pimpinellifolium*, *S. hirsutum*, *S. hirsutum* f. *glabratum*, *S. peruvianum*, *S. chilense* (Sotirova & Bogatzevska, 1994; Somodi et al., 1996; Scott et al., 1995, 1997, 2001, 2003).

In this research we aimed to study the disease reaction of interspecific hybrid tomato lines derived from crossing with wild species. Additionally, their use as resistant donors towards *X. vesicatoria* (races T1, T2, T3), *X. gardneri* and P. *syringae* pv. *tomato* (races R0, R1) in the tomato breeding program has been assessed.

Materials and Methods

This study was conducted under:

• controlled conditions at the Institute of Soil Science, Agrotechnologies and Plant Protection (ISSAT) "N. Pushkarov" – Sofia, Bulgaria;

• field conditions at the Vegetable Crop Research Institute (VCRI) "Maritsa" – Plovdiv, Bulgaria, during 2015-2017.

Plant material

A total of 15 interspecific hybrid tomato lines were used derived form crosses with the following wild species from genus *Solanum:* L1622 (*S. cheesmaniae*), L1052 and L1056 (*S. pimpinellifolium),* L1930, L1787 and L1791 (*S. racemigerum*), L1927, L1921, L1934, L1929 and L1165 (*S. chilense*) and L1655, L1923, L1781 and L1047 (*S. hirsutum*).

Bacterial strains

X. vesicatoria – T1 strain 24t; T2 strain 53t and T3 strain 30t; *X. gardneri* strain 67t; *P. syringae* pv. *tomato* – strain 31pt –R0; strain 27 pt – R1 (strains belong to pathology collection of the Plant Protection Department of N. Pushkarov Institute of Soil Science, Agrotechnologies and Plant Protection).

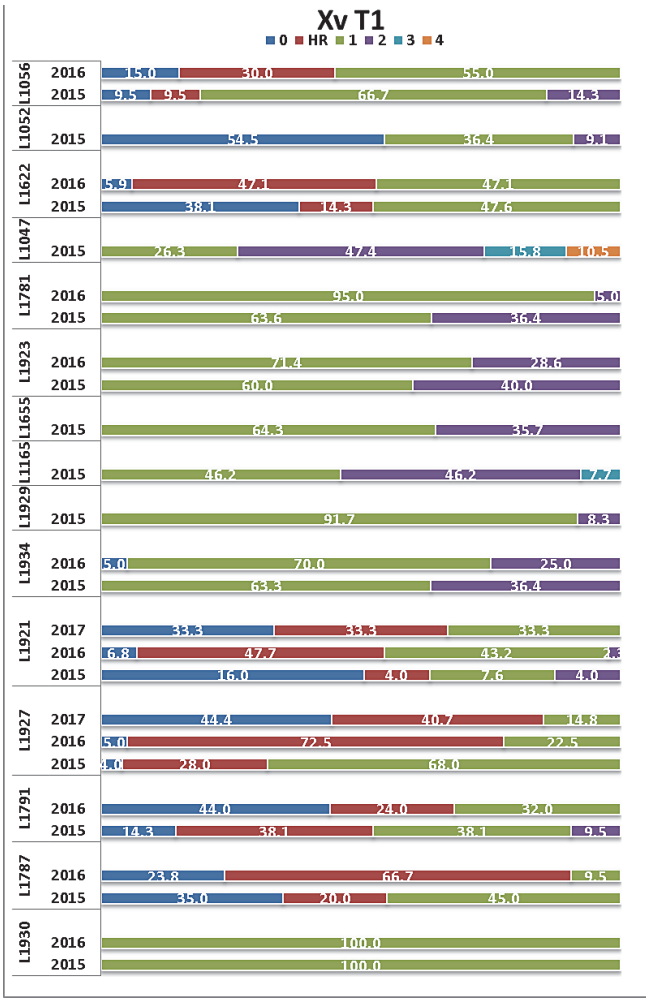

Fig. 1a. Distribution of the plants according to the rate of infestation to *Xanthomonas vesicatoria* race T1, in %

Inoculation *in vivo*

Tomato plants in the phase of 5-6 true leaves of each line (20 pieces or more) were vacuum infiltrated with bacterial suspension in concentration 10^8 cfu/ml from 36 h culture of *X. vesicatoria* (races T1, T2 and T3), *X. gardneri* and 10^4 cfu/ml (24 h culture) of *P. syringae* pv. *tomato* (races R0,R1). The inoculated plants of each line and bacteria were grown in nutrient solution in laboratory conditions in room temperature (Bogatzevska, 2002).

Disease estimation

Disease estimation hypersensitive reaction HR on plants was recorded after 24 h; the number of the spots necessary to diagnose bacterial spot and speck on the leaves was registered 4-5 days after the infiltration. The mean scope of infection (ms) was calculated by the scale of Sotirova and Beleva (1975) for *X. vesicatoria, X. gardneri* and for *P. syringae* pv. *tomato* by the scale of Chambers and Merriman (1975).

The classification of the lines in groups was made on the basis of ms: immune – 0 (I); resistant: 0.01 – 0.6 (R); moderately sensitive: 0.61 – 1.49 (MS); sensitive: 1.50 – 2.99 (S); highly sensitive over 3 (SS). Healthy plants and with (HR) were selected and transmitted to be grown on the experimental field at Maritsa VCRI. Seeds were obtained for further screening.

Results and Discussion

The research of our group focuses on the understanding of genetic and molecular mechanisms underlying disease re-

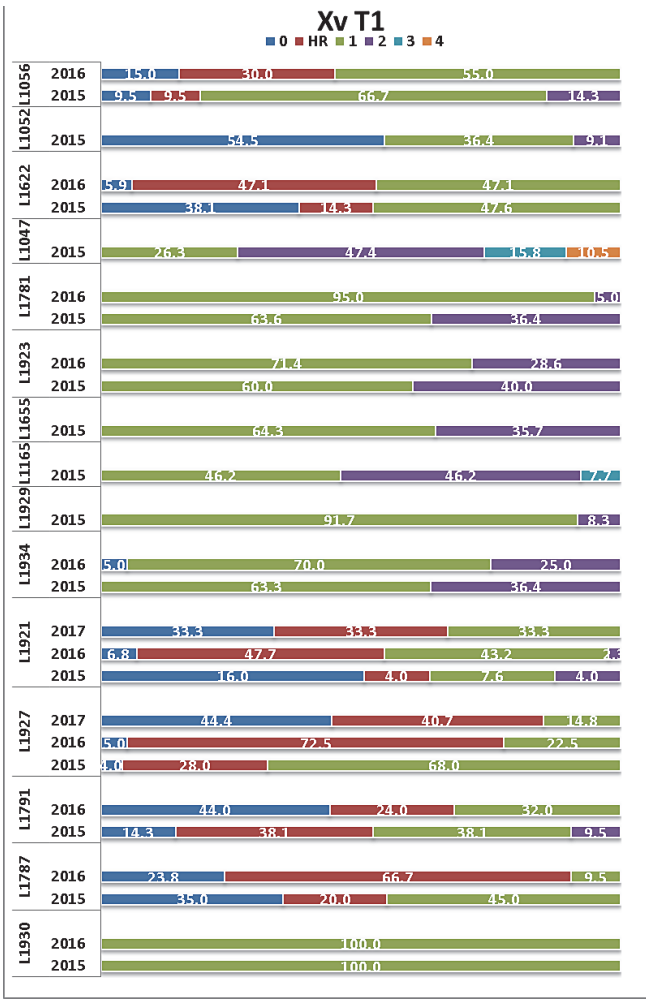

Fig. 1b. Distribution of the plants according to the rate of infestation to *Xanthomonas vesicatoria* race T2 in %

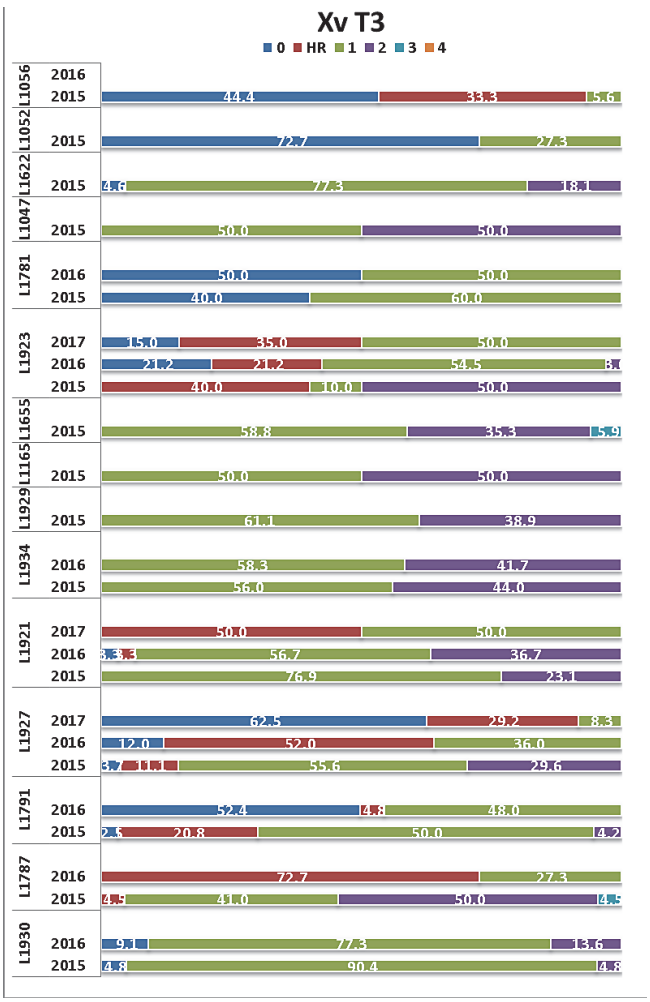

Fig. 1c. Distribution of the plants according to the rate of infestation to *Xanthomonas vesicatoria* race T3 in %

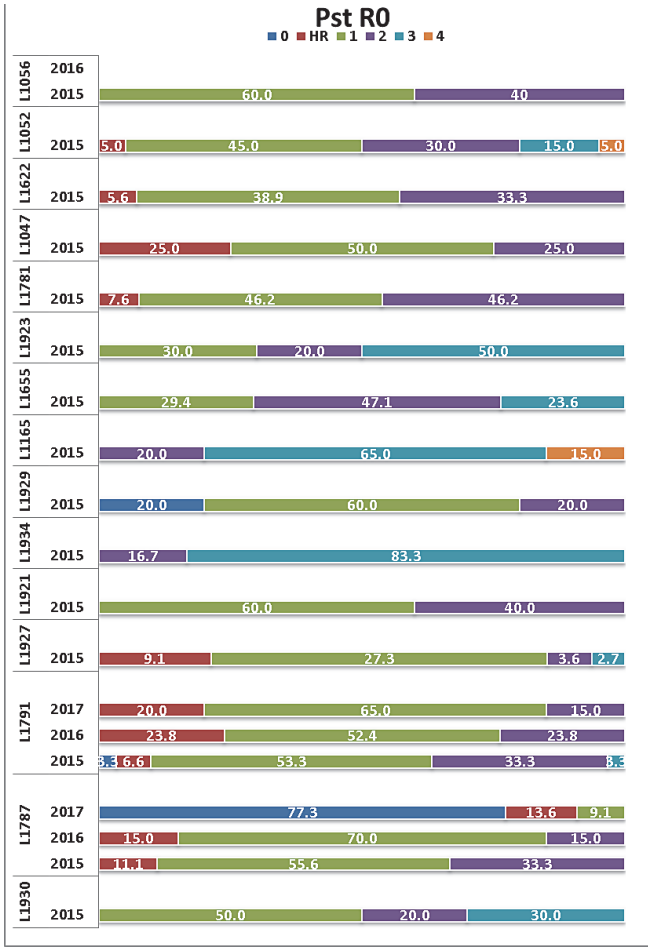

Fig. 2a. Distribution of the plants according to the rate of infestation to *Pseudomonas syringae* pv. *tomato* race R0, in %

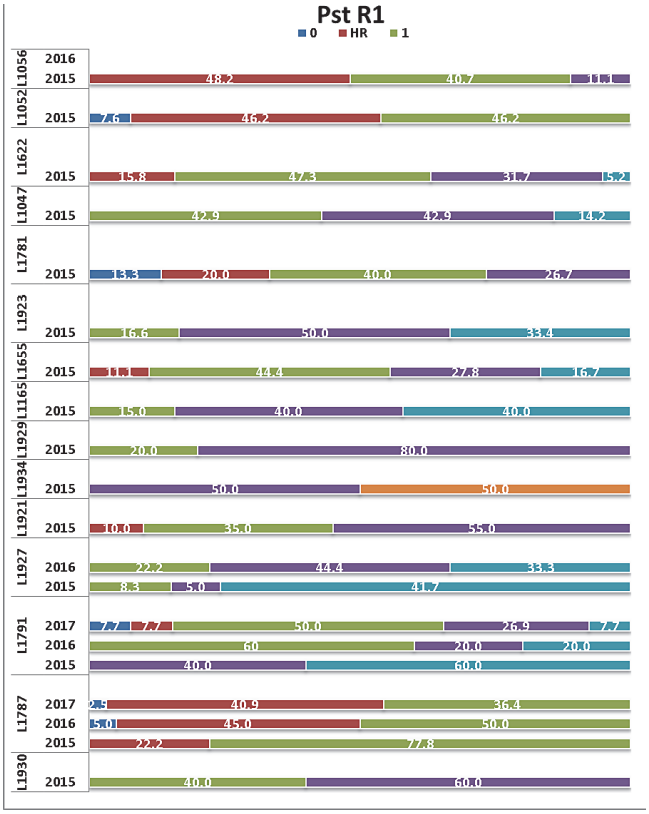

Fig. 2b. Distribution of the plants according to the rate of infestation to *Pseudomonas syringae* pv. *tomato* race R1, in %

sistances in tomato against bacteria pathogens. In this study we screened interspecific hybrid tomato lines derived from crosses between *S. lycopersicum* and wild species, including *S. cheesmaniae, S. pimpinellifolium, S. racemigerum, S. chilense* and *S. hirsutum* against different casual pathogens of bacterial spot and spec diseases. We found that plants were characterized by different degrees of resistance to the races of the causal agents *X. gardneri* (Table 1), *X. vesicatoria* (Fig. 1 a, b, c) and *P. syringae* pv. *tomato* (Fig. 2 a, b).

Plants with immune or highly sensitive reaction wеre not recorded in any of the lines. Those with sensitive and highly sensitive reaction are of no interest to the selection and therefore they are not subject of the discussion bellow. A differential level of resistance in the studied tomato lines, infected with different races of *X. vesicatoria* was established (Fig. 1 a, b, c). Resistant genotypes were characterized by a high percentage of healthy and HR plants which possessed disease symptoms of a single leaf spot (measured with a rate of 1). The lines of interest to use further in the breeding program are the ones resistant to the races of *X. vesicatoria*: T1-L1622 (*S. cheesmaniae*), L1052 (*S. pimpinellifolium*), L1787 and L1791 (*S. racemigerum*); T2- L1791 (*S. racemigerum*), L1052 and L1056 (*S. pimpinellifolium*); T3 – L1052 (*S. pimpinellifolium*), L1781 (*S. habrochaites = hirsutum*) and L1791 (*S. racemigerum)*.

Our screening identified two lines with a multiple resistance: L1052 (*S. lycopersicum* x *S. pimpinellifolium*) and L1791 (*S. lycopersicum* x *S. racemigerum*). The resistance identified is most likely incorporated from the wild species *S. pimpinellifolium* and *S. racemigerum,* known to be resistant to bacterial spot and speck. An individual plant selection of the healthy plants was performed and the ones having HR in order to increase and stabilize the resistance to races of *X. vesicatoria* in lines L1787 and L1791 (T1,

Table 1. Evaluation of the resistance to *X. gardneri* of lines obtained as a result of interspecific hybridization

№	Lines	Seeds with origin from plants with HR	*X. gardneri*							
			Number of plants	0	HR	1	2	3	4	ms
1	L 1930	XvT2	20	2	7	9	2	0	0	0.65
		Xg	24	2	12	10	0	0	0	0.42
		Xg	21	10	5	6	0	0	0	0.29
2	L 1787	XvT2	21	2	6	13	0	0	0	0.65
		Xg	22	9	5	8	0	0	0	0.36
		Xg	26	16	8	2	0	0	0	0.08
3	L 1791	XvT2	20	3	6	11	0	0	0	0.55
		Xg	26	10	6	10	0	0	0	0.38
		Xg	20	9	8	3	0	0	0	0.15
4	L 1927	XvT1	23	0	9	8	6	0	0	0.87
		Xg	23	2	4	16	1	0	0	0.78
		Xg	20	4	4	12	0	0	0	0.60
5	L 1921	XvT1	22	5	5	8	4	0	0	0.73
		Xg	20	9	6	5	0	0	0	0.25

T2, T3); L1927, L1921 (T1, T3); L930 (T2), L923, L1781 (T3). Different wild species (*S. racemigerum, S. chilense* and *S. hirsutum*) incorporated in the interspecific hybrid tomato lines pedigree conditionthe resistance to races T1, T2 and T3 of *X. vesicatoria* (Fig. 1 a, b, c). Line L1791 was resistant to *X. gardneri* infection (Table 1). All of the five lines included in the research were moderately sensitive with mean score of 0.65 to 1.21. The selection of healthy plants with HR lead to a decrease in the susceptibility of L1930, L1921 and L1787, which from moderately resistant improved to resistant ones. Our finding that lines L1791, L1927, L1921 possessed resistance to *X. vesicatoria* and *X. gardneri* confirms the presence of genes for resistance transferred from *S. racemigerum* and *S. chilense* into their genome.

Different degree of the mean disease reaction was found within the interspecific hybrid tomato lines after artificial inoculation with races of *P. syringae* pv. *tomato* (Fig. 2 a, b). Line L1781 (*S. habrochaites*) was resistant to race R0, and L1052 (*S. pimpinellifolium*) of race R1. The subsequent selection of healthy and HR plants resulted in a decrease of the mean disease reaction core in L1787 (*S. lycopersicum* x *S. racemigerum*). Conducting a consistent selection of individual plants without symptoms and/or those with HR stabilized the resistance in L1787. In line L1791, the selection reduces the mean disease score only slightly, but does not lead to resistance. The lines having a resistance to *X. gardneri* and *X. vesicatoria* (races T1, T2, T3) responded specifically to the infection of *P. syringae* pv. *tomato* in our study. Individual selection against a targeted disease/pathogen in the intraspecific hybrid tomato lines crossed with *S. racemigerum* lead to stabilization and enhancement of the resistance to *X. gardneri, X. vesicatoria* and *P. syringae* pv. *tomato*. These lines are newly developed tomato hybrids with good agro-economic qualities and complex resistance to the causal agents of bacterial spot and speck. Wild species of the genus *Solanum* can be cross-breeding with *S. lycopersicum*, though sometimes achieving the intraspecific hybrids proved to be very difficult (Spooner et al., 2005).

All members of the *Eulycopersicon* subgenus, including *S. lycopersicum* are self-compatible species and easily cross-breeding the cultural tomato. With few exceptions (*S. habrochaites = L. hirsutum f. glabratum*), representatives of the *Eriopersicon* subgenus are self-incompatible and often exhibit unilateral incompatibility in cross-breeding with *S. lycopersicum* (Robertson & Labate, 2007). This hampers the transfer of R genes from wild species into domesticated tomato. Carriers of resistance to causative agents of bacterial spot and speck are known to be *L. pimpinelifolium*, *L. peruvianum*, *L. hirsutum, L. hirsutum f. glabratum* (Scott & Jones, 1989; Scott et al., 1989, 1997; Minsavage et al. 1990; Somodi et al., 1996; Sotirova & Bogatzevska, 1994, 1998; Krause et al., 2001). The resistance found is associated with HR of plants to *X. vesicatoria* (Scott et al., 2001). A high level of resistance to T1 and T3 of *X. vesicatoria* has been found in tomato lines containing a three genomes hybrid (*L. esculentum* x *L. chilense* LA460) x *L. peruvianum* var. *humifusum* PI 127829 and *L. esculentum* x *L. pimpinellifolium* PI 126925 (Ivanova & Bogatzevska, 2006). Reliable sources of resistance to *X. vesicatoria* race T1, T3 are

L. pimpinellifolium LA121 and *L. chilense* CGN15531. Another suitable donor of the R genes is *L. pimpinellifolium* LA121 (*Eulycopersicon*) due to its easy crossbreeding with the cultural tomato, whereas there is unilateral interspecies incompatibility in *L. chilense* (*Eriopersicon*) (Ivanova & Bogatsevska, 2007). *S. pimpinellifolium* appeared to be resistant to *X. gardneri* in both greenhouse and field trials (Liabeuf et al., 2015).

Race R0 is controlled by the Pto-1 gene in *L. pimpinellifolium*. Resistance to race R1 is found in *L. chilense* and *L. pimpinellifolium* (Stamova et al., 1990). Two other Pto 3 and Pto 4 genes have been identified *in L. hirsutum* var. *glabratum* that control resistance to both races (Stockinger & Walling, 1994). Both lines *Solanum neorickii* LA1329 and *S. habrochaites* LA1253 are resistant to *P. syringae* pv. *tomato* race 1, which are also resistant to bacterial infection as adult plants (Hassan et al., 2017).

Conclusions

Development of intraspecific hybrid tomato lines (crosses between *S. lycopersicum* and *S. chilense*, *S. pimpinellifolium* or *S. racemigerum)* is a prerequisite for improving the resistance to *X. vesicatoria* and *X. gardneri*. Two lines – L1052 (*S. lycopersicum* x *S. pimpinellifolium*) and L1791 (*S. lycopersicum* x *S. racemigerum*) were found to be resistant to the races of *X. vesicatoria* in this study. Performed sequentially individual plant selection in L1787 (*S. racemigerum*), L1927 and L1921 (*S. chilense*) reduced the mean disease score and stabilized resistance to the races of *X. vesicatoria.* Two lines L1787 and L1791 were found to be resistant to X. *gardneri* are *S. Racemigerum* in this study. The line L1787 (*S. racemigerum*) was found to have multiple resistance to *X. gardneri*, the races of *X. vesicatoria* and *P. syringae* pv. *tomato*. Positively differentiated impact on resistance to *P. syringae* pv. *tomato* is present in the genome of *S. pimpinellifolium* (L1052-R1), *S. chilense* (L1930-R1) and *S. hirsutum* (L1781-RO). This is the first study reporting on the influence of wild specie *S. racemigerum* on the resistance of interspecific hybrid tomato lines towards *X. gardneri* races of *X. vesicatoria* and *P. syringae* pv. *tomato.*

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