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Sensitivity of tomato (*Solanum lycopersicum*) cultivars from Turkey to bacterial speck (*Pseudomonas syringae* pv. *tomato*)

Ali Turgut and Hüseyin Basim*

Department of Plant Protection, Faculty of Agriculture, University of Akdeniz, 07058, Antalya, Turkey.

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The susceptibility of 93 different tomato cultivars that are commonly grown in greenhouses and field in the western Mediterranean region of Turkey have been assessed for resistance to bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato* strains. The disease severity indexes (DSI) varied between zero and four for the tomato cultivars. Seven tomato cultivars showed hypersensitive reactions against strains of *P. syringae* pv. *tomato* carrying the *avrpto1* gene. Six of these seven tomato cultivars had the *PTO* gene coding for resistance against bacterial speck. The *PTO* sequences from the tomato cultivars Atalay, Party, Petrus, Piccadilly, Prenses and Tyty had similarity of 94, 93, 94, 92, 95 and 94%, respectively, with the *PTO* sequence of *Lycopersicon esculentum* VFNT Cherry (AF220603), and had similarity of 98, 98, 96, 95, 100 and 98%, respectively, with the *PTO* sequence of *Lycopersicon pimpinellifolium* Rio Grande 76R (AF220602). These findings indicate a considerable variation in bacterial speck resistance and will aid in the choice of parental lines for breeding new tomato cultivars with resistance to bacterial speck.

Key words: *avrpto*, Mediterranean region, polymerase chain reaction, *PTO*.

INTRODUCTION

Bacterial speck disease was first reported by Bryan (1933) in the USA. In the following years, the occurrence and importance of bacterial speck disease increased, with epidemics occurring in several places around the world, and new occurrences of the disease are continually being reported in different places worldwide to date.

The typical symptoms of bacterial speck disease can be found on leaves, stems, petiole, pedicles and sepals. The symptoms of the disease on leaves are marked by small dark brown/black spots surrounded with a green halo. In the progressive stage, the halos become clear, followed by the specks uniting in most conditions (Young et al., 1986), which causes the leaves to die. The phytotoxin Coronatine produced by many strains of this pathogen creates chlorotic halos around the specks. Spots on the flowers are not as clear as the spots on the leaves. However, the disease affects the first flowers, which prevents flowering and can cause considerable losses in yield (Young et al., 1986). The symptoms of the disease found on the fruits are somewhat superficial, with slightly raised black specks that are approximately 1.5 mm in diameter (Young et al., 1986). Fruit shapes are deformed by uniting of the specks on the fruits. As the spotted fruits remain small in correlation with disease severity, the market value of the fruits decreases (Goode and Sasser, 1980).

In appropriate conditions, bacterial speck disease can expeditiously spread to other plants in the field in a short time (McCarter et al., 1983). When appropriate conditions for the disease exist, the largest economical losses occur in the tomato seed and tomato seedling industry

*Corresponding author. E-mail: hbasim@akdeniz.edu.tr. Tel: 90-242-3102432. Fax: 90-242-2274564.

Table 1. *P. syringae* pv. *tomato* strains used in this study.

Strain	Origin	Reference
0853-td1	Turkey	Basim et al., 2004
0843-td5	Turkey	Basim et al., 2004
2533-td20	Turkey	Basim et al., 2004
0433-td28	Turkey	Basim et al., 2004
0433-td31	Turkey	Basim et al., 2004
1033-td34	Turkey	Basim et al., 2004
1833-td35	Turkey	Basim et al., 2004
1833-td37	Turkey	Basim et al., 2004
2943-tt41	Turkey	Basim et al., 2004
0143-td47	Turkey	Basim et al., 2004
1933-td49	Turkey	Basim et al., 2004
0753-td50	Turkey	Basim et al., 2004
1153-td48	Turkey	Basim et al., 2004
1434-td1	Turkey	Basim et al., 2004
1434-td2	Turkey	Basim et al., 2004
2034-td3	Turkey	Basim et al., 2004
0504-td4	Turkey	Basim et al., 2004
1044-td5	Turkey	Basim et al., 2004
1044-td6	Turkey	Basim et al., 2004
Pst006A	Canada	D. A. Cuppels
Pst03T12	Canada	D. A. Cuppels
Pst04T5	Canada	D. A. Cuppels
3374	Bulgaria	T. N. Donev
301591	Japan	T. Sato, NIAS
BPIC 315	Greece	A. S. Alivizatos

(Psallidas, 1988).

The first determination of this disease in Turkey was made by Saygili (1975) in the Aegean region and Cinar (1977) in the east Mediterranean region. The bacterial speck disease in the western Mediterranean region has occurred in commercial vegetable seedling production companies and caused great seedling losses, as reported by Basim et al. (2004).

In Turkey, 58 of a total of 90 commercial seedling companies are located in Antalya. During the spring months of 2002 and 2003, a serious epidemic of bacterial speck disease occurred in trade seedling companies in southwest Turkey. The epidemic was severe, especially in the western Mediterranean region of Turkey. The epidemic in the spring months of 2002 and 2003 caused approximately 20 to 25% seedling losses in seedling companies. The outbreak in 2003 caused an approximate 5% loss in 142 commercial greenhouses in the region. The greenhouse tomato production was not affected by the epidemic and disease symptoms on the fruit were not observed (Basim, 2004).

Physical precautions, cultural precautions, healthy seed usage, certified seed usage, biological control agents, thermal soil applications, several types of chemical preparations and resistant variety usage are important for challenging the pathogen. However, the use of antibiotics and copper compounds does not effectively control the disease as reported earlier (Jones and Jones, 1989; Silva and Lopes, 1995). The use of resistant tomato cultivars is one effective way to control the disease.

Some tomato cultivars that are resistant to bacterial speck disease need a functional *PTO* gene to provide resistance against the disease (Martin et al., 1993a; Riley and Martin, 2001). *PTO* is a member of a multiple gene family, and it is located in a 400-kb region on the fifth chromosome of the tomato genome (Martin et al., 1994). *PTO* is a gene that encodes a serine threonine protein kinase, which provides resistance against *Pseudomonas syringae* pv*. tomato* strains that carry the *avrPto1* effector gene (Carland and Staskawicz, 1993; Martin et al., 1993b).

The objective of this study was to assess the resistance levels of bacterial speck of tomato germplasm used in the production of greenhouse and field tomatoes in the west Mediterranean region of Turkey. This is the first research on determining susceptibility levels to bacterial speck of tomato germplasm in the region.

MATERIALS AND METHODS

Bacterial cultures

In this study, 25 different *P. syringae* pv. *tomato* strains were used (Table 1). The bacterial cultures used in this study were stored in 30% glycerol (Sigma-Aldrich Chemie GmbH, Eschenstrasse 5 82024 Taufkirchen Germany) in nutrient broth (Merck KGaA, Frankfurter Str. 250 64293 Darmstadt Germany) medium at -86°C.

Plant materials

The cultivars of tomatoes used in this study were obtained from companies producing commercial tomato seedlings in Antalya province. A total of 93 popular tomato cultivars including the Ontario 7710 cultivar, which is resistant to *P. syringae* pv. *tomato*, were tested. The Ontario 7710 cultivar supplied by the C.M. Rick Tomato Genetics Resource Center in California was used as a control cultivar.

The tomato seedlings were grown in peat medium until the seedlings reached the four real leaves stage at 50 to 70% relative humidity and 21 to 27°C in the greenhouse.

Detection of the *avrPto1* gene

The following primers (Iontek Ltd.Şti, Ali Rıza Gurcan Cad. Cirpici Yolu No:1/410 34010 Merter Istanbul) were used to amplify the *avrPto1* gene by PCR: 5'-CCATGGGAAATATATGTGTCGGCGG-3' and 5'-CTGGAGTCATTGCCAGTTACGGTACGG-3' (Techne TC-512 Gradient Thermal Cycler) (Chang et al., 2001). The PCR programme consisted in 1 cycle at 94°C for 5 min; 40 cycles of 30 s at 92°C, 30 s at 55°C, and 30 s at 70°C; and 1 cycle at 72°C for 10 min (Chang et al., 2001).

The PCR products were electrophoresed at 70 V in a 1% agarose (Merck KGaA, Frankfurter Str. 250 64293 Darmstadt Germany) gel for 150 min. Tris-acetate-EDTA (TAE) (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was used in the

electrophoresis process and in preparation of the agarose gel. After electrophoresis, the PCR products were stained with 0.5 μg/ml ethidium bromide (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) for 30 min and were imaged in ultraviolet light (Vilber Lourmat SR 12575).

Inoculation of the bacterial pathogen

The *P. syringae* pv. *tomato* strain (0853-td1) was used to inoculate the plants. The bacterial strains were grown in nutrient agar (Merck KGaA, Frankfurter Str. 250 64293 Darmstadt Germany) medium for 48 h. The inoculum absorbance was set up as follows: $A_{600} = 0.3$ at an equilibrium of 1 x 10^8 cfu/ml in a deionised water suspension. A suspension containing approximately 1 x 10^8 cfu/ml bacteria prepared from bacterial cultures was sprayed on healthy tomato plants, then incubated at 90 to 95% relative humidity for 24 h. The plants were then transferred to a growth chamber having 50 to 70% relative humidity at 25 ± 1°C ambient temperature for progression of symptoms. During the incubation process, plants were exposed to 4,850 lux of illuminance for 12 h each day.

Evaluation of disease severity

The typical symptoms of bacterial speck disease include a number of necrosis-shaped specks on the leaves, changing colour from dark brown to black, and this change is part of the criteria for determining the severity of disease. The disease symptoms can be observed by the second day. The disease progression in the tomato cultivars was followed by counting the specks 4, 7, 14, 21, 28 and 35 days after inoculation. The evaluation of whether the plants were sensitive, tolerant or resistant to the disease was carried out according to the scale of Chambers and Merriman (1975): Infection class 0 = no lesions; infection class 1 = 1-10 lesions per plant; infection class 2 = 11-20 lesions per plant; infection class 3 = 21-40 lesions per plant; and infection class 4 = more than 40 lesions per plant.

The values of the disease severity index (DSI) were calculated by counting the specks 14 days after inoculation. Five replicates were considered in a completely randomised design. The DSI value was calculated from the sum of the data classified by the Chambers and Merriman scale acquired from five replicates divided by the replication number for each variety. If the calculated DSI values did not have a normal distribution, they were controlled by the Kolmogorov-Smirnov test statistic. The use of nonparametric methods has been approved because normal distributions in carried transformation procedures cannot be shown without the use of the Kolmogorov-Smirnov test. The Kruskal-Wallis H test was used whether there were any important differences between the tomato cultivars. The Mann-Whitney U test statistic was used to investigate the differences between the tomato cultivars.

A profile analysis technique, which is a special condition of multivariate analysis of variance (MANOVA) was used in the analysis of repeat measure data, and used to analyse the differences between the infection classes. The following tested hypothesis was used: H_0: $C\mu_1 = C\mu_2 = ... = C\mu_k$ (Ricklefs, 1967; Tsoularis and Wallace, 2002; Narinc et al., 2010). The data acquired at 4, 7, 14 and 21 days after inoculation were used to determine the progression of the disease over time. A logistic model, which is a nonlinear regression model, was used to analyse the disease progression dependence on time (Zwietering et al., 1990). The mathematical expression of the three-parameter logistic growth model used is as follows: $Y = \beta_0 (1 + \beta_1 \exp\text{-}\beta_2 t)^{-1}$; where, β_0 is the asymptotic (steady state) number of specks; β_1 is a scaling parameter (constant of integration); and β_2 is the instantaneous growth rate (per day) (Narinc et al., 2010). All the statistical analyses were carried out using SPSS 17 (SPSS Inc.).

Detection of the *PTO* gene

The tomato cultivars found to be resistant based on bacterial inoculation and statistical analyses were tested for the presence of *PTO*. A combined system (Qbiogene/BIO 101 FastDNA molecular isolation kit and Thermo FastPrep FP120A-230) (Qbiogene Inc., 205-10340 De La Côte De Liesse Rd. Lachine, Quebec H8T 1A3) was used to isolate genomic DNA using 180 mg of fresh leaf tissue from the different tomato cultivars. A spectrophotometer (Thermo NanoDrop ND-1000) was used to determine the purity and concentration of DNA isolated from the plant materials. The genomic DNA was diluted with sterile deionised water to a final concentration of 100 ng/μl.

SSP17 (5'-GGTCACCATGGGAAGCAAGTATTC-3') and JCP32 (5'-GGCTCTAGATTAAATAACAGACTCTTGGAG-3') primers were used to determine the presence of the *PTO* gene using a previously described PCR method: 1 cycle at 94°C for 5 min; 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 90 s; and 1 cycle at 72°C for 10 min (Rose et al., 2007). The PCR products (15 μl) were electrophoresed in a 1% agarose gel in TAE buffer at 80 V for 120 min. The PCR products were stained with 0.5 μg/ml ethidium bromide for 30 min and imaged in ultraviolet light.

Hypersensitive reaction test

The presence of a hypersensitive reaction was evaluated in the resistant tomato cultivars with the *avrPto1* gene as determined in the present study. Tomato leaves were inoculated with bacterial suspensions by infiltrating the bacteria into the leaf mesophyll spaces with a 1-ml plastic syringe and a 27-gauge needle. The bacteria concentration in the suspension (containing sterile deionised water) was approximately 10^9 cfu/ml, which was determined by measuring the optical density with a spectrophotometer. For the hypersensitive test, 25 different *P. syringae* pv. *tomato* strains (Table 1) were used. The plants were incubated in a growth chamber, which was programmed to maintain the relative humidity at 50 to 70% and the temperature at 25 ± 1°C for 72 h. The Ontario 7710 variety, which is resistant to bacterial speck was used as the control tomato variety.

Sequencing of the *PTO* gene

The presence of the *PTO* gene in the resistant tomato cultivars was verified by sequencing the PCR product amplified using the JCP32 and SSP17 primer set. Sequence analysis was performed by the RefGen Gene Research and Biotechnology Company. Two gene sequences belonging to each resistant tomato variety were aligned using the BioEdit Sequence Alignment Editor Program. Aligned gene sequences were compared using the nucleotide basic local alignment search tool (BLAST) to related sequences of *Lycopersicon esculentum* VFNT Cherry (AF220603) and *Lycopersicon pimpinellifollium* Rio Grande 76R (AF220602). The Nucleotide BLAST (Megablast) programme is optimised for highly similar sequences.

RESULTS

Presence of *avrPto1*

A 495-bp DNA fragment (Figures 1a and b) was observed after PCR and electrophoresis in all 25 *P. syringae* pv. *tomato* strains (Table 1).

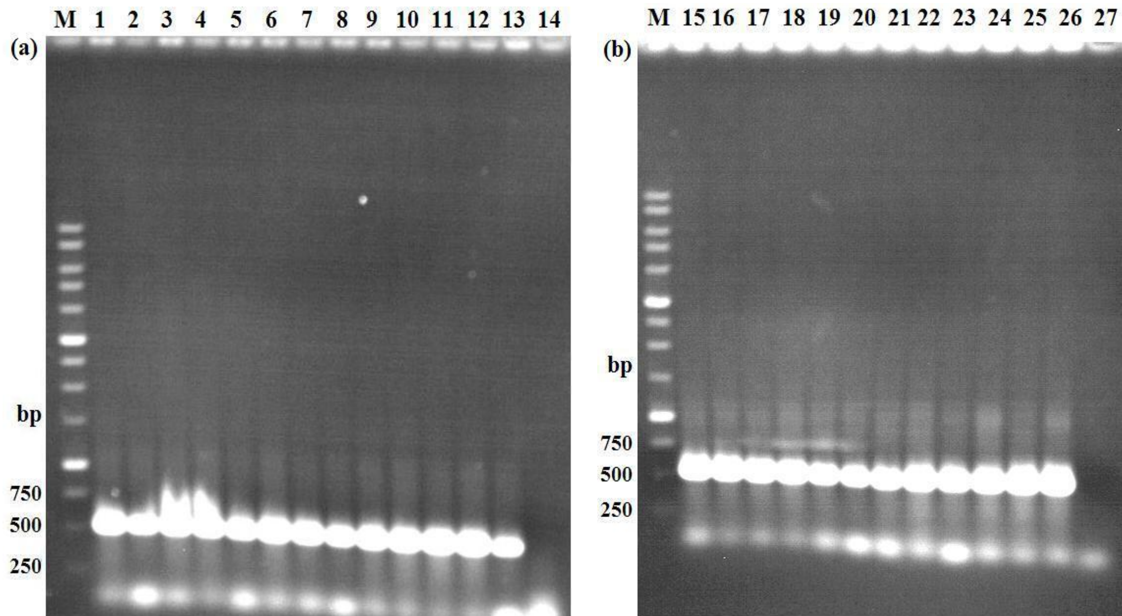

Figure 1. PCR amplification of the *avrPto* gene (495 bp) of *P. syringae* pv*. tomato* strains. **(a)** Lane M, 1-kb marker; lane 1, 0853-td1; lane 2, 0843-td5; lane 3, 2533-td20; lane 4, 0433-td28; lane 5, 0433-td31; lane 6, 1033-td34; lane 7, 1833-td35; lane 8, 1833-td37; lane 9, 2943-at41; lane 10, 0143-td47; lane 11, 1933-td49; lane 12, 0753-td50; lane 13, 1153-at; lane 14, negative control. **(b)** Lane M, 1-kb marker; lane 15, Akd Fide; lane 16, Akd Fide1; lane 17, Akd Fide2; lane 18, Kumluca; lane 19, Tomato1; lane 20, Tomato3; lane 21, Pst006A; lane 22, Pst03T12; lane 23, Pst04T5; lane 24, 3374; lane 25, 301591; lane 26, BPIC 315; lane 27, negative control.

Evaluation of disease severity and progression

Approximately 48 h after inoculation, the typical bacterial speck symptoms appeared, and the specks became more visible and larger over time. The first symptoms were dependent on the plant genotype, and they had differences in terms of number of specks and halo diameter. The speck diameters were relatively small in the infection classes 1 and 2 because the disease was less severe. However, the specks on the leaves continued to expand and were classified in infection classes 3 and 4 because the disease was more severe. Despite the change in infection class severity, cholorosis was dependent on the halo in the tomato cultivars classified in infection classes 1 and 2, which were clearly reduced.

The disease quickly progressed after the first symptoms were observed. The number of necrosis-shaped specks formed during the first four days and doubled by the end of the first week. The number of necrosis-shaped specks approximately doubled during the first week on the leaves within the same period. After the second week, the number of necrosis-shaped specks was slightly increased in all the tomato cultivars classified in infection classes 1 and 2 and in only some of the tomato cultivars classified in infection class 3. The number of necrosis-shaped specks decreased for some cultivars because the specks were close to each other, causing the number to decrease when the specks united.

Data collected from some sensitive tomato cultivars 21 days after inoculation were inconsistent due to deformed leaves covered with specks that were close to each other and became confluent, preventing correct counts of the specks. Inconsistency was found for almost all the data, except for some cultivars that were resistant or tolerant to the disease 28 and 35 days after inoculation. However, the counting was continued for 21 days after inoculation to obtain sufficient data to be used in determining the disease progression over time with nonlinear regression analysis. Statistically significant differences ($P < 0.0001$) among the infection classes were found using a parallel test, suggesting that a single progression curve could not represent all infection classes. Thus, separate development curves were created for each infection class (Figure 2).

Although, the different tomato cultivars demonstrated different levels of resistance against the disease, the use of many different cultivars made it difficult to analyse the raw data and impossible to effectively distinguish the cultivars. Therefore, the data obtained 14 days after inoculation were analysed according to the Chambers and Merriman (1975) scale and were separated into five different levels. DSI value was calculated for each tomato variety using the averages of the data.

Data collected 14 days after inoculation were used because secondary infections on the tomato cultivars

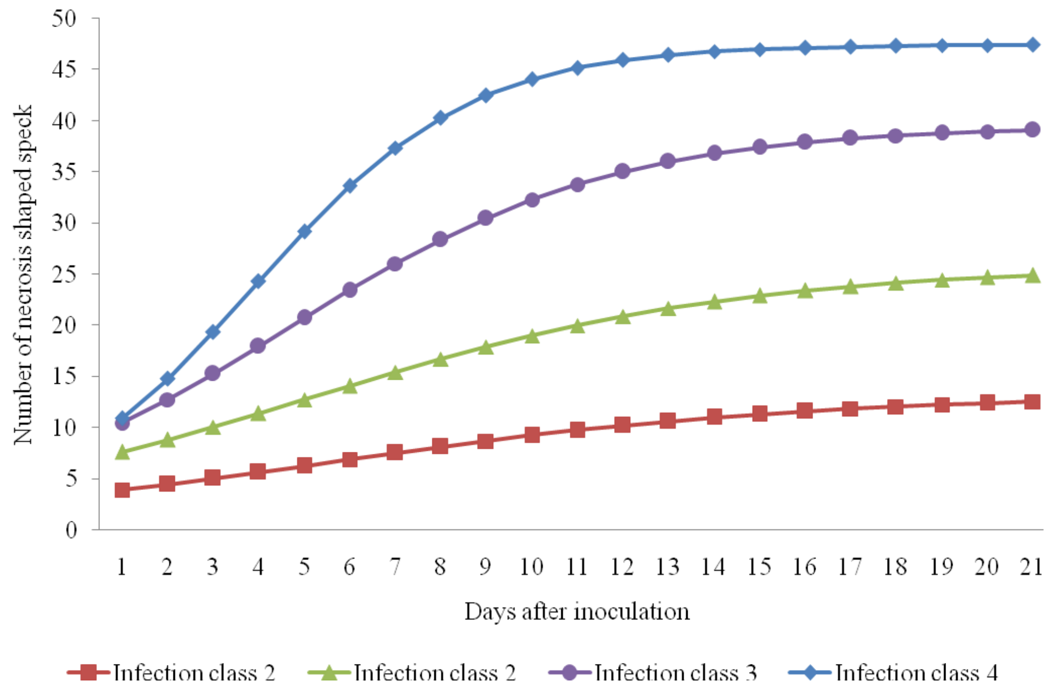

Figure 2. Logistic model curves for infection classes of the bacterial speck disease.

appeared later on. The secondary infections increased the number of necrosis-shaped specks on the plant, causing them to be close to each other, which deformed the structure of the leaves, thus preventing a healthy count and skewing the data from normal levels. Moreover, the variations in the tomatoes did not skew the data in the following weeks, resulting in no significant differences in the obtained data (P <0.05).

There were differences in the resistance levels among the 93 different cultivars, as found by the Kruskal-Wallis H test (P <0.05). The Mann-Whitney U test was performed to elucidate which variety caused the differences, and it demonstrated that the tomato cultivars conglomerated into 12 different groups, except for the group of plants that had no infection symptoms.

According to the Chambers and Merriman scale, infection class 1 included the highest resistance level among the various tomato cultivars, and infection classes 3 and 4 contained the cultivars that had a high severity of infection. The DSI averages of the five different infection classes were 0, 1.25, 2.05, 2.92 and 3.88, which showed the highest resistance among the classes (P < 0.5).

The Mann-Whitney U test indicated that the cultivars with no symptoms of the infection in addition to the (a) Petrus and Prenses cultivars with a high resistance against the infection were significantly different from the other groups. In addition to these cultivars, other cultivars had different reactions of resistance (b-l).

A logistic model curve demonstrating a reverse relationship between the infection progression and time is shown in Figure 2.

Detection of *PTO*

The presence of the *PTO* gene in the five different tomato cultivars classified as: (a) in infection class 0 by statistical analysis, which corresponded to the plants showing no symptoms related to the disease, was determined by PCR using the SSP17 and JCP32 (Rose et al., 2007) primers. The 963-bp fragment (Figure 3) from the resistant plants is compatible with the results of Rose et al. (2007). The *PTO* gene was also detected in eight tomato cultivars (b) classified in infection class 1. However, most of the susceptible tomato cultivars that were classified in infection classes 3 and 4 (b-m) amplified no fragment.

Hypersensitive reaction test

The 25 different *P. syringae* pv. *tomato* strains (Table 1) caused hypersensitive reactions in the Ontario 7710 control variety and in the tomato cultivars found to contain the *PTO* gene.

Hypersensitive reactions in the resistant plants were observed approximately 24 h after inoculation. The occurrence of a hypersensitive reaction in the resistant cultivars also confirmed the strains characterised by infection class 0.

Sequencing of *PTO*

Sequencing and BLAST analysis of *PTO* found in the

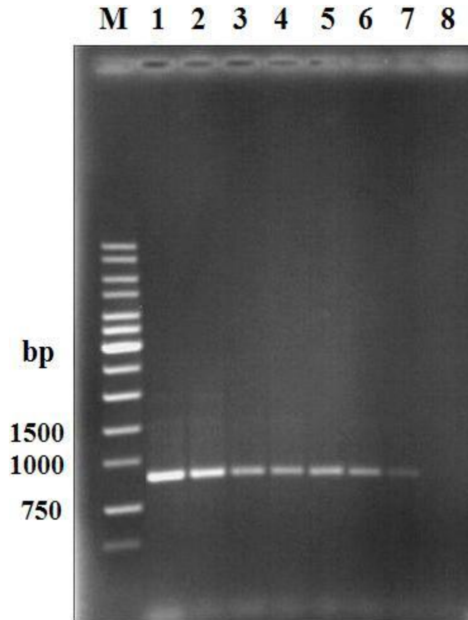

Figure 3. Amplification of the *pto* gene (963 bp). Lane M, 1-kb DNA marker; lane 1, Ontario 7710; lane 2, Atalay; lane 3, Party; lane 4, Petrus; lane 5, Piccadilly; lane 6, Prenses; lane 7, Tyty; lane 8, negative control.

Atalay, Party, Petrus, Piccadilly, Prenses and Tyty cultivars showed 94, 93, 94, 92, 95 and 94% similarity with *L. esculentum* VFNT Cherry (AF220603), respectively and 98, 98, 96, 95, 100 and 98% similarity with *L. pimpinellifolium* Rio Grande 76R (AF220602), respectively.

DISCUSSION

The resistance reaction levels to strains of the bacterial speck pathogen *P. syringae* pv. *tomato* of 93 different tomato cultivars preferred by tomato producers in greenhouse production in the West Mediterranean region of Turkey were determined. Most of the tomato seeds for greenhouse production in Turkey are imported from Israel, Holland, Spain, the USA, Germany and France; although many commercial Turkish seed companies also produce tomato seeds in Turkey. In this case, farmers from different countries, including Turkey, use the same or similar tomato cultivars used in this study for greenhouse tomato production. The disease severity indexes (DSI) varied between zero and four among the different cultivars. The Yeniceri variety was classified as infection class 0, which was referred to as a resistant class. The cultivar did not have the *PTO* gene, but the other four cultivars classified as infection class 0 had the resistance gene. On the other hand, the Prenses and Petrus cultivars, classified as infection class 1 with small necrosis-shaped specks and halo formations (Table 2), had the *PTO* gene. The Selin, Vitamin, Deniz, Atabey RN, İkram, and Polyana cultivars were classified as infection class 1, but these cultivars did not have the *PTO* gene, which may be explained by the involvement of a secondary defence mechanism which has not been identified yet. Disease progress in the tomato cultivars was also noticeably different. Kozik (2002) reported that several necrosis-shaped specks were observed in tomato cultivars with the *PTO* gene. Differences in the level of resistance to the disease, disease progression over time, types of necrosis and halo formations occurring on all of the cultivars suggest that the cultivars may have different defence mechanisms.

Common morphological features among the seedlings of the resistant cultivars were not observed. The only observed common feature of the cultivars was their fruiting structure, which was a cluster. Tomatoes from these types of cultivars are harvested by cutting the entire tomato cluster, with four to six small fruits in each cluster. Therefore, the resistant cultivars may have several common sequences coding for the fruit structure in the tomato plant.

Pitblado and Kerr (1980) reported the bacterial speck disease resistance levels of 40 tomato cultivars in Canada, and they also reported the disease resistance of wild tomato relatives, such as *Lycopersicon hirsutum* var. *glabratum* and *L. pimpinellifolium*, and several cultivars belonging to the same line, such as Ontario 7710, Ontario 7611, Ontario 782 and Farthest North.

Kozik (2002) determined the resistance levels of 17 cultivars including Ontario 7710 against bacterial speck disease. In addition to the Ontario 7710 variety, a wild variety, *L. hirsutum*, is also resistant to the disease. The resistance is due to the hybridisation of a sensitive variety, A100, with Ontario 7710.

Shenge et al. (2007) evaluated the resistance reactions to bacterial spot and bacterial speck diseases for several local tomato cultivars in Tanzania, and they found that the Tonquay and BSS 436 cultivars were resistant to bacterial speck disease and sensitive to bacterial spot disease. In the present study, the resistant cultivars were also found to be sensitive to different domestic and foreign strains of *Clavibacter michiganensis* subsp. *michiganensis* and *Xanthomonas axonopodis* pv. *vesicatoria*, which are causal agents of tomato bacterial canker and leaf spot disease, respectively.

Rose et al. (2007) amplified a 963-bp fragment of the *PTO* gene from tomato plant DNA by PCR using the SSP17 and JCP32 primers. The results from the present study (Figure 1) are in agreement with those reported by Rose et al. (2007). The *PTO* sequence was found in the Atalay, Party, Petrus, Piccadilly, Prenses and Tyty cultivars and showed 94, 93, 94, 92, 95 and 94% similarity with the *PTO* sequence of *L. esculentum* VFNT Cherry (AF220603), respectively, and 98, 98, 96, 95, 100

Table 2. Number of plants in infection classes and disease severity indexes of tested tomato cultivars.

Variety	Number of plants in infection class					DSI	Variety	Number of plants in infection class					DSI	Variety	Number of plants in infection class					DSI
	0	1	2	3	4			0	1	2	3	4			0	1	2	3	4	
Ontario 7710	5					0	Yağmur			1	4		2.8^{h-i-j}	Hadino				1	4	3.8^{l}
Atalay	5					0	4413				5		3^{i-j}	Hamlet				1	4	3.8^{l}
Party	5					0	Alsancak RN				5		3^{i-j}	Han				1	4	3.8^{l}
Piccadilly	5					0	Altess				5		3^{i-j}	M-09				1	4	3.8^{l}
Tyty	5					0	Aspava				5		3^{i-j}	Milas				1	4	3.8^{l}
Yeniçeri	5					0	Bobo				5		3^{i-j}	Newton				1	4	3.8^{l}
Petrus		5				1b	Cibellia/7314				5		3^{i-j}	Nun 3080				1	4	3.8^{l}
Prenses		5				1b	Crisol				5		3^{i-j}	Redo				1	4	3.8^{l}
Selin		3	2			1.4^{b-c}	Kardelen				5		3^{i-j}	Yıldız				1	4	3.8^{l}
Vitamin		3	2			1.4^{b-c}	Klass				5		3^{i-j}	2242 Lavinia					5	4^{l}
Deniz		2	3			$1.^{6c-d}$	Mondi				5		3^{i-j}	2246					5	4^{l}
Atabey RN		1	4			1.8^{c-d-e}	Pascal				5		3^{i-j}	Alex					5	4^{l}
İkram		1	4			1.8^{c-d-e}	Pelin				5		3^{i-j}	Aristo					5	4^{l}
Polyana		1	4			1.8^{c-d-e}	Töre				5		3^{i-j}	Bali					5	4^{l}
Grendal			5			2^{d-e-f}	Tuana				5		3^{i-j}	Bolkar					5	4^{l}
Kıymet			4	1		2.2^{e-f-g}	Yeni Talya				5		3^{i-j}	Diamond					5	4^{l}
M-28			4	1		2.2^{e-f-g}	Zorro				5		3^{i-j}	Durinta					5	4^{l}
Orient			4	1		2.2^{e-f-g}	Alsancak				4	1	3.2^{j-k}	Export					5	4^{l}
Pegasus/At-03			4	1		2.2^{e-f-g}	Bandita				4	1	3.2^{j-k}	İnci					5	4^{l}
Victory			4	1		2.2^{e-f-g}	Helena				4	1	3.2^{j-k}	İzmir					5	4^{l}
Malike			3	2		2.4^{f-g-h}	Karakaya RN				4	1	3.2^{j-k}	M-79					5	4^{l}
Beril/7314			2	3		2.6^{g-h-i}	Süvari				4	1	3.2^{j-k}	M-89					5	4^{l}
Ilgın			2	3		2.6^{g-h-i}	Allegro				2	3	3.6^{k-l}	Manyla					5	4^{l}
Klasdo			2	3		2.6^{g-h-i}	Clotilde				2	3	3.6^{k-l}	Maya					5	4^{l}
Melis			2	3		2.6^{g-h-i}	Depar				2	3	3.6^{k-l}	Mirella					5	4^{l}
Multy			2	3		2.6^{g-h-i}	İdalyo				2	3	3.6^{k-l}	Nakış					5	4^{l}
575			1	4		2.8^{h-i-j}	Linda				2	3	3.6^{k-l}	Posseidon					5	4^{l}
Alexey			1	4		2.8^{h-i-j}	Titanic				2	3	3.6^{k-l}	Sarafin					5	4^{l}
Alida			1	4		2.8^{h-i-j}	2247				1	4	3.8^{l}	Vo-5656					5	4^{l}
Gökçe/191			1	4		2.8^{h-i-j}	Astona				1	4	3.8^{l}	Vuslat					5	4^{l}
M-16			1	4		2.8^{h-i-j}	Astona RN				1	4	3.8^{l}							
Sümela			1	4		2.8^{h-i-j}	Belen				1	4	3.8^{l}							

and 98% similarity with the *PTO* sequence of *L. pimpinellifolium* Rio Grande 76R (AF220602), respectively. The variation in the *PTO* sequences detected in this study may be explained by the presence of natural variation, which is already present in wild-type tomato plants, as reported by Rose et al. (2005).

To evaluate whether a plant is resistant, it is essential that the plant has the resistance gene or genes and that the pathogen has an avirulence gene corresponding to the resistance gene. Otherwise, resistance mechanisms will not be induced, regardless of the number of resistance genes that the plant carries. The resistance of tomato cultivars carrying the *PTO* gene to bacterial speck disease depends on the plant recognising *P. syringae* pv. *tomato*. The recognition can only occur when the pathogen has the avirulence *avrPto1* gene. The results regarding the amplification of *avrPto1* sequences from the *P. syringae* pv. *tomato* strains in this study agree with results previously reported by Chang et al. (2001). All the *P. syringae* pv. *tomato* strains in this study have the *avrPto1* gene. *P. syringae* pv. *tomato* strains with the *avrPto1* gene are classified as race 0, and *P. syringae* pv. *tomato* strains without the *avrPto1* are classified as race 1. The existence of *P. syringae* pv. *tomato* race 1 strains was determined by Lawton and MacNeill (1986) in Canada and Bogatsevska et al. (1989) in Bulgaria. Abak et al. (1990) reported that all the *P. syringae* pv. *tomato* strains isolated from diseased plant samples in Turkey were classified as race 0. Thus, the identification of the strains used in this study as race 0 further confirms the strains in Turkey to be race 0.

Stockinger and Walling (1994) isolated a *PTO*4 gene controlling race 1 and a *PTO3* gene controlling race 0 in *L. hirsutum* var. *glabratum*. Transferring the combination of resistant genes, *PTO*3 and *PTO*4, to tomato cultivars would thus be advantageous for resistance to bacterial speck.

The controlled conditions created in greenhouse production are highly conducive to plant diseases and pests. While plant production continuously increases, economic losses related to pests and diseases caused by fungi, viruses and bacteria are also increasing. Bacterial diseases have been given preference over other diseases due to the high speed of disease spread, product losses and decrease in product quality. As most of the greenhouse products grown in Turkey are intended for export, the quality losses due to bacterial diseases will be economically harmful leading to important yield losses.

Expansion of the gene pool used in the development of resistant cultivars together with technological advances will accelerate the gene flow from wild tomato relatives to new cultivars, which will allow new cultivars to be developed to control bacterial speck disease in the future. Considering that the products grown in the western Mediterranean region in Turkey are generally exported to the world market, it is clear that the products are important to Turkey from the point of view of greenhouse tomato and tomato seedling production in this region.

Resistant cultivars are one of the most important tools for the integrated disease management of bacterial speck disease, and these cultivars are deficient in Turkey. The resistant cultivars against bacterial speck disease determined in this study have an important role in fulfilling this deficiency. The cultivars tested in this study are not only important for agriculture in the west Mediterranean region but also for agriculture in Turkey, where most of the companies producing commercial tomato seedlings in Turkey are located in Antalya.

In addition to determining the resistant cultivars, the disease resistance levels in the tomato cultivars commonly grown in this region were determined. The determination of alternative cultivars can be used for protection against the disease. Bacterial speck disease, which cannot be effectively controlled by chemical treatment, can be controlled, or at least the product loss caused by the disease can be reduced, by using resistant tomato cultivars.

Many different commercial copper mixes and antibiotics have been used as bactericides to control Bacterial Speck. European Good Agricultural Practice (EUREGAP) protocols only allow tolerable residue limits of pesticides on vegetables. Increased awareness of environmental and health risk problems concerning chemical pesticides has led to a search for resistant cultivars to be used for the management of plant diseases. The use of resistant cultivars may be the most effective approach for disease management because of the sustainability and eco-friendly nature of this technique.

Consequently, in this study, the determination of the *PTO* gene in some tomato cultivars in Turkey will help to develop new resistant cultivars by transferring the gene from resistant cultivars to other cultivars by classical breeding or modern biotechnological methods.

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