Reaction of Some Popular Hybrid Tomato Cultivars Grown in Aegean Region to Bacterial Speck Disease and Determination of Disease Incidence in Şahnalı, Aydın

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Accepted for publication October 10, 2013

ABSTRACT

In this study, reaction of some popular hybrid tomato cultivars grown in Aegean region was tested to bacterial speck disease of tomato and disease incidence was determined in Şahnalı village in Aydın. A virulent isolate of *Pseudomonas syringae* pv. *tomato* B07-1 was spray inoculated to 6-8 leaves-old seedlings of cultivars of Beril, Dorit, 5656, Marmara, Newton, Selin, 144, in growth chamber conditions. Reaction of cultivars was evaluated with a disease severity ranking scale from zero to four. Cultivar 144, showed the most resistant reaction and ranked 1.13 for the disease severity while cultivar Newton, ranked 2.92 showing the most susceptible reaction to the disease, in the first experiment. In the second experiment, similar results were obtained for 144 (1.39) and Newton (2.42). In this study, cultivars 144 and Marmara showed highly resistant, Beril and Selin moderately resistant, Dorit and 5656 susceptible and Newton highly susceptible reaction to bacterial speck disease. A disease survey was conducted in 2011 and 2013 in Şahnalı village located nearby to main tomato production areas in Aydın and field-tomato cultivation is commonly practiced. When the disease occurred in the surveyed areas, incidence was 100 % in 2011 and 7 % in 2013.

Keywords: Bacterial speck disease, hybrid tomato cultivars, cultivar reaction, *Pseudomonas syringae* pv. *tomato*

INTRODUCTION

Bacterial speck caused by the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Pst) is an economically important disease of tomato. Disease occurs in tomato grown areas worldwide (Goode and Sasser, 1980). Above parts of tomato plants are affected by the pathogen causing black specks on leaves, stems and fruits. At early growth stages of plants, infections could cause heavy yield losses (Yunis et al., 1980). The pathogen is mainly seed-borne (Bashan et al., 1982). Disease can be controlled by copper applications (Yunis et al., 1980, Goode and Sasser, 1980) however copper resistant isolates of the pathogen were also reported (Mellano and Cooksey, 1988; Benlioğlu and Benlioğlu, 1998).

In Turkey, disease was first reported in Aegean region in 1975 (Saygılı, 1975) and subsequently was found in Mediterranean region in 1977 (Çınar, 1977). Disease causes outbreaks sporadically in Turkey (Şahin, 2001; Basim et al., 2004; Aysan et al., 2007). In 1999 and 2000, field grown tomatoes were heavily infected in Eastern Anatolian region (Şahin, 2001). In 2002 and 2003, seedling industry in the Western Mediterranean region of Turkey was affected due to 20-25 % losses in transplants caused by the disease (Basim et al., 2004).

Recently Turgut and Basim (2013) investigated reaction of 93 tomato cultivars grown in Western Mediterrenean region in Turkey to bacterial speck disease and seven cultivars found to be resistant to *P. syringae* pv. *tomato*. Aynı literatüre iki kere değinilmiş gerek olmadığını düşündüm

In Aydın, disease occurs in field-grown fresh and processing tomatoes almost every year (Z. Özdemir, unpublished data) however there is no record of disease incidence. Majority of field tomato production is mainly located through the border of the Great Meandros River in Aydın. Şahnalı village located bordering the Great Meandros River selected for the survey where field tomato production for fresh market and processing is practiced commonly and ecologically a representing area of major tomato grown fields in Aydın. In Şahnalı, copper applications are the only method used to control the disease (İ. Kavas, personal communication). Resistant cultivar use is one of the most efficient cultural methods in control of bacterial speck (Fallik et al., 1983; Abak et al., 1990; Lamichhane et al., 2010, Turgut and Basim, 2013). However resistant cultivar use by the growers is not practiced as a disease control method in Şahnalı. On the other hand, bacterial speck disease reaction of cultivars grown in the region is unknown. Therefore in order to recommend use of resistant cultivars to the growers, reaction of cultivars to the disease should be investigated. In this study, we have chosen as plant material several popular hybrid cultivars that grown in Aegean region. Especially, cultivars Dorit and 5656 are widely grown in Aydın for several years (Z. Özdemir, unpublished data) and there are no reports of reaction of these cultivars to bacterial speck disease.

The aim of this study is to evaluate the reaction of commonly grown hybrid cultivars in Aegean region against *Pseudomonas syringae* pv. *tomato* and determine recent incidence of the disease in Şahnalı village in Aydın.

MATERIALS AND METHODS

Plant material and growth conditions

Tomato cultivars; Beril, Dorit, 5656, Marmara, Newton, Selin and 144 were used for inoculations (Table 1). Seedlings were obtained from a seedling company. One week after seedlings received, plants were transferred to plastic pots in 4 cm diameter in size and grown in sterilized sand: torf: perlit (1:1:1) mix. Plants were kept at 26°C for 16 h light and 8 h dark conditions in growth chamber.

Table 1. Cultivars used in this study and their type of cultivation.

Cultivar	Type of Cultivation	Seed Company
Dorit	Field	Antalya Tarım
Beril	Field	RitoTohum
Marmara	Field	YükselTohum
Newton	Greenhouse	Syngenta
Selin	Field	Zeralim
144	Greenhouse	Hazera
5656	Field	Hazera

Bacterial culture and inoculum preparation

Pseudomonas syringae pv. *tomato* (Pst) isolate B07-1 were used for inoculations. *Pseudomonas syringae* pv. *tomato* B07-1, was isolated from processing tomato cultivar 513 grown in Belevi, İzmir in 2007 from a disease survey and identified by pathogenicity tests on tomato, Gram staining, hypersensitive reaction on tobacco and by ITS-PCR using Space F-B/Space R primers (Özdemir, 2003). Recently this isolate was used in inoculation studies of tomato cultivars by Acıpehlivan (Acıpehlivan, 2012). Bacterial culture was grown in tryptic soy agar (TSA) for 48 h at 26°C. Colonies were transferred to 50 ml of tryptic soy broth (TSB) and shaken overnight at 26°C. Liquid culture was centrifuged at 6000 rpm at 4°C for ten min and supernatant was discarded and TSB added to pellet and this step was repeated twice. Bacterial pellet was suspended in sterile distilled water to obtain inoculum. In order to

determine amount of inocula, Mc Farland standard 0.5 was used. Dilution series were also prepared in TSB medium for live bacterial count. Experiments were repeated twice. The amounts of inoculum in the first and the second experiment were $0.3x10^8$cfu/ml and $0.6x10^7$cfu/ml, respectively.

Inoculation of plants

Inoculations were carried out on plants at 6 to 8 leaves growth stage. Inoculum was applied to above parts of the plants by a hand sprayer until run off. Only sterile distilled water was sprayed for control plants. Seven plants were used per cultivar for inoculations and five plants were used per cultivar for water control. After inoculations, plants were covered with plastic bags and were kept at 28°C under 16 h light conditions in growth chamber. Bags were removed after sixty four hours. Plants were observed for disease symptoms.

Data analyses

A disease severity ranking scale was done based on our preliminary studies that in growth chamber conditions, in an infected leaf, disease was observed as high as 25 %. According to this ranking, each leave was evaluated as follows: 0: No symptoms; 1: 1-3 specks; 2: 4-10 specks; 3: 5-10 % and 4: 10-25% (Figure 1). Data were taken from three leaflets of the most infected three leaves of each plant. In order to check disease development, rankings were taken three times with weekly intervals. First disease rankings were recorded two weeks after observation of symptoms in all cultivars. Re-isolations from infected leaves were done in King B medium.

Data were analyzed by one way analysis of variance (ANOVA). Multiple comparisons of means were analyzed by Waller –Duncan test by SPSS13 statistical program (SPSS Inc., Chicago, IL, USA).

Figure 1. Disease severity ranking used for the evaluation of bacterial speck disease of tomato (0: No symptoms; 1: 1-3 specks; 2: 4-10 specks; 3: 5-10 % and 4: 10-25% of infected leaf area)

Disease survey

A disease survey was conducted in May 27th in 2011 and in May 17th in 2013 and in the field-grown tomato production areas in Şahnalı village in Aydın. Surveyed areas were shown in Table 4 and 5. At least a hundred plants was evaluated randomly for the presence of the disease in a tomato field. Samples from infected leaves, stems and fruits were taken and the pathogen was isolated in King B medium. Reisolated strains were pathogenicity tested in cultivar H-2274.

RESULTS AND DISCUSSION

In this study, we tested the reaction of commonly grown hybrid cultivars in Aegean region to bacterial speck disease of tomato. Disease symptoms were observed in all cultivars after seven days of inoculations. Cultivar Newton showed the most susceptible reaction and 144 showed the most resistant reaction to the disease at both experiments (Table 2 and 3). In the first experiment, cultivars Dorit and 5656 were found susceptible to the disease (Table 2). These cultivars were the most commonly grown cultivars in the surveyed region. Beril and Selin showed moderately resistant reaction and cultivar Marmara showed resistant reaction in the first experiment. In the second experiment, cultivars Dorit and Beril were not tested for disease reaction because seedlings of these cultivars were commercially unavailable during the experiment as they are early season cultivars. Nevertheless, reactions of those cultivars to bacterial speck were previously tested by Acıpehlivan (2012) that cultivars' reactions were similar as in the first experiment of this study. Non-inoculated and sterile distilled water inoculated control plants showed no disease symptoms. In the second experiment, Marmara and 144 were the most resistant cultivars that ranged 0.58 to 0.38 and to 1.42 to 1.39 between first and last rankings, showing almost no symptoms to one to three specks per leaf in average. On the other hand, the most susceptible cultivar Newton ranked between 1.30 and 2.42, showing one to three specks to almost five percent disease per leaf in average during the disease development (Table 3).

In a recent study of Turgut and Basim (2013), cultivars grown for field and greenhouses in Western Mediterranean region of Turkey was tested for the bacterial speck disease resistance and found 7 cultivars resistant out of 93 cultivars tested. Cultivars Beril and Selin were also included in their study and Selin was grouped in class 1; classified as highest resistance level and Beril was found to be in class 2, showing higher susceptibility than Selin according to disease severity index used in the disease evaluation (Turgut and Basim, 2013). In this study, we found that Selin is slightly more susceptible than Beril according to our disease severity ranking scale (Table 2).

Survey results indicate that the disease was observed in most of the tomato fields in 2011. Bacterial speck disease was not observed in some areas however when the disease occurred incidences were as high as 100 percent in the surveyed areas in 2011 (Table 4). In contrast, in 2013, disease was found only in one field with 7 percent disease incidence (Table 5) in Şahnalı, although surveyed area is much smaller than as in 2011.

This study shows that disease incidence vary in years as the disease occurs sporadically (Şahin, 2001; Basım et al., 2004; Aysan et al., 2007) and cultivars grown in the area is susceptible to the disease.

Table 2. Disease evaluation of hybrid tomato cultivars inoculated with *Pseudomonas syringae* pv. *tomato* B07-1 (Experiment 1).

Cultivar	Disease Severity		
	Ranking I[1]	Ranking II	Ranking III
Newton	2.63 a[2]	2.83 a	2.92 a
Dorit	2.11 b	2.37 b	2.46 b
5656	1.84 b	2.08 c	2.24 b
Selin	1.79 c	2.05 c	2.24 b
Beril	1.25 d	1.46 d	1.57 c
Marmara	0.89 e	1.21 e	1.30 c
144	0.71 e	0.97 e	1.13 d

[1]Disease rankings were taken three times per weekly intervals. First reading was made two weeks after the first observation of symptoms in all cultivars.

[2]Different letters indicate statistically significant differences (P =0.05)

Table 3. Disease evaluation of hybrid tomato cultivars inoculated with *Pseudomonas syringae* pv. *tomato* B07-1 (Experiment 2).

Cultivar	Disease Severity		
	Ranking I[1]	Ranking II	Ranking III
Newton	1.30 a[2]	1.96 a	2.42 a
5656	1.12 b	1.85 a	2.31 a
Selin	1.09 b	1.63 b	1.82 b
Marmara	0.58 c	1.22 c	1.42 c
144	0.38 d	0.84 d	1.39 c

[1]Disease rankings were taken three times per weekly intervals. First reading was made two weeks after the first observation of symptoms in all cultivars.
[2] Different letters indicate statistically significant differences (P =0.05)

Table 4. Incidence of bacterial speck disease in field grown tomatoes in Şahnalı in 2011.

Area (da)	Disease incidence (%)	Cultivar
20	0	Albeni
15	0	Dorit and 5656
10	100	Dorit
10	100	Dorit
19	100	5656

Table 5. Incidence of bacterial speck disease in field grown tomatoes in Şahnalı in 2013.

Area (da)	Disease incidence (%)	Cultivar
3	7	Dorit
2	0	Dorit
6	0	Dorit
4	0	Dorit

ACKNOWLEGMENTS

Authors thank İlknur Kavas (Turkish Ministry of Agriculture, in Şahnalı in Aydın) for her help in disease surveys.

ÖZET

Ege Bölgesinde Yetiştirilen Bazı Güncel Hibrit Domates Çeşitlerinin Domates Bakteriyel Benek Hastalığına Karşı Reaksiyonlarının ve Hastalığın Aydın Şahnalı Köyünde Bulunma Oranının Belirlenmesi

Bu çalışmada, Ege Bölgesi'nde yetiştirilen bazı güncel hibrit domates çeşitlerinin bakteriyel benek hastalığına karşı reaksiyonları test edilmiş ve hastalığın Aydın ili Şahnalı köyünde bulunma oranı belirlenmiştir. İklim odası koşullarında, virülent bir izolat olan *Pseudomonas syringae* pv. *tomato* BO7-1, 6-8 yapraklı Beril, Dorit, 5656, Marmara, Newton, Selin, 144 çeşitlerine püskürtme şeklinde uygulanmıştır. Çeşitlerin reaksiyonu 0-4 hastalık şiddeti skalasına göre değerlendirilmiştir. İlk denemede, hastalık şiddeti 1.13 bulunan 144 çeşidi, en dayanıklı çeşit reaksiyonu gösterirken hastalık şiddeti 2.92 bulunan Newton çeşidi hastalığa karşı en hassas çeşit reaksiyonu göstermiştir. İkinci denemede 144 (1.39) ve Newton (2.42) çeşitleri için benzer sonuçlar elde edilmiştir. Bu çalışmada, bakteriyel benek hastalığına karşı 144 ve Marmara çeşitleri yüksek dayanıklı, Beril ve Selin orta dayanıklı, Dorit ve 5656 hassas ve Newton ise çok hassas reaksiyon göstermiştir. Hastalık bulunma oranını tespit etmek için 2011 ve 2013 yılında domates üretiminin yaygın olarak yapıldığı ve domates üretiminin en fazla olduğu alanlara yakın olan Aydın ili Şahnalı köyünde sörvey yapılmıştır. Hastalığın görüldüğü sörvey alanlarında hastalık bulunma oranı 2011 yılında %100 ve 2013 yılında % 7 olarak tespit edilmiştir.

Anahtar Kelimeler: Bakteriyel benek hastalığı, hibrit domates çeşitleri, çeşit reaksiyonu, *Pseudomonas syringae* pv. *tomato*

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