DETECTION OF *XANTHOMONAS AXONOPODIS* pv. *VESICATORIA* IN NATURALLY INFECTED PEPPER SEEDS IN TURKEY

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SUMMARY

Bacterial spot, caused by *Xanthomonas axonopodis* pv. *vesicatoria*, is one of the most important diseases of pepper in Turkey, where the disease has been epidemic in the last six years in the eastern Mediterranean region. The incidence and importance of natural infection in pepper seeds produced in the region was investigated in the present study. A total of 29 seed samples were tested by immunofluorescence assay using a commercial specific antibody, semi-selective isolation on medium Tween B, and a seedling screening tests, where plantlets were examined for typical spot symptoms on cotyledons 7-14 days after emergence. Pathogen populations were found to be in the range of 5×10^1-5×10^4 cells/g seed when using semi-selective medium Tween B. The diseased seeds as determined in the seedling screening ranged from 7 to 36%; these figures agreed with numbers found in the Tween B medium test. The strains isolated on Tween B were identified by PCR using primer designed on the *hrp* gene specific for *Xanthomonas* spp., and pathogenicity on pepper plants. Twenty-one out of 29 seed samples were found to be contaminated by *Xanthomonas axonopodis* pv. *vesicatoria*. On the basis of these findings, infested seeds must be seen as a major source of inoculum for this disease in the region

Key words: *Xanthomonas axonopodis* pv. *vesicatoria*, pepper, seed, epidemiology, IFAS, PCR.

INTRODUCTION

Bacterial leaf spot of pepper, caused, by *Xanthomonas axonopodis* pv. v*esicatoria* (Doidge) Dye, has been identified as the major bacterial disease of peppers in the eastern Mediterranean region of Turkey (Aysan and Sahin, 2003). The disease is also a problem on tomato in western Mediterrenean region (Basim *et al*., 2004). Bacterial spot infections are found in fresh-marketed sweet pepper growing areas in the vicinity of Ceyhan district, and in processing pepper-producing areas in Karaisali and Ceyhan districts of Adana.

The province and central districts of Osmaniye in Turkey were determined to have 52, 91 and 100% disease prevalence in 2002, 2003 and 2004, respectively (Mirik *et al*., 2005). All above-ground plant parts on pepper are susceptible to the disease. The first symptoms appear on leaves as small, angular spots with a yellow halo. Leaves heavily infected at an early stage become deformed and drop prematurely. Blister-like fruit lesions and stem lesions are observed in late summer, and affected fruits may not be marketable.

The pathogen can survive in/on dry seeds for 10 years and on plant debris in soil for a year (Bashan *et al*., 1982). The bacterium overwinters in/on infected plant debris (Peterson, 1963; Jones and Scott, 1986; Buonaurio *et al*., 1994) and in/on contaminated seeds (Goode and Sasser, 1980; Diab *et al*., 1982). It is possible to minimize yield losses from light and local infections by using pathogen-free seeds and seedlings, crop rotations, resistant cultivars, sanitation and spraying copper compounds. However, standard application of copper compounds gives poor control because of the presence of copper resistant strains of the bacterium in the region (Mirik *et al*., 2007). Pepper growers who usually obtain seeds from their own harvested crop are not aware of the epidemiology of the disease, and have no understanding about spread and transmission of the pathogen from their own material.

Bacterial spot may be caused by two different organisms (*X. axonopodis* pv. v*esicatoria* and/or *X.* v*esicatoria*) as has been found in many tomato and/or pepper growing areas in the world (Louws *et al*., 1995). A clear understanding of the identity of strains occurring in pepper in eastern Turkey and the role of contaminated seed in primary inoculum sources of the disease, is necessary in order to develop effective management where bacterial spot is serious. The aim of this work was to identify bacteria obtained from seed lots produced from growers' harvested material in this region, and to characterize the bacterial strains using pathological, physiological, serological and molecular tests.

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MATERIALS AND METHODS

Seed lots. During the years 2002, 2003 and 2004, 29 different local seed samples were obtained from pepper growers of the provinces of Adana (Ceyhan, Karaisali, Salbas, Pirili, Kuzgun, Kuyucu, Örcün, Beydemir and Güvenç villages) and Osmaniye (Sunbas, Armaganlı, Mehmetli villages). The cultivar name of local seed samples was identified by growers as "Karaisali salcalik".

Detection and isolation of *X. axonopodis* pv. *vesicatoria* strains from seed sources. The pathogen was detected as described by McGuire and Jones (1989), using a semi-selective medium (Tween B), immunofluorescence assay and examination for typical symptom development on cotyledon leaves within 7-14 days after artificial inoculation.

Twelve grams of pepper seeds per sample were placed in a 500 ml Erlenmeyer flask containing 100 ml of sterile peptone buffer (5.30 $g \cdot l^{-1}$ KH_2PO_4, 8.61 $g \cdot l^{-1}$ $NaHPO_4$ and 1 $g \cdot l^{-1}$ Bacto peptone, using distilled water). Suspensions of the seeds were shaken at 200 rpm at 4°C for 3 h. Thereafter, the seeds were shaken for 1 h at 200 rpm at room temperature and centrifuged for 15 min at 10,000 *g*. The pellet was re-suspended in 5 ml sterile peptone buffer and the suspension serially diluted six fold (each step 1:10). From each undiluted and diluted sample 100 µl was spread on the surface of three Tween B plates which were then incubated at 28°C for 4-5 days. Light yellow colonies surrounded by a clear zone were obtained, and transferred to yeast dextrose calcium carbonate agar medium for identification.

The serially twice-diluted (10^{-2}) aliquots were tested by IFAS using *X. axonopodis* pv. v*esicatoria*-specific antibody (Loewe, Germany Cat. No. 07331) according to Schaad (1978) and EPPO PM7/23(1) (EPPO, 2004). IFAS tests were performed in the Plant Protection Research Institute in Adana.

A total of 600 pepper seeds from each seed lot were sown in six different plastic trays (20x10 cm diameter), a hundred seeds per tray. The trays contained sterile soil, sand and vermiculite mixture (1:1:1 v/v/v) and were maintained in a controlled climate chamber at 28±2°C for 7-14 days at 70% relative humidity and

Table 1. Pepper seed lots collected in 2002-2004 at Adana and Osmaniye, disease severity in a seedling test, and identification of 37 strains isolated from these seedlots as *Xanthomonas axonopodis* pv. *vesicatoria.*

No. of seed lot	Location	Diseased/Total seedlings	Severity (%)	Cells/g seed	IFAS	PCR on *hrp*-genes	Pathogenicity test
SL-1	Ceyhan	102/414	24.64	5.10^2	+	+	+
SL-2	Ceyhan	84/588	14.29	8.10^1	+	+	+
SL-3	Karaisali	108/522	20.07	5.10^1	+	+	+
SL-4	Karaisali	0/534	-	-	-	-	-
SL-5	Karaisali	0/486	-	-	-	-	-
SL-6	Salbas	48/588	8.17	5.10^2	+	+	+
SL-7	Salbas	0/462	-	-	-	-	-
SL-8	Salbas	0/576	-	-	-	-	-
SL-9	Pirili	114/432	26.39	3.10^3	+	+	+
SL-10	Pirili	102/588	17.34	2.10^2	+	+	+
SL-11	Kuzgun	42/588	7.14	3.10^2	+	+	+
SL-12	Kuzgun	108/426	25.35	1.10^2	+	+	+
SL-13	Kuzgun	0/488	-	-	-	-	-
SL-14	Kuyucu	114/444	25.68	1.10^3	+	+	+
SL-15	Kuyucu	0/542	-	-	-	-	-
SL-16	Kuyucu	72/594	12.12	2.10^3	+	+	+
SL-17	Örcün	42/426	9.86	3.10^2	+	+	+
SL-18	Örcün	68/588	11.57	1.10^2	+	+	+
SL-19	Beydemir	150/420	35.71	5.10^4	+	+	+
SL-20	Beydemir	84/420	20.00	2.10^4	+	+	+
SL-21	Güvenç	60/470	12.76	8.10^2	+	+	+
SL-22	Güvenç	132/470	28.08	5.10^1	+	+	+
SL-23	Sunbas	72/414	17.40	9.10^1	+	+	+
SL-24	Sunbas	0/498	13.25	1.10^2	+	+	+
SL-25	Armaganlı	144/462	31.17	5.10^2	+	+	+
SL-26	Armaganlı	48/570	8.42	3.10^2	+	+	+
SL-27	Armaganlı	0/542	-	-	-	-	-
SL-28	Mehmetli	90/570	15.78	2.10^2	+	+	+
SL-29	Mehmetli	0/588	-	-	-	-	-

8000 lux in a 16h/8h day/night cycle. After germination, infected seedlings were recorded and the pathogen was isolated from them. Surface-sterilized small pieces of leaf spots from diseased seedlings were macerated in one ml of sterile distilled water. A loopful of suspension was streaked on a semi-selective medium (Tween B) plate and incubated at 25°C for 3-14 days.

Identification of strains and final diagnosis. Bacterial strains obtained from Tween B medium and infected seedlings were screened by a pathogenicity test (Sahin and Miller, 1996) on pepper plants cv. Bursa yaglik (a local cultivar). Pathogenic strains were further characterized by amplification of the 355 bp portions of *hrp* gene by classical PCR (Leite *et al*., 1994), determination of pectolytic or amylolytic activity (Bouzar *et al*., 1994) and ELISA with a set of *X. axonopodis* pv. v*esicatoria* specific monoclonal antibodies Xv1, 5, 6, and 10, Xv8, 15 and 30 specific for *X. vesicatoria* (Bouzar *et al*., 1994).

RESULTS AND DISCUSSION

As reported in Table 1, 21 of 29 pepper seed samples were found to be contaminated by a bacterium with light yellow colonies surrounded by a clear zone on Tween-medium B. A total of 37 bacterial strains were isolated from samples that were collected from 12 different locations in Adana and Osmaniye. Pathogen populations on Tween B medium ranged from $5x10^1$ to 5×10^4 cells/g seed. In immunofluorescence screening, the same 21 samples showed typical positive cells at a serum dilution of 10^3. When pepper seeds were sown in trays, diseased seedlings were observed again in the same 21 seed samples within 7-14 days after artificial inoculation. Disease severity was recorded as 7-36% for these seed lots.

In the pathogenicity tests, pepper seedlings inoculated with bacterial suspensions of the 37 strains isolated on Tween-B medium developed typical bacterial spot symptoms 5 to 10 days after inoculation. No symptoms appeared on negative control plants. Using the RST 9 and RST 10 primers for *hrp*-genes, a specific band at 355 bp was obtained by PCR for these 37 strains as reported by Leite *et al*. (1994, 1995). None of the strains was pectolytic or amylolytic. All strains reacted with the *X. axonopodis* pv. v*esicatoria*-specific monoclonal antibodies Xv1, 5, 6 and 10 but not with the *X. vesicatoria*-specific MABs Xv8, 15 and 30. It seem then plausible to conclude that bacterial spots on sweet and processing pepper in the eastern Mediterranean region of Turkey are caused by *X. axonopodis* pv. v*esicatoria*. Our results show that at least 72% of seed lots tested (21/29 samples) were contaminated with this pathogen.

We have shown that bacterial spot disease of pepper, caused by *X. axonopodis* pv. v*esicatoria,* is a serious problem in commercial pepper fields in the Turkish provinces of Adana and Osmaniye and that disease spread within the field increases with high temperature and high humidity. In addition, this study provides evidence that *X. axonopodis* pv. v*esicatoria* contaminated seeds may be a significant primary inoculum source in disease outbreaks in Adana and Osmaniye. Since it is difficult to control bacterial spot on pepper in the field, further investigations are necessary to develop effective management that considers contaminated seeds as the primary inoculum source of the disease. Use of pathogen-free seed and/or seed treatments, strict hygiene and good cultural practice are recommended for effective management of bacterial spot disease in the pepper growing areas of Turkey.

ACKNOWLEDGEMENTS

This study was supported by grant No. ZF.2002.BAP.62 from Cukurova University, Turkey. We are grateful to Prof. Dr. O. Cinar (University of Cukurova, Turkey) for critical reading of the manuscript and to the Adana Plant Protection Research Institute for the immunofluorescence study.

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Received February 27, 2008
Accepted March 5, 2009