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RESEARCH PAPER

Determination of *Phytophthora* Species Causing Root and Crown Rot on Tomatoes Grown in Antalya Province and Reactions of Some Tomato Genotypes against *Phytophthora nicotianae*

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Abstract

Antalya province is the main center of vegetable production in Türkiye. Tomato comes first in terms of crops cultivated under greenhouse. *Phytophthora* species causing root and crown rot are among the factors negatively affecting tomato yield and quality. This research aimed to determine the prevalence of root and crown rot of tomatoes grown in Antalya province and to identify *Phytophthora* species causing disease. During surveys performed in 170 tomato greenhouses, plant and soil samples were taken from the areas where root and crown rot, stem blight, and drying symptoms were observed. Disease prevalence and incidence in the investigated greenhouses were 25.88% and 4.87%, respectively. *Phytophthora* symptoms were not observed in the greenhouses in Demre and Kepez districts, while the highest disease prevalence was found in Elmalı district with 75%. Eighty of 84 *Phytophthora* isolates were identified as *P. nicotianae* and four as *P. capsici*, according to their cultural, morphological and molecular characterisation. Virulence of 18 selected isolates were determined by using stem inoculation technique and all isolates caused lesions with different lengths on tomato stems. The reactions of 22 tomato genotypes in the gene pool of BATEM against *P. nicotianae* were also investigated and the genotype DT-15 was found as the most susceptible genotype with the largest lesions, while A-286 was the most resistant genotype. This study formed the basis for further studies on tomato breeding and integrated disease management.

1. Introduction

Tomato (*Solanum lycopersicum* L.), belonging to Solanaceae family, is an important vegetable both for fresh consumption and as an agricultural raw material. Tomato production constitutes about 55% of the total vegetable production of Antalya province and is an important source of income for the farmers (Karaköse et al., 2022). Plant diseases cause significant economic losses in agriculture and pose a major threat to global food security (Kroon, 2010). Pests and diseases can also cause yield and quality losses in tomato production worldwide. Tomato is susceptible to more than 200 diseases and yield losses can reach 70-95% (Lukyanenko, 1991; Ma et al., 2023).

Phytophthora genus has many pathogenic species and is among the most important plant pathogens all over the world (Erwin and Ribeiro, 2005; Brasier et al., 2022; Giachero et al., 2022). These species cause various destructive diseases on many plant species, from vegetable or ornamental plants to fruit and forest trees. Most species cause root or crown rot on plants (Agrios, 2005; Erwin and Ribeiro, 2005). Infected plants show drought and nutrient deficiency symptoms at the beginning, then the plants quickly weaken and become vulnerable to attack by other pathogens.

Phytophthora root and crown rot destroys its hosts in almost all parts of the world, having waterlogged soils with relatively low (15-23°C) temperatures (Agrios, 2005). Diseases caused by *Phytophthora* species have become more important with the increasing trade in plant materials, especially with the ornamental plant trade (Cacciola and Gullino, 2009; Ebrahimzadeh and Dolar, 2019).

Various studies showed that *P. nicotianae (=P. parasitica*), *P. capsici, P. cryptogea, P. arecae, P. citricola, P. mexicana, P. erythroseptica, P. cactorum*, *P. drechsleri* (Blancard, 2012) and *P. syringae* (Hyder et al., 2019) cause root and crown rot on tomatoes. *Phytophthora nicotianae* has a very wide host spectrum. Since its first description on tobacco was in 1896, it has been reported to cause root rot, crown rot, leaf blight, stem canker, tip blight and fruit rot on about 255 plant species from 90 families (Erwin and Ribeiro, 2005; Cline et al., 2008; Minuto et al., 2008; Gilardi et al., 2013; 2014; Gupta et al., 2022).

Tomato production, made in Antalya for many years, has become possible in all seasons with the increase of highland greenhouse cultivation in the province. Root and crown rots are the most important diseases threatening tomato production. These diseases caused by soil-borne pathogens are common in tomato growing areas. *Phytophthora* species are the most important group of agents related to root and crown rot. However, since their isolation is difficult and require special media, they cannot be isolated from diseased plants. Considering that the disease is caused by different pathogens, unnecessary fungicide applications are made. Thus, the disease cannot be controlled successfully. Studies on root and crown rot disease caused by *Phytophthora* species on tomato plants are very limited in Türkiye. Since they are not considered as significant pathogens of tomatoes, studies on the breeding of resistant cultivars against this group of pathogens have been neglected. No detailed research has been done on the reactions of tomato genotypes against *Phytophthora* species. In this study, surveys were performed in the tomato-growing areas of Antalya province and plant and soil samples were collected. *Phytophthora* species were isolated from the collected samples by using selective media and identified according to their morphological and molecular features. Virulence variations among species and isolates were also determined by pathogenicity tests and reactions of tomato genotypes against the most common species were determined.

2. Material and Methods

2.1. Field studies

Surveys were performed in Aksu, Alanya, Demre, Elmalı, Finike, Gazipaşa, Kaş, Kepez, Korkuteli, Kumluca and Serik districts of Antalya province, where tomato cultivation is common and over 500 hectares land, during 2019-2021 vegetation period. According to the simple random sampling method, selected greenhouses were examined for disease symptoms and root and crown rot prevalence and incidences were determined (Bora and Karaca, 1970). A total of 170 greenhouses were investigated and soil and plant samples with root and crown rot, wilting and drying symptoms were collected. Samples were brought to the Mycology Laboratory of the Plant Health Department of the Batı Akdeniz Agricultural Research Institute (BATEM) and investigated for the presence of *Phytophthora* species.

2.2. Isolation of *Phytophthora* species from plant and soil samples

The roots of the diseased plants were washed under tap water and small tissue pieces with lesions taken from the roots, crown and stem were directly transferred onto a selective medium (PARP-Corn meal agar amended with pimaricin, ampicillin, rifampicin and pentachloronitrobenzene) (Jeffers and Martin, 1986). Cultures were incubated at 20±1°C in the dark for 2-3 days. Colonies were then examined under a microscope and agar plugs with coenocytic mycelia were cut from the edges of the colonies and transferred to carrot juice agar (CA) (200 ml boiled carrot juice, 800 ml distilled water and 20 g agar) (Kurbetli et al., 2020). The baiting technique was used to isolate *Phytophthora* species from the soil samples. Green tomato fruits and fresh tomato leaves were used as traps. Small tissue pieces taken from the fruits and leaves with lesions were then transferred onto selective medium as mentioned above.

2.3. Identification of the *Phytophthora* isolates

The *Phytophthora* isolates were identified according to their colony types, and morphology and sizes of sporangia (Erwin and Ribeiro, 2005; Gallegly and Hong, 2008). Soil extract (1.5%) or rain water was used to induce sporangia formation (Jeffers, 2006) and carrot juice agar amended with β-sitosterol (30 mg L^{-1}) to induce a sexual structures (Latorre et al., 2001; Gallegly and Hong, 2008). The presence and morphology of hyphal swellings and chlamidospores were investigated in two week-old cultures. The growth of heterothallic species at 35°C on carrot agar and potato dextrose agar was investigated after 5-7 days of incubation. Colony morphologies of the isolates on different media (carrot agar, V8 juice agar, malt extract agar, cornmeal agar and potato dextrose agar) were also determined.

2.4. DNA isolation, PCR and DNA sequencing

Isolates were grown on carrot agar at 24±1°C in the dark for one week. Mycelia were taken by a

sterile scalpel, transferred to Eppendorf tubes and crushed using the TissueLyser instrument (Qiagen, Tokyo, Japan). DNA was extracted by using the Wizard Genomic DNA Kit (Promega, Madison, WI, USA) according to the manufacturer's instructions. DNA sequence of the internal transcribed spacer (ITS) regions of the isolates was amplified by PCR by using universal primers ITS4 (R 5' TCC TCC GCT TAT TGA TATGC 3') and ITS6 (F 5' GAA GGT GAA GTC GTA ACA AGG 3') (White et al., 1990; Kroon et al., 2004). DNA sequences of the PCR products were analyzed by BMLABOSİS (Ankara-Türkiye) and compared with the sequences listed in GenBank (NCBI-National Center for Biotechnology Information) to verify the morphological identifications of the isolates. DNA sequences were submitted to GenBank.

2.5. Pathogenicity test

Virulence of the *Phytophthora* species was determined by stem inoculation technique. A total of 18 isolates, 15 of which were *P. nicotianae* (Dalpem, Dalbel-1, Dtur-5, Dtur-6, Dakadlı-1, Dakadlı-2, Dakgökdere-2, Dal7-3, Dkare-1, Dkare-4, Dkumsyn, Dgazi, DSermer-1, DSermer-2, DKO 20-4) and 3 *P. capsici* (DTur-1, DTur-3, and DTur-4) were used in the test. Stems of tomato plants (cv. Batem Özçelik) with 6-7 leaves were decapitated by a sterile scalpel and 2.5 mm agar discs taken from one-week-old pathogen culture were placed on the injury. Then the inoculums were covered with aluminum foil to keep the inoculum on the stem and maintain humidity on the inoculation site. Sterile agar discs were used for control plants. Plants were incubated at 24±1°C and isolates causing lesions on tomato stems 5 days after inoculation were determined as pathogens. Lengths of the lesions were measured and small pieces taken from the lesions were transferred onto a selective medium to reisolate the pathogens (Pochard et al., 1976; Messaouda et al., 2015).

2.6. Determination of the reactions of tomato genotypes

To determine the susceptibilities of tomato genotypes against *Phytophthora* root and crown rot, 22 genotypes in the gene pool of BATEM (DT-7, DT-9, DT-15, DT-31, DT-50, DT-62, DT-90, DT-233, DT-253, DT-257, DT-284, DT-289, DT-296, DT-630, BH-4, TY-83, TY-84, YS-580, YS-583, FHG-470, A-286, Batem Özçelik) were used. *Phytophthora nicotianae* isolate Dakadlı-1, which was found as the most virulent isolate in the pathogenicity test, was inoculated to the genotypes by the stem inoculation technique. Plants were grown at 24±1°C in greenhouse conditions and lesion lengths were measured every 5 days after inoculations. In addition, necrosis progression (mm day^{-1}) was found by dividing the differences between two successive measurements by days between them (Sağır, 1984; Sağır and Yıldız, 1988) and statistically evaluated. Death started in susceptible genotypes 10 days post inoculation (dpi) due to the necrosis covering whole plant. Since the sensitivity levels of tomato genotypes began to dicriminate 10 dpi, genotypes were classified as resistant, moderately resistant, susceptible and very susceptible, based on the necrosis progressions in this period (Göçmen, 2006).

2.7. Statistical analyses

Obtained data were subjected to one-way ANOVA and means were compared by Tukey's HSD test using SPSS (Version 23.0) program.

3. Results and Discussion

During surveys, investigations were made in 170 tomato greenhouses and plant and soil samples from the areas with root and crown rot symptoms were studied. Mean disease prevalence was 25.88% and incidence was 4.87% in the surveyed greenhouses. There was no plant with disease symptoms in the greenhouses in Demre and Kepez districts, while disease prevalence and incidence were highest in Elmalı district (Table 1). In Elmalı and Korkuteli districts, tomato production is mainly

Table 1. Number of tomato greenhouses investigated in Antalya province, greenhouses infected with root and crown rot disease, disease prevalence and incidence rates.

Districts	Number of greenhouses surveyed	Number of greenhouses with disease symptoms	Disease prevalence (%)	Disease incidence (%)
Aksu	35	7	20.00	1.86
Alanya	10	3	30.00	3.08
Demre	11	-	-	-
Elmalı	12	9	75.00	4.88
Finike	10	2	20.00	4.55
Gazipaşa	7	1	14.29	0.12
Kaş	17	5	29.41	3.38
Kepez	10	-	-	-
Korkuteli	12	8	66.67	3.41
Kumluca	23	2	8.70	0.41
Serik	23	7	30.43	2.71
Total/Mean	170	44	25.88	4.87

performed in plateaus during summer and period of time is not sufficient for solarization and soil fumigation. The reason why disease prevalence was higher in these areas may be the lack of pre-plant applications preventing soil-borne pathogens. Results of the study made by Perez et al. (2004) supported this thought. They applied metham sodium and solarization in the seed beds after inoculations of *P. nicotianae* and *R. solani.* As a result of the experiment, disease symptoms were not observed in these seed beds, while high rates of pathogens were observed in the controls.

As a result of isolations, a total of 84 *Phytophthora* isolates, 53 of them from the plant and 31 from the soil samples, were obtained. Isolates were evaluated according to their cultural and morphological criterias and 80 of them were identified as *P. nicotianae (= P. parasitica)*, and the remaining 4 were *P. capsici.* Identification of *Phytophthora* species are rather difficult due to the morphological similarities among the species (Waterhouse et al., 1983). Therefore, identifications were supported by molecular techniques. As a result of the comparison of the ITS sequences of our isolates with those of the *P. nicotianae* and *P. capsici* isolates in the GenBank, they showed 99-100% similarity. Our isolates were registered in the GenBank with the accession numbers OQ415883 (*P. nicotianae*) and OQ415886 (*P. capsici*).

Phytophthora nicotianae and *P. capsici* were isolated both from the plant and soil samples in the same greenhouses as only species and found virulent in the pathogenicity test. However, *P. nicotianae* was more common with a 95.24% isolation rate. Our results are compatible with the previous findings reporting these species as pathogens affecting tomato (Kırbağ and Turan, 2006; Gilardi et al., 2013). Bolkan (1985) mentioned that *Phytophthora* root rot caused by *P. capsici* or *P. parasitica* was an important disease in tomato-growing areas in California, and *P. parasitica* was responsible for more than 85% of the disease. Similarly, Colla (2012) stated that *P. capsici* and *P. nicotianae* were commonly found in tomato areas, but *P. nicotianae* was more important. *P. nicotianae* was also reported as the dominant species in Brazil, Egypt, Tunisia and South Africa (Panabières et al., 2016). In Türkiye, symptoms of root and crown rot disease caused by *P. nicotianae* have especially been observed during the early season in tomato areas and the damage caused by this pathogen has constantly been increasing (Altın et al., 2018). Some researchers noted that the root infections related to *P. nicotianae* were more severe in summer and early autumn (Alvarez et al., 2009). However, some others indicated that the pathogen caused severe epidemics on grafted tomatoes especially spring and summer months (Minuto et al., 2008; Garibaldi and Gullino, 2010). *Phytophthora nicotianae* susceptibility was observed on rootstocks of *S. lycopersicum* × *S. hirsutum* and other *S. lycopersicum* hybrids (Gilardi et al., 2011). In our surveys, the pathogen was similarly isolated from the grafted tomato plants.

As a result of necrosis length measurements made at 5 dpi, it was observed that the pathogen started to reproduce and form necrotic areas in the plant tissues. Due to necrosis covering the whole plant, death started at 10 dpi, and on the 20th day, necrosis covered all over the stems and susceptible plants died. However, the necrosis progression rate slowed or stopped in the resistant and moderately resistant genotypes. Differences among the genotypes in terms of mean necrosis lengths, measured during 20 days, were statistically significant (Table 2).

DT-15 was found as the most susceptible tomato genotype with the largest lesions, while A-286 was the most resistant one. Necrosis progression was also higher on the genotype DT-15 in the first 10 dpi, then it slowed and stopped after 15 days (Table 3). Similar to the necrosis lengths, daily necrosis progression was also slower on genotype A-286, especially during the first 10 dpi.

According to the classification made at 10 dpi, when the sensitivity levels of tomato genotypes began to discriminate, DT-9 and DT-15 genotypes were classified as highly susceptible, while Batem Özçelik genotype was susceptible. DT-7, YS-583, FH6-470 and A-286 genotypes were classified as resistant, and the remaining genotypes were moderately resistant (Table 4).

Control of soil-borne pathogens like *Phytophthora* species is rather difficult, since they can survive in soil and have wide host ranges. Chemical control has negative side effects on the environment and human health, while physical methods are more expensive and laborius (Ma et al., 2023). Therefore, the development of resistant cultivars against *Phytophthora* root and crown rot disease will make a significant contribution to tomato breeders and growers (Bolkan, 1985). In fact, it is focused on the selection and breeding of resistant cultivars against the disease, in many countries. However, disease symptoms and genetic factors controlling pathogen resistance can vary depending on the region and the virulence of the pathogen (Naegele, 2013).

4. Conclusion

This research showed that root and crown rot diseases caused by *Phytophthora* species are common in the tomato greenhouses in Antalya province, with the highest prevalence rate in Elmalı district. As a result of the isolations made from the plant and soil samples, *P. nicotianae* was found to be the most common agent causing the disease. A greenhouse trial showed differences among the resistance levels of 22 tomato genotypes found in the gene pool of BATEM, against the pathogen. The results obtained in this study will contribute to the integrated management of the disease by

Table 2. Mean lesion lengths on tomato genotypes 5-20 days after inoculation with *Phytophthora nicotianae*.

Tomato genotypes	Lesion length (mm)			
	5th day	10th day	15th day	20th day
DT-7	20.30 ce*	30.20 df	36.70 fh	41.60 eg
DT-9	35.27 a	80.47 a	87.73 a	87.73 a
DT-15	35.67 a	81.40 a	94.91 a	94.91 a
DT-31	32.07 ab	52.00 bc	65.73 b	72.73 b
DT-50	31.53 ab	48.60 bc	58.80 be	65.93 bd
DT-62	28.20 abc	47.73 bc	56.60 be	61.27 bd
DT- 90	31.27 ab	51.00 bc	58.27 be	63.47 bd
DT- 233	27.53 ad	43.53 bd	50.60 cf	56.20 ce
DT-253	31.13 ab	51.47 bc	63.00 bd	71.87 b
DT-257	30.13 ab	50.33 bc	62.33 bc	72.60 b
DT-284	33.53 a	49.27 bc	60.13 be	69.60 bc
DT-289	24.13 bd	40.40 ce	49.60 df	55.87 ce
DT- 296	28.87 ab	47.67 bc	63.00 bd	72.67 b
DT-630	29.67 ab	46.20 bc	59.07 be	68.20 bc
BH-4	31.07 ab	47.67 bc	59.20 be	67.07 bd
TY-83	31.07 ab	48.47 bc	60.60 be	67.40 bd
TY-84	31.27 ab	50.07 bc	63.40 bd	71.53 b
YS-580	29.80 ab	48.13 bc	62.20 bd	72.33 b
YS-583	24.60 bd	39.40 ce	46.80 eg	52.93 df
FH6-470	19.27 de	27.33 ef	34.47 gh	39.80 fg
A-286	15.13 e	23.13 f	30.13 h	35.13 g
BATEM Özçelik	30.93 ab	56.00 b	64.40 bc	70.87 b

* Means in the same column shown by the same letters are not statistically different from each other according to Tukey test (P=0.05).

Table 3. Necrosis progression on the tomato genotypes measured for 20 days in 5-day intervals following inoculation with *Phytopthora nicotianae*.

Tomato genotypes	Necrosis progression (mm day^{-1})			
	0-5 days	5-10 days	10-15 days	15-20 days
DT-7	4.05	1.99	1.29	0.99
DT-9	7.05	9.04	1.45	0.00
DT-15	7.13	9.15	2.70	0.00
DT-3	6.41	3.99	2.75	1.40
DT-50	6.31	3.41	2.04	1.43
DT-62	5.64	3.91	1.77	0.93
DT- 90	6.25	3.95	1.45	1.04
DT- 233	5.51	3.20	1.41	1.12
DT-253	6.23	4.07	2.31	1.77
DT-257	6.03	4.04	2.40	2.05
DT-284	6.71	3.15	2.17	1.89
DT-289	4.83	3.25	1.84	1.25
DT- 296	5.77	3.76	3.07	1.93
DT-630	5.93	3.31	2.57	1.83
BH-4	6.21	3.32	2.31	1.57
TY-83	6.21	3.48	2.43	1.36
TY-84	6.25	3.76	2.67	1.63
YS-580	5.96	3.67	2.81	2.03
YS-583	4.92	2.96	1.48	1.23
FH6-470	3.85	1.61	1.43	1.07
A-286	3.03	1.52	1.40	1.00
BATEM Özçelik	6.19	5.01	1.68	1.29

preventing unnecessary fungicide use, and will ensure the protection of environment and human health.

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Table 4. Susceptibilities of the tomato genotypes according to the necrosis regression rates on the 10th day after inoculation.

Tomato genotypes	Mean necrosis regression rate (mm day^{-1})	Sensitivity level
DT-7	1.99	Resistant
DT-9	9.04	Highly susceptible
DT-15	9.15	Highly susceptible
DT-31	3.99	Moderately resistant
DT-50	3.41	Moderately resistant
DT-62	3.91	Moderately resistant
DT- 90	3.95	Moderately resistant
DT- 233	3.20	Moderately resistant
DT-253	4.07	Moderately resistant
DT-257	4.04	Moderately resistant
DT-284	3.15	Moderately resistant
DT-289	3.25	Moderately resistant
DT- 296	3.76	Moderately resistant
DT-630	3.31	Moderately resistant
BH-4	3.32	Moderately resistant
TY-83	3.48	Moderately resistant
TY-84	3.76	Moderately resistant
YS-580	3.67	Moderately resistant
YS-583	2.96	Resistant
FH6-470	1.61	Resistant
A-286	1.52	Resistant
BATEM Özçelik	5.01	Susceptible

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