Prevalence, isolation and identification of bacterial canker pathogens on sweet cherry trees in Tekirdag

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Abstract

Sweet cherry (*Prunus avium* L.) is one of the most important fruit trees grown in Tekirdag. Bacterial canker which reduces the yield and quality of sweet cherry fruit and causes death of trees was investigated. For this purpose, a survey study was conducted in 2012-2013, and 129 plant tissue samples were collected from symptomatic trees. As a result of survey studies, bacterial canker was determined in all orchards, and the disease prevalence rate was determined as 17 to 57%. Severity of disease was measured as 20 to 85% in Tekirdag province depending on orchards. Totally, 41 bacterial strains of *Pseudomonas syringae* were determined by using classical and molecular identification tests, 23 out of 41 yielding. The identity of the 23 strains *Pseudomonas syringae* pv. *morsprunorum* and 18 strain *Pseudomonas syringae* pv. *syringae* were confirmed by applicans of the expected size 650 bp and 752 bp, respectively.

Keywords: *Pseudomonas syringae* pv. *syringae*, PCR, sweet cherry

INTRODUCTION

Cherry is an important fruit with a production of 480.748 t year^{-1} in Turkey and first place in the world (Anonymous, 2013). Bacterial canker and blast caused by *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum* are a major problem in Tekirdag. The disease was severe mostly in Tekirdag and its provinces in recent years. The canker on branches or trunks causes the most serious damages.

The symptoms of the disease on cherry are blossom blight, dieback, bacterial spots on leaves and fruits, canker on branch and trunks. Substantial gummy occurs on the bark surface and around the canker margins. Underneath the outer bark, cankers are reddish brown and reddish scratches or fleck in the phloem extend beyond the margins of the canker, and canker may griddle and kill entire branches or if on the trunk they can kill the entire tree. The root system of the diseased tree is usually not affected. Branches affected by bacterial canker may fail to grow in the spring. Buds that fail to open are brown and usually exudate droplets of gum. In some cases, blossoms turn brown after they open and the bud and spur also may be affected, forming a small canker that exudes gum. Young green fruit is occasionally affected by the pathogens. They develop small, dark brown, canker lesions that leave the fruit severely deformed.

The objective of the research was to determine the prevalence and identification of bacterial canker agent in Tekirdag. The collected plant samples in the survey were examined, and the pathogenic bacteria were isolated. The isolated strains were identified by pathogenicity test, morphological, physislogical, biochemical tests and by PCR tests.

MATERIALS AND METHODS

The samples were randomly obtained by collecting young infected stems and branches of sweet cherry according to Lazarov and Grigorov (1961). The number of healthy and infected fields was recorded for disease prevalence in Tekirdag.

The collected plants were placed in paper bag and transported to the laboratory for isolation and identification of the causal organism. Surfaced-sterilized small pieces of lesion

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area were macerated in one mL of sterile distilled water. A loopful of suspension was streaked on the King's B medium into petri plates and incubated at 25°C for 2-3 days. Single fluorescent or non-fluorescent representative colonies of the predominant morphological types of bacterial isolates were re-streaked on the new King's B (KB) plates and incubated at 25°C for 2 days. The pure single colonies obtained were grown on KB plates and stored at 4°C or in 20% glycerol at -80°C until further identification.

Pathogenicity tests were carried out as described by Lelliott and Stead (1987). All of the strains were inoculated into 1-year-old shoots of cherry. Bacterial suspensions (10^8 cfu mL^{-1}) of all bacterial strains were injected in three replicates into the bark of each test plant using a sterile needle. Bacterial canker and necrotic area development was observed up to 15-20 days after inoculation when bacterial strains were re-isolated from symptomatic plants. Sterile distilled water and reference strain NCPPB2307 of *P. syringae* pv. *syringae* was used as negative and positive controls, respectively.

All morphological properties on KB (King et al., 1954) media and biochemical and nuhitional tests were used to identity the bacteria i.e., Gram reaction, levan production on 5% sacchrarose nutrient agar (SNA) oxidase and catalase reaction, gram reaction, gelatin hydrolase and HR test on tobacco leaves (Schaad et al., 2001). All tests were repeated twice with three replicate. PCR assays were performed using *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum* specific primers B1 (5'-CTTTCCGTGGTCTTG ATGAGG-3')-B2 (5'-TCGATTTTGCCGTGATGAGTC-3') (Bereswill et al., 1994) and CFLF (5'-GGCGCTCCCTCGCACTT-3')-CFLR (5'-GGTATTGGCGGGGGTGC-3') designed by Abbasi et al. (2013), respectively.

RESULT AND DISCUSSION

Disease prevalence was 35.6% Naip, 33.3% Memer, 17.4% Center, 38.5% Kumbağ, 25.0% Karahisarlı, 20% Çanakcı and 57.1% Barbaros. Disease severity was recorded 20-85% in Tekirdag.

Forty-one isolates from naturally infected plants were isolated and subjected to biochemical and pathogenicity tests. All strains were florescent on KB. On KB medium, most of the isolates grew slowly. Small, greyish-white colonies as reported previously (Lelliott and Stead, 1987). The reaction of *P. syringae* pv. *syringae* strains was positive for levan production; hypersensitive reaction on tabacco, gelatin hydrolysis, aesculine hydrolysis and negative for oxidase reaction, pectolitic activity, arginine dehydrolase, tyrosine, tartaric acid. The reaction of *Pseudomonas syringae* pv. *morsprunorum* strains was positive for levan production, hypersensitive reaction on tabacco, tyrosine, tartaric acid and negative for oxidase reaction, pectolotic activity, arginine dehyrolase, gelatin hydrolysis, aesculin hydrolysis. In the pathogenicity tests, 1-year-old shoots of cherry inoculated with bacterial suspensions of the all strains developed typical bacterial canker symptoms 30-35 days after inoculations. No symptoms appeared on negative control plants.

The identification of the 23 isolates was confirmed as *P. syringae* pv. *morsprunorum* by amplification of the expected size 650 bp (Figure 1) in accordance with the previously reported ones (Abbasi et al., 2013). The identification of the 18 isolates was confirmed as *P. syringae* pv. *syringae* by amplification of expected size 752 bp (Figure 1) in accordance with the previously reported ones (Bereswill et al., 1992). Results of PCR were identified as *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum.*

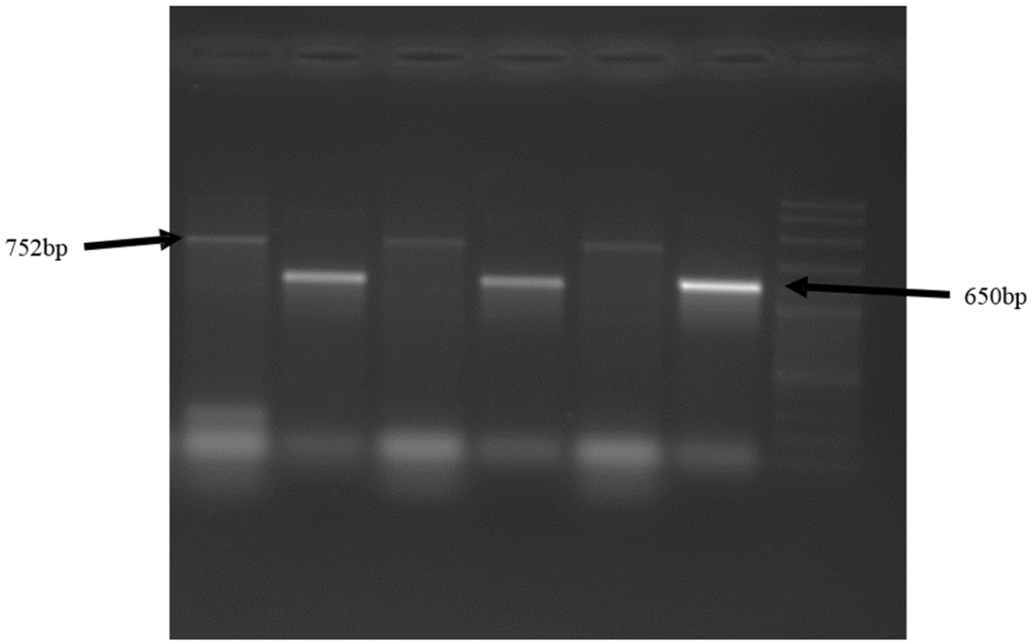

Figure 1. PCR product of different *P. s.* pv. *syringae* and *P. s.* pv. *morsprunorum* strains generated with CFLF-CFLR and B1-B2 primers. Line 1: 100 bp DNA marker, Line 2: Naip 4/1 strain, Line 3: Naip 11/1 strain, Line 4: Mermer 4/1 strain, Line 5: Karahisarlı 3/1 strain, Line 6: Barboros 3/1 strain.

Literature cited

Abbasi, V., Rahimian, H., and Tajick-Ghanbari, M.A. (2013). Genetic variability of Iranian strains of *Pseudomonas syringae* pv. *syringae* causing bacterial canker disease of stone fruits. Eur. J. Plant Pathol. *135* (*2*), 225–235 http://dx.doi.org/10.1007/s10658-012-0095-1.

Anonymous. (2013). www.tuik.gov.tr.

Bereswill, S., Pahl, A., Bellemann, P., Zeller, W., and Geider, K. (1992). Sensitive and species-specific detection of *Erwinia amylovora* by polymerase chain reaction analysis. Appl. Environ. Microbiol. *58* (*11*), 3522–3526 PubMed.

Bereswill, S., Bugert, P., Völksch, B., Ullrich, M., Bender, C.L., and Geider, K. (1994). Identification and relatedness of coronatine-producing *Pseudomonas syringae* pathovars by PCR analysis and sequence determination of the amplification products. Appl. Environ. Microbiol. *60* (*8*), 2924–2930 PubMed.

Fahy, P.C., and Hayward, A.C. (1983). Media and methods for isolation and diagnostic tests. In Plant Bacterial Diseases, P.C. Fahy, and G.J. Persley, eds. (Academic Press), p.337–378.

Janse, J.D. 2006. Phytobacteriology. Principles and Practice (Wallingford, UK and New York, USA: Oxford Press), p.208–209.

Klement, Z., Rudolph, K., and Sands, D.C. (1990). Methods in Phytobacteriology (Budapest: Akademia Kiado), pp.XIV+568.

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature *178* (*4535*), 703. PubMed http://dx.doi.org/10.1038/178703a0

Lazarov, A., and Grigorov, P. (1961). Karantina na Rastenijata (Sofia: Zemizdat), pp.258.

Lelliott, R.A., and Stead, D.E. (1987). Methods for the Diagnosis of Bacterial Diseases of Plants (Oxford, UK: Blackwell Scientific Publications), pp.vii+216.

Schaad, N.W., Jones, J.B., and Chun, W. (2001). Laboratory Guide for Identification of Plant Pathogenic Bacteria (St. Paul, MN, USA: The American Phytopathological Society), pp.371.