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Determination of the Resistance Reactions of Some Tomato Cultivars against Bacterial Speck Disease

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ABSRACT

Pseudomonas syringae pv. *tomato* is the causative agent of the bacterial speck disease of tomato (*Lycopersicon lycopersicum*), a disease that occurs worldwide and causes severe reduction in fruit yield and quality. Disease resistance conferred by the *pto* gene, encodes a serine–threonine protein kinase, is one of the first R-genes to be cloned and sequenced. In this research, the resistance reactions of 50 different tomato cultivars which are grown commonly in Central Anatolia against *P. s.* pv. *tomato* causal agent of bacterial speck disease were determined. Six-week-old plants were inoculated by spraying of *P. s.* pv. *tomato* strains, YA-1 and YA-2 (10^8 CFU ml^{-1}), until leaf surfaces were uniformly wet. After inoculation, the plants were incubated at 25±1 °C in 65-75 % relative humidity with a 12 h photoperiod and the disease progress occurred on the seedling leaves by *P. s.* pv. *tomato* was followed by counting the dark brown-black leaf necroses in 21st days after inoculation of the seedlings. Each experiment was performed at least three times and control plants were sprayed with sterile distilled water. The results of resistance reactions on plants were evaluated according to Chambers and Merriman scale. The resistance levels of the cultivars were statistically determined by using ANOVA variance analyze and Duncan multiple range tests. Presence of *pto* gene in the tomato cultivars was verified by using the primers SSP17 and JCP32 (a 963 bp DNA fragment) by PCR and the gene was determined in 15 different tomato cultivars. The determination of the *pto* gene in some tomato cultivars may help to develop new resistant cultivars and to reduce of disease severity.

1. Introduction

Tomato (*Lycopersicum esculentum* Mill.) is one of the most important greenhouse and field-grown vegetables in Turkey, with a production of 10.745.600 tons in 2009 (FAO 2011). Tomato is an important fresh fruit in Turkey's export, and ranks third after citrus and stone fruits with a 14% share (Yucel et al. 2008).

The bacterial pathogens, *Clavibacter michiganensis* subsp. *michiganensis*, *Xanthomonas axonopodis* pv. *vesicatoria*, *Ralstonia solanacearum*, *Pseudomonas syringae* pv. *tomato*, *P. corrugata*, *P. viridiflava*, *P. cichorii*, *P. mediterranea*, *P. fluorescens*, *Pectobacterium carotovorum* subsp. *carotovorum*, *Dickeya chrysanthemi*, cause important yield loses in greenhouses and field grown tomatoes in Turkey (Demir 1990; Cınar and Aysan 1995; Aysan 2001; Ustun and Saygili 2001; Sahin et al. 2002; Basim et al. 2004; Sahin et al. 2004; Saygili et al. 2004; Basim et al. 2005; Sahin et al. 2005; Aysan et al. 2006).

Bacterial speck caused by *Pseudomonas syringae* pv. *tomato* (Okabe) is an economically important disease that presents a serious problem to tomato growers in many areas of the world (Pitblado and Kerr 1980). The disease attacks stems, buds, flowers, leaves and fruits, causing reduction of yield and sometimes leading to death of tomatoes plants (Louws et al. 2001; Preston Louws, 2000). Coronatine which is produced by the pathogen, causes chlorotic halos around the specks (Young et al. 1986). In favorable conditions, bacterial speck disease can fastly spread in the field in a short time

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(McCarter et al. 1983) and causes important economic losses in tomato seedlings and mature plants (Psallidas 1988).

The pathogen was determined first time by Saygili (1975) in the Aegean region and Cinar (1977) in the east Mediterranean region. In Turkey, severe outbreaks of bacterial speck on tomatoes have been reported during the spring months of 2002 and 2003 in the western Mediterranean region of Turkey, causing lower fruit productivity and quality of cultivars (Basim 2004). In Eastern Anatolia region, crop losses were determined about 20% as a result of the survey (Sahin, 2001). This is related to the susceptible tomato cultivars grown and to the lack of effective chemical control of the disease.

Its control is based on application of copper compounds which are not enough effective and highly depend on cultivar, environmental conditions and as well as inoculum potential. Therefore breeding of tomatoes for genetic resistance against bacterial speck seems to be very important and promising perspective. However, progress in development new highly productive and resistant cultivars depends on the availability of an effective technique to identify resistant germplasm and progeny at the seedling growth stage (Kozik and Sobiczewski 2008). Plants have evolved complex mechanisms to recognize, and defend themselves against many potential pathogens (Lamb et al. 1989; Lamb 1994). In many plant-pathogen interactions, recognition of pathogen is mediated by a plant disease resistance (R) gene that responds to the presence of corresponding avirulence (*avr*) gene in the pathogen (Keen et al. 1993; Staskawicz et al. 1995).

Resistance to the disease has been reported previously (Gitaitis et al. 1982, Pilovsky and Zutra, 1982, Pitblado and Kerr, 1980; Yunis et al., 1980). It has also been found that a single dominant (Pitblado and Kerr 1980, Pilovsky and Zutra 1982) and incompletely dominant gene *pto* (Carland and Staskawicz 1993; Kozik 2002) is responsible for resistance to bacterial speck. An R gene to the pathogen, *Pto*, was originally discovered in a wild-type species of tomato *Lycopersicon pimpinellifolium*, and has subsequently been introgressed into many cultivated tomato (*L. esculentum*) cultivars by backcrossing.

The *Pto* locus confers resistance specifically to *P. s.* pv. *tomato* strains that express the avirulence gene *avrPto* (Ronald et al. 1992; Martin et al. 1993). *Pto* is located in a 400-kb region on the fifth chromosome of the tomato genome (Martin et al. 1994). *AvrPto* was the first Avr protein for which a corresponding plant resistance gene was identified the *pto* gene of tomato, which encodes a protein kinase (Martin et al. 1993). 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). It was found that resistance of *Lycopersicon esculentum* 'Ontario 7710' to bacterial speck is determined by a single dominant gene (Pitblado and MacNeill, 1983).

The objective of this study was to determine the *pto* gene, confers resistance against *P. syringae* pv. *tomato*, in some tomato cultivars. This is the first study on determining susceptibility levels of tomato cultivars to bacterial speck disease in Central Anatolia region of Turkey.

2. Material and Methods

2.1. Plant materials and growing conditions

The tomato cultivars used in this study were obtained from commercial companies producing tomato seed or seedling in Konya and Antalya provinces. The study was carried out on a total of 50 tomato cultivars, grown in Turkey (Aynaz, Çiğdem, Gözde, Gülhan, Gümrük, Diamond, Ebia, Erdem, Falcon, Hamlet, Impala, Kardelen, Kokpit, Konya, Kutlu, Marmara, Mete, Natura, Otranta, Oturak, Reyhan, Rio Grande, Super Standart, Şimşek, Tueza, Verdi, 144, 4F, M-16, T2, T3, T6, T7, TY9, TY10, TY13, OD1101, OD1102, OD1103, OD1104, OD1105, OD1106, OD1107, OD1108, OD1109, OD1110, OD1111, OD1112, OD1113 and H-2274, and in addition as positive control the Ontario 7710 cultivar, which is resistant to *P. s.* pv. *tomato*. Twenty saplings from each cultivar (20 plants for each *P. s.* pv. *tomato* strain) were transplanted into plastic pots of 20 cm diam filled with 3 kg of soil, and they were grown for 20 d at 25 ± 2°C, 65–75% relative humidity (RH) and under 12.000-14.000 Lux from tungsten-filament lamps for a 16-h photoperiod. After transplantation, the plants were fertilized once a week (each pot) with 3 g ammonium sulfate, 3 g diammonium phosphate, 3 g potassium sulfate, and 10 ml of a liquid fertilizer having 0.05% Mn, Cu, Zn, B, and Mo (Kacar and Katkat, 1999). The soil used in the experiments is characterized by 1.9% total soil organic matter, 0.08% total salts, 63% soil saturation percentage, 8.1% lime (CaCO3) with soil pH of 7.8 in distilled water (1.5 v/v). The plots were trickle irrigated as needed. In addition, sulfur dust (Thiovit, 80% microlized sulfur, Syngenta) was applied once (4 g l^{-1} water) for powdery mildew and mite control.

2.1. Bacterial strains

Sources of the reference strains of *Pseudomonas syringae* pv. *tomato* tested in this study were given Table 1. The strains, YA1 and YA2, were found to be the most virulent among other tested in our previous studies. The bacterial strains used in this study were stored in 30% glycerol in nutrient broth (Merck, Germany) medium at 30°C. Before inoculation of tomato plants, the ability of YA1 and YA2 strains to induce hypersensitivity reaction (HR) on tobacco plants (*Nicotiana tobacum* cv. Benthamiana) were checked according to the method of Klement (1963) and these strains were used in all tests. Negative and positive control plants were sprayed with sterile distilled water and reference strains. HR tests were fulfilled by injecting suspensions of test bacteria using a 0.46-mm-diam (26-gauge) hypodermic syringae at a concentration of 10^8 CFU ml^{-1} or water (control) into the 8 week-old tobacco plants.

2.3. Detection of the avrPto1 gene

Aliquots (100 µl) of the all stock bacterial strains were plated in triplicate onto KB agar plates and incubated for 48 hr, washed individually three times, each time with 1 ml of SDW. The combined washes from each plate (total volume 3 ml) were used for PCR analysis. The bacterial pellets obtained by centrifugation at 10,000 g for 5 min were suspended in 800 µl of extraction buffer (100 mM Tris-HCl pH 8.0, 0.5 M NaCl, 50 mM EDTA, 1% SDS) and incubated at 65ºC for 30 min. Then, 400 µl of potassium acetate 5 M was added in each sample and the mixture was incubated on ice for 20 min. After centrifugation at 10,000 g for 10 min, 40 mg ml^{-1} of RNase at 37ºC was added, followed a phenol/chloroform/isoamyl alchool (25:24:1) extraction, and finally an ethanol precipitation. The obtained pellet of each sample was suspended in Tris-EDTA (TE) buffer (Goncalves and Rosato 2002; Nunes et al. 2008). In this study, all PCR amplifications were carried out in 0,5 ml thin wall PCR tubes, in a final volume of 25 µl and Mastercycler, Eppendorph thermocycler. Reaction mixtures contained the following ingradients at the given final concentrations: target DNA 2 µl, PCR Master Mix (0.05 unit / µl *Taq* DNA, 4 mM $MgCl_2$, 0,4 mM dATP, 0,4 mM dCTP, 0,4 mM dGTP and 0,4 mM dTTP) 12,5 µl, Forward primer 2 µl, Revers primer 2 µl, sterile distilled water 6,5 µl.

The primers, avrpto1F 5'-CCATGG-GAAATATATGTGTCGGCGG-3' and avrpto1R 5'-CTGGAGTCATTGCCAGTTACGGTACGG-3', were used to amplify the *avrPto1* gene by PCR (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). Tris-acetate-EDTA (TAE) was used in the electrophoresis process and in preparation of the agarose gel. The PCR products were electrophoresed at 80 V in a 1% agarose gel. After electrophoresis, the PCR products were stained with 0.5 µg/ml ethidium bromide for 15 min and were imaged by transilluminator.

Table 1
List of reference strains of *Pseudomonas syringae* pv. *tomato*

Strain No	Source	Country
PST2	Prof. Dr. Hatice Ozaktan (Ege University)	Turkey
YA1	Prof. Dr. Yesim Aysan (Cukurova University)	Turkey
YA2	Prof. Dr. Yesim Aysan (Cukurova University)	Turkey
RK351	Assoc.Prof. Dr. Recep Kotan (Ataturk University)	Turkey
PstKkkb28	Assist. Prof. Dr. Kubilay K. Bastas (Selcuk Univ.)	Turkey
PstKk324	Assist. Prof. Dr. Kubilay K. Bastas (Selcuk Univ.)	Turkey
PSTb25	West Mediterranean Agricultural Research Inst.	Turkey
NCPPB3160	National Collection of Plant Pathogenic Bacteria	England

2.4. Detection of the pto gene

DNA was isolated using a CTAB method (Doyle and Doyle 1987) from 2 g of leaf tissue collected from each plant. The DNA was resuspended in 300-1000 ml TE to a final concentration of 100 ng μl^{-1} with a Biophometer Plus (Eppendorph, Germany). DNA of reference strain, NCPPB3160, was used as the positive control and sterile distilled water as negative control. Alleles of *pto* from each species were amplified by PCR using the primers SSP17 (GGTCACCATGGGAAGCAAGTATTC) and JCP32 (GGCTCTAGATTAAATAACAGACTCTT-GGAG). 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 and 1000 bp marker (Fermantas; 100bp Plus DNALadder SM1153) were electrophoresed in 1,5% agarose gel in 1×TBE buffer and analyzed by transilluminator (Vilber Lourmat) and Quantity One Imaging and Analysis PDQest 2-D Gel Analysis Software, User Guide for Version 4.1 Windows after ethidium bromide staining (Sambrook et al. 1989).

2.5. Inoculation of the pathogen

Bacterial suspensions prepared from growing colonies on King's B medium (KB) at 25-27°C and were diluted in sterile distilled water (SDW) to give an absorbance of 0.15 at 660 nm. From viable plate counts this represented 10^8 CFU ml^{-1}. Inoculum was maintained on ice and was used for plant inoculation within 2 h. of dilution. Six-week-old plants were inoculated by spraying of *P. s.* pv. *tomato* YA-1 and YA-2 strains until leaf surfaces were uniformly wet. Control plants were sprayed with sterile distilled water. After inoculation the plants were kept under a plastic cover for 3 days to obtain a relative humidity of 100%. Afterwards the covers were taken out and the humidity fluctuated between 60 and 75%. The temperature set points were 27ºC by day and 21ºC by night.

2.6. Determination of disease severity (DS)

The dark brown lesions of bacterial speck on leaves per plant were counted in 21st day after inoculation of the seedlings and plants were classified using Chambers and Merriman scale (1975) as follows: 0 = no lesions, 1 = 1-10 lesions per plant, 2 = 11-20 lesions, 3 = 21-40 lesions and 4 = more than 40 lesions per plant. Data were

collected as means overall leaves on the plants within each cultivar in a completely randomised design. The DS value was calculated from the sum of the data classified by the Chambers and Merriman scale obtained from six replicates divided by the replication number for each cultivar (Eenink 1981). According to the scale, data were classified in 5 resistance class: Resistant; R, Moderately Resistant; MR, Moderately Susceptible; MS, Susceptible; S and Highly Susceptible; HS.

2.7. Re-isolation of P. s. pv. *tomato from the plants*

After ratings were taken from the inoculated plants, diseased and symptomless plants were randomly selected and sampled at the point of inoculation. The samples were surface disinfested by immersion in 0.5% sodium hypochlorite for 3 min, cut into small segments, and soaked 1 h in SDW. The liquid was then streaked on KB media. The isolates were identified using the tests described by Lelliott and Stead (1987) and Schaad et al. (2001): Gram reaction, fluorescence on KB medium, oxidative-fermentative metabolism of glucose (O/F test), levan formation, oxidase activity, potato rot, arginine-dehydrolase activity, tobacco hypersensitivity, starch hydrolysis, gelatin liquefaction aesculin hydrolysis catalase activity NH_3 production, nitrate reduction, acid production from sorbitol, mannitol, inositol, erythritol and L-lactate, and the ice nucleation test.

In PCR assay, specific oligonucleotid primers (Pst1; 5'GGCGCTCCCTCGCACTT3' and Pst2; 5'GGTATTGGCGGGGGTGC'3) were used primers specific for detection of *Pseudomonas syringae* where the expected PCR products are 650-bp. PCR primers used in all experiments were synthesized by Thermo-Fermentas, Life Technologies, USA. The amplification conditions were: initial denaturation at 93°C for 2 min, followed by 37 cycles of denaturation at 93°C for 2 min, annealing at 67°C for 1 min, and extension at 72°C for 2 min. Analysis of PCR products was performed in 1,5% agarose gel (Bereswill et al. 1994; Milijasevic et al. 2009).

2.8. Data analysis

Data were subjected to analysis of variance, and differences between means were compared by MINITAB ver. 14 (State College, PA, USA) statistical program. The means (expressed as percent disease) were used to determine significant treatment differences. Data were analyzed using MSTAT software (Michigan State University, MI, USA) and the differences between treatments were determined by LSD New Multiple Range Test (MSU 1986).

3. Results

The resistance reaction levels to 2 strains of the bacterial speck pathogen *P. s.* pv. *tomato* of 50 different tomato cultivars used by tomato producers in greenhouse and field production in the Central Anatolia region of Turkey were determined. The remarkable results were obtained between presence/absence of *pto* gene and disease severity levels.

3.1. Detection of Pto and avrPto1

A 963-bp DNA fragment was obtained by PCR using the specific SSP17 and JCP32 primers in 15 tomato cultivars (Çiğdem, Gözde, Gülhan, Ebia, Impala, Konya, Kutlu, Natura, 144, T3, T6, OD1101, OD1104, OD1105 and OD1111) and the positive control cv. Ontario 7710 (Figure 1 and Table 1).

The presence of the *avrPto1* was searched in *P. s.* pv. *tomato* strains, YA-1, YA-2, PstKkkb28, NCPPB3160, and 495-bp DNA fragment was obtained by PCR using the specific *avrPto1* primers (Figure 1).

3.2. Evaluation of disease severity

Data collected from tomato cultivars in 21st day after inoculation shown statistically significant differences ($p<0.01$) with regard to the disease severites (Table 2). According to the Chambers and Merriman scale, resistance class R included the highest resistance level among the various tomato cultivars, and class 4 contained the cultivars that had a high severity of infection.

Only positive control cv. Ontario 7710 did not show any disease symptoms and placed in the class 0 as resistant. Fifteen different tomato cultivars containing *pto* gene were classified in MR (Kutlu, Gülhan, 144, OD1101, OD1104, OD1105, OD1111, Çiğdem, Impala) and MS (T3, T6, Konya, Ebia, Natura, Gözde) with small necrosis-shaped specks and halo formations. Some cultivars (Aynaz, Erdem, OD1108, OD1109, OD1110, H-2274, Diamond, Falcon, Oturak, Super Standart, Gümrük) were classified in susceptibility classes (S and MS) and they did not have *pto* gene (Table 2).

3.3. Re-isolation of the bacterial strains

The results of conventional bacteriological identification tests are given in Table 3. All strains were Gram-negative, fluorescent on KB medium, and metabolized glucose oxidatively. The investigated strains formed the levan type of colonies on NSA, were oxidase- and arginine-dehydrolase-negative and potato rot-negative. Other biochemical and physiological characteristics of all strains were as follows: catalase-positive; gelatin and aesculin hydrolysis-positive; starch hydrolysis-negative; NH_3 production-positive; nitrate reduction-negative; and acid production from sorbitol, mannitol, and inositol-positive. Based on these characteristics, the isolated strains were identified as *P. s.* pv. *tomato*.

The identity of strains isolated from diseased tomato plants was confirmed using the PCR protocol and primer set designed by Bereswill et al. (1994). PCR products of expected size (650-bp) specific for *P. s.* pv. *tomato* were amplified from all investigated strains previously identified by conventional methods as *P. s.* pv. *tomato*, as well as from the reference strain NCPPB3160.

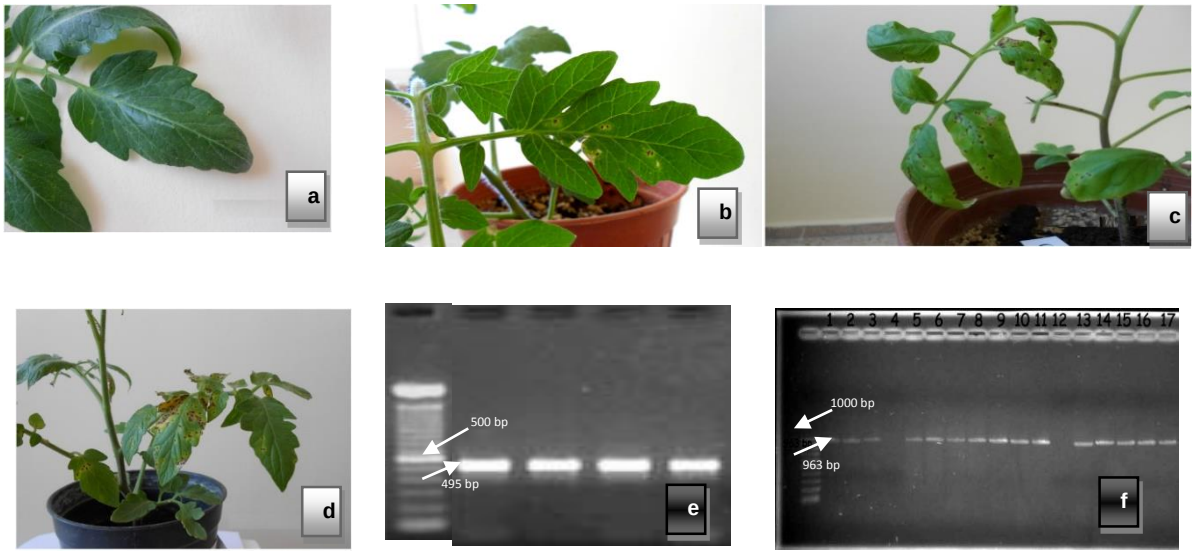

Figure 1
Bacterial speck disease severities on tomato cultivars and detection of pto and avrpto1 genes by PCR assays: bacterial speck symptoms on a) cv. OD1111, b) cv. Gülhan, c) cv. T3, d) cv. Gözde, e) PCR amplification of the *avrPto1* gene (495 bp) of *P. syringae* pv. *tomato* strains: Lane M, 3-kb marker; lane 1, YA-1; lane 2, YA-2; lane 3, PstKkkb28; lane 4, NCPPB3160; f) PCR amplification of the *pto* gene (963-bp) using SSP17 and JCP32 primers: Lane M, 1-kb marker; Lane 1, Ebia; Lane 2, Çiğdem; Lane 3, OD1101; Lane 4, Kokpit; Lane 5, Impala; Lane 6, Natura; Lane 7, 144; Lane 8, Gülhan; Lane 9, OD1105; Lane 10, Gözde; Lane 11, T3, Lane 12, Mete; Lane 13, Konya; Lane 14, T6; Lane 15, OD1104; Lane 6, OD1111; Lane 17, Kutlu

4. Discussion

Bacterial speck disease has been a serious problem on tomatoes since it was first reported by Saygili (1975) and Cinar (1977) in Turkey (Aysan et al. 1995). Cultivar resistance is the most desirable within combat strategies to the disease. A large variation in virulence of *P. s.* pv. *tomato* isolates may affect the differences in plant-pathogen interaction and make evaluation for bacterial speck resistance difficult (Mitchell et al. 1983; Bashan et al. 1978; Kozik and Sobiczewski 2000). Although molecular markers for resistance genes against bacterial speck have been found (Martin et al. 1991; Carland and Staskawicz 1993) researchers are still searching for other alternative methods which would eliminate difficulties in determination of resistant to *P. s.* pv. *tomato* genotypes.

Pto confers disease resistance to *P. s.* pv *tomato* carrying the cognate *avrPto* gene. *P. s.* pv. *tomato* strains with the *avrPto1* gene are classified as race 0, and the strains without the *avrPto1* are classified as race 1 (Martin et al. 1993). Although there is a direct interaction between *AvrPto* and *Pto* in resistant plants (Scofield et al. 1996; Tang et al. 1996), the cellular target of *AvrPto* in susceptible plants appears to be quite distinct. In Turkey, all the *P. s.* pv. *tomato* strains isolated from diseased plant samples were identified as race 0 (Abak et al. 1990). Therefore we perefered to use the *P. s.* pv. *tomato* race 0 in our experiments.

Resistance of tomato plants to the bacterial pathogen *P. s* .pv. *tomato* race 0 is controlled by the locus Pto. Our initial goal was to determine whether an avirulence gene in *P. s.* pv. *tomato* race 0 strains was responsible for limiting disease on *pto*-containing tomato cultivars. Previous studies have shown that resistance to bacterial speck in 'Ontario' is controlled by a single dominant gene (Pilovsky and Zutra 1982) or semi-dominant gene (Carland and Staskawicz 1993; Kozik 2002). 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 are in agreement with those reported by these researchers.

In this study, fifteen different tomato cultivars containing *pto* gene were classified in MR and MS. This may be explained by the involvement of a secondary defence mechanism which has not been identified yet. Kozik (2002) reported that several necrosis-shaped specks were observed in tomato cultivars with the *pto* gene. Differences between the varieties in terms of plant resistance to disease susceptibility in the genetic structure of the plant are sometimes associated with one or a few genes (monogenic) and administer, and sometimes many genes (polygenic) is known to be controlled by (Roberts, 2002). Managed by a single gene studies of breeding for resistance, are easier to get than others, and the result is quite simple (Geiger 1989). In *L. hirsutum*

var. *glabratum*, *pto*3 gene is responsible from controlling race 0 (Stockinger and Walling 1994). This data may be used to improve new resistance genotypes to bacterial speck. Sowing or planting of disease-resistant varieties and in some cases even completely eliminated, reduced chemical applications is an advantage (Hammond-Kosack and Jones 1996).

Table 2

Agronomic characteristics and breeding type of tomato plants used in the experiments, presence of *pto* gene in the cultivars, disease severity index caused by *P. s.* pv. *tomato* strains (YA-1 and YA-2) and resistance classes for the disease

No	Cultivar name	Plant Characteristics		Breeding	Presence of *pto* gene	DSI with YA-1	Resistance Class for YA-1	DSI with YA-2	Resistance Class for YA-2
		Fruit shape	Cultivation						
1	Kutlu	G	D	F	+	17±1 hıjkl	MS	4.33±1.155 qr	MR
2	Gülhan	G	D	F	+	7±1 stu	MR	3.33±0.577 qr	MR
3	Aynaz	G	ID	F	-	29.33±2.082 b	S	23±2 c	S
4	Erdem	SOG	D	F	-	23±2.646 de	S	20.67±1.528 cde	S
5	Mete	SOG	ID	G	-	18±1 ghıjk	MS	11.33±1.528 klmn	MS
6	TY13	SOG	ID	G	-	23.67±1.528 cde	S	22±2 cd	S
7	TY10	SOG	ID	G	-	12±2 nopq	MS	12.67±1.155 jklmn	MS
8	T3	SOG	ID	G	+	13.33±1.528 lmnop	MS	15.67±2.082 ghıj	MS
9	TY9	B	ID	G	-	23.33±3.055 de	S	20±2 cdef	MS
10	T6	SOG	ID	G	+	14±1 lmno	MS	51±1 a	HS
11	OD1106	SOG	D	F	-	5.33±1.528 uv	MR	5.67±1.528 pqr	MR
12	OD1112	SOG	D	F	-	13±1 mnop	MS	5.67±1.528 pqr	MR
13	OD1105	SOG	D	F	+	9.67±0.577 pqrst	MR	2.33±1.155 r	MR
14	OD1111	SOG	D	F	+	3±1 v	MR	5±1 qr	MR
15	OD1101	SOG	D	F	+	5±1 uv	MR	12.33±1.155 jklm	MS
16	OD1102	SOG	D	F	-	18.33±1.528 ghıj	MS	12.33±1.528 jklm	MS
17	OD1103	SOG	D	F	-	8.33±0.577 qrstu	MR	6.67±1.528 opq	MR
18	OD1104	SOG	D	F	+	5±1 uv	MR	2.33±0.577 r	MR
19	OD1107	SOG	D	F	-	12±2 nopq	MS	6.33±0.577 pq	MR
20	OD1108	SOG	D	F	-	34.67±2.309 a	S	33±1 b	S
21	OD1109	SOG	D	F	-	37.33±2.517 a	S	16.67±1.528 fghı	MS
22	OD1110	SOG	D	F	-	21±1 efg	S	14.33±2.082 hıjk	MS
23	OD1113	SOG	D	F	-	16±1 ıjklm	MS	9±1.732 nop	MR
24	H-2274	SLG	D	F	+	30±1 b	S	31.33±1.155 b	S
25	Diamond	G	ID	F-G	-	22.67±7.767 ef	S	12.67±1.528 jklmn	MS
26	M-16	G	ID	G	-	13.33±2.082 lmnop	MS	4.33±0.577 qr	MR
27	Rio Grande	G	D	F	-	21.67±2.517 cde	MS	34.67±1.155 b	S
28	Tueza	G	ID	G	-	11.67±1.528 nopqr	MS	22.67±1.528 cd	S
29	Falcon	G	D	F-G	-	27.33±2.517 bc	S	16±2.646 ghıj	MS
30	Konya	G	D	F	+	16.33±0.577 ıjklm	MS	11.33±1.155 klmn	MS
31	Kardelen	G	ID	F	-	6±1 tuv	MR	13±1 ıjklm	MS
32	Marmara	SO	D	F	-	7.33±1.528 stu	MR	10.33±1.155 lmno	MS
33	Oturak	G	D	F	-	30.33±0.577 b	S	21±1 cd	MS
34	Ebia	G	D	F	+	13.67±0.577 lmno	MS	15±1hıjk	MS
35	Hamlet	G	ID	G	-	5.33±0.577 uv	MR	4.67±1.528 qr	MR
36	Reyhan	G	ID	G	-	13±1 mnop	MS	13.33±0.577 hıjkl	MS
37	Çiğdem	G	ID	G	+	18.67±3.055 ghıj	MS	15.33±2.309 ghıj	MS
38	Verdi	G	ID	G	-	16.33±1.528 ıjklm	MS	14.67±1.155 hıjk	MS
39	Natura	SOG	ID	F	+	14±2 lmno	MS	15.67±2.082 ghıj	MS
40	Otranta	SOG	D	F	-	14.33±2.082 klmno	MS	15.33±2.082 ghıj	MS
41	Super Standart	G	D	F	-	26.67±1.528 bcd	S	22.67±1.528 cd	S
42	Gözde	G	D	F	+	19±2 fghı	MS	16±2 ghıj	MS
43	144	G	ID	F	+	8±1 rstu	MR	4.33±1.155 qr	MR
44	Impala	SOG	ID	F	+	5.67±1.528 uv	MR	9.33±1.155 mnop	MR
45	T-7	B	ID	G	-	15±1 jklmn	MS	13.33±1.528hıjkl	MS
46	Şimşek	SOG	ID	G	-	18.33±1.155 ghıj	MS	17±2 efgh	MS
47	T-2	B	ID	G	-	20.33±1.528 efgh	MS	23±2 c	S
48	Gümrük	G	ID	G	-	26.67±3.055 bcd	S	11.33±3.512 klmn	MS
49	Kokpit	G	ID	G	-	15±1.732 jklmn	MS	16±2.646 ghıj	MS
50	4F	B	ID	G	-	10.67±2.517 opqrs	MS	19±3.606 defg	MS
						16.133 A		14.780 B	

G; Globe, SOG; Slightly Oval Globe, SLG; Slightly Long Globe, SO; Slightly Oval, B; Beef, D; Determinant, ID;Indeterminant, F; Field, G; Greenhouse, F-G; Field and Greenhouse, DSI; disease severity index, R: Resistant; MR: Moderately Resistant, MS: Moderately Susceptible, S: Susceptible, HS: Highly Susceptible

Data in this study revealed that plants that carry resistant gene *pto* to bacterial speck can be found in all of the tested populations, but genetic backgrounds of the families were different and depended on the homo/heterozygous status of resistant gene *pto*. Generally, if backcross breeding is to be successful, the genotype of the recurrent parent must be recovered in its essential plant and fruit features. The results also revealed that backcross pedigree programs coupled with a particularly high intensity of selection for bacterial speck resistance and the type of recurrent parent made variation among methods insignificant.

Table 3
Biochemical, physiological and PCR tests to identification of re-isolated *P. s.* pv. *tomoto* strains from tomato plants

Tests	Re-isolated strain YA1	Re-isolated strain YA2	Reference strain NCPPB3160	*P. s.* pv. *syringae*
Gram reaction	-	-	-	-
Oxidative/Fermentative reaction (O/F)	O	O	O	O
Fluorescens pigment on KB	+	+	+	+
Levan type colonies on NSA	+	+	+	+
Oxidase	-	-	-	-
Arginine dehydrolase	-	-	-	-
Pectolytic activity on potato	-	-	-	-
Catalase	+	+	+	ND
Gelatin hydrolisis	+	+	+	+
Aesculin hydrolisis	+	+	+	+
Starch hydrolysis	-	-	-	ND
NH_3 production	+	+	+	ND
Nitrate reduction	-	-	-	ND
Acid production from				
Sorbitol	+	+	+	+
Mannitol	+	+	+	+
Inositol	+	+	+	+
Erthritol	-	-	-	+
L-lactate	-	-	-	+
PCR (650-bp product by Pst1 and Pst2 primer set)	+	+	+	-

ND; not determined, (+): positive reaction, (-): negative reaction

This study is the first to quantify levels of bacterial speck resistance in some native and common tomato cultivars in Central Anatolia. Based on these results, it should be possible for a breeder to make progress in improving the resistance level by selecting parents based on phenotype. To successfully breed resistant cultivars of tomatoes, more extensive surveys of existing cultivars, breeding materials, and perhaps wild species are needed to better identify sources of resistance. The use of resistant cultivars may be the most effective approach for the disease management because of the sustainability and eco-friendly nature of this technique.

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