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First Report of Fruit Infections of *Clavibacter michiganensis* subsp. *michiganensis*, on Processing Tomato in Turkey

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Abstract: Detection of *Clavibacter michiganensis* subsp. *michiganensis* was studied on naturally infected fruits of processing tomatoes grown in Izmir, Turkey. This is a first report of tomato fruit infections proved to pathogenicity for this pathogen in Turkey.

Key words: Bacterial canker, *Clavibacter michiganensis* subsp. *michiganensis*, tomato, fruit infection

INTRODUCTION

Clavibacter michiganensis subsp. *michiganensis* (Cmm), causal agent of bacterial canker of tomato, is an important seed-borne pathogen. Although regulated strictly by quarantine procedures, disease observed sporadically and its occurrence has been reported from countries[1,2].

Tomatoes are mainly grown for processing in Izmir province in the Aegean Region of Turkey, in which many tomato processing plants are located. Historically about five years ago processing tomato production has started in Izmir and vicinity (Pervin Tuskan, personal communication) and becoming second most important production area after the Marmara Region of Turkey. In this study, during a survey of bacterial tomato diseases in vicinity of Izmir, in July 2004; in Torbali, a county of Izmir, bacterial canker disease was detected on processing tomato cultivar NDM 447 and detection studies of fruit infections of Cmm was performed.

MATERIALS AND METHODS

Bacterial isolation: Isolations were made from diseased plants; stems and necrotic fruits, non-necrotic minute starting fruit spots. NBY medium (8 g nutrient broth, 2 g yeast extract, 2 g K_2HPO_4, 0.5 g KH_2PO_4, 2.5 g glucose, 15 g agar, 1 mL of 1 M sterile $MgSO_4.7H_2O$ L^{-1}, pH 7.2) was used for isolations. Infected tissues were surface sterilized in 2% a.i. sodium hypochlorite for 1.5-2 min, rinsed twice in sterile distilled water and dried in sterile filter paper. Surface sterilized tissues were placed in NBY plates and incubated at 25-26°C. Forty eight hours later plates were observed for bacterial growth and isolated bacteria were further purified in fresh NBY plates. Isolated bacteria were Gram stained twice from 24 h nutrient agar cultures.

Pathogenicity tests: Standard processing tomato cultivars Rio Grande and H-2274 were grown in commercial potting soil (Plantaflor Profi Type 3, Plantaflor Humus, Germany) in vials and after about one week later were transferred foam glasses (7-cmm in dia.). Five week-old plants (3-5 leaf stage) were inoculated for pathogenicity. Inoculum was prepared as in Giatitits[3] with slight modifications. Overnight nutrient broth cultures were centrifuged at 2000 rpm for 15 min at 4°C and supernatant was discarded. Bacterial pellet was resuspended in sterile 0.85% NaCl. Inoculum density was adjusted spectrophotometrically at 600 nm wavelength to 0.1 giving approximately 1.2-2.4x10^8 cfu mL^{-1} on dilution platings. Inoculation was done by cutting first true leaf with scissor dipped to inoculum. Between inoculations, scissor was surface sterilized with alcohol and flamed. Inoculated plants were placed in a growth room with 12 h light and 12 h dark conditions. Temperatures fluctuated in growth room as 22°C at dark and 27°C at light conditions. Humidity was around 40-62%. *Clavibacter michiganensis* subsp. *michiganensis* ICMP 2550 was used as positive control and 0.85% NaCl was used as negative control in pathogenicity tests. Two to three plants per isolate were used in all inoculations. Inoculations were repeated twice in cultivar Rio Grande and once in H-2274. Plants were observed periodically for symptom development until three weeks after inoculation. Reisolations were made from infected plants.

Hypersensitivity tests (HR) on four-o' clock (*Mirabilis jalapa*) plants: Four-o'clock plants (mixed colors, Royalfleur Co., France) were inoculated with 2x10^8 cfu mL^{-1} inoculum (measured spectrophotometrically at optical density 600 nm to 0.1) grown overnight in 5 mL nutrient broth cultures. Inoculum was infiltrated to under leaves as in Giatitis[3] except a needless syringe was used. Nutrient broth was used as

negative control and Cmm ICMP 2550 was used as positive control. Presence or absence of HR was determined after 48 h of inoculation. Experiment repeated twice.

RESULTS AND DISCUSSION

Thirteen isolates were obtained from infected plant parts; stems and fruits. Colonies were yellow, round on NBY medium and all were Gram positive. Symptoms of the disease on processing cultivar NDM 447 were curled leaves and severe wilting of plants (Fig. 1). Fruit symptoms were observed mainly on immature green fruits easily dropping of the peduncles. There were two stages of fruit symptoms. Minute pinpoint sized specks which can be easily neglected by naked eye hereafter called non-necrotic fruit spots were apparently initial stages of 'bird's eye' necrotic spots. Also typical bird's eye lesions were observed (Fig. 2).

Identity of the isolated bacteria was confirmed with HR test on four-o'clock plants and pathogenicity tests on processing tomato cultivars. Pathogenicity tests of 1 stem, 2 non-necrotic fruit spot and 9 necrotic, bird's eye lesion fruit isolates were all positive showing wilting 10-13 days after inoculation. Symptoms in inoculated plants were moderate to severe canker formation on inoculation point and on stems, wilting of one side of leaves and later wilting of whole plant (Fig. 3). Reisolations were made and typical yellow round colonies of Cmm were detected on NBY plates. During reisolations, brown discoloration of vascular tissue was observed on longitudinal sections of plants.

Although Sahin[2] and Aksoy[4] mentioned the presence of bird's eye lesions in their first reports and dissertations, respectively in Turkey, they have not proven these lesions to pathogenicity. In this study fruit symptoms in starting stage and as bird's eye lesions were proven to be caused by the pathogen *Clavibacter michiganensis* subsp. *michiganensis* in Turkey. The fruit lesions were observed on processing tomato cultivar NDM 447 which is an imported cultivar from Japan. Although inoculum source is not known, the pathogen most probably transported either via infected seeds or during transplant production practices, seedlings became contaminated by the pathogen.

Fig. 1: Field symptoms of bacterial canker on processing tomato cultivar NDM 447. Photograph was taken in July, 2004

Fig. 2: Bird's eye lesions on processing tomato cultivar NDM 447, fruits were collected from infected plants

Fig. 3: Pathogenicity test of fruit isolate of Cmm on processing cultivar H-2274. One-sided wilting of leaves are typical

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