

## SHORT COMMUNICATION

COPPER-RESISTANT STRAINS OF *XANTHOMONAS AXONOPODIS* pv. *VESICATORIA* (Doidge) Dye IN THE EASTERN MEDITERRANEAN REGION OF TURKEYM. Mirik<sup>1</sup>, Y. Aysan<sup>2</sup> and O. Cinar<sup>2</sup><sup>1</sup>Department of Plant Protection, Namık Kemal University, Tekirdag 59030, Turkey<sup>2</sup>Department of Plant Protection, University of Çukurova, Adana 01330, Turkey

## SUMMARY

Bacterial spot, caused by *Xanthomonas axonopodis* (syn: *campestris*) pv. *vesicatoria* is a widespread disease of processing pepper in Adana and Osmaniye (eastern Mediterranean Turkey). According to our and pepper growers' observations, chemical control by copper compounds has not been effective on the disease in commercial pepper fields for the last three years. Therefore, sensitivities to copper and streptomycin among strains were tested under *in vitro* conditions. In the summers of 2002-2004, sixty-seven bacterial strains were isolated from diseased pepper plants in the region. The strains were identified as *Xanthomonas axonopodis* pv. *vesicatoria* by morphological, physiological and biochemical tests, hypersensitive reaction on tobacco (*Nicotiana tabacum* cv. Samsun N), pathogenicity tests on pepper plants (a local cultivar, Bursa Yaglik), das-ELISA and amplification of the 355 bp *hrp* genes by PCR. Of the 67 strains, all were found to be tolerant to 100 µg ml<sup>-1</sup> of copper sulfate and 7% were resistant to streptomycin (20 µg ml<sup>-1</sup>).

*Key words:* pepper, bacterial spot, copper tolerance, *Xanthomonas axonopodis* pv. *vesicatoria*.

One of the major agricultural areas for processing pepper in Turkey is located in the eastern Mediterranean region (Adana and Osmaniye cities). More than 11000 da in Adana and Osmaniye are planted to local processing pepper cultivars (Karaisali and Bursa yaglik) each year. Bacterial spot disease, caused by *Xanthomonas axonopodis* (syn: *campestris*) pv. *vesicatoria* (Doidge) Dye (*Xav*), is an important disease of pepper (*Capsicum annuum* L.) in the region (Aysan and Sahin, 2003) with 52-100% prevalence in pepper fields (Mirik *et al.*, 2005). Wet springs and summers are ideal for growth and dissemination of the bacteria.

Copper-containing compounds have been routinely applied to control the disease for more than 20 years in

the region, but this has not been effective in commercial pepper fields for the last three years, based on our and growers' observations. Use of streptomycin on transplants/plants is not allowed in Turkey. Tolerance to streptomycin and copper compounds for *Xav* has been reported in Mexico (Adaskaveg and Hine, 1985), in USA (Ritchie and Dittapongpithch, 1991; Sahin and Miller, 1996) and Barbados (Ward and O'Garro, 1992) but not in Turkey. The objective of this work was to determine the sensitivity to copper and streptomycin sulfate of *Xav* strains collected during the years 2002-2004 in the eastern Mediterranean region.

During the 2002, 2003 and 2004 growing seasons plants infected with bacterial spot were collected from the region. Surface-sterilized small pieces of leaf spots were macerated in one mL of sterile distilled water. A loopful of suspension was streaked onto yeast dextrose calcium carbonate (YDC) agar in Petri plates and incubated at 25°C for 24-48 h. A total of 67 strains isolated in the study were selected for further tests. All strains isolated were tested for pathogenicity on pepper plants, cv Bursa yaglik. Foliage of 4-week-old plants was sprayed with a suspension (10<sup>8</sup> colony-forming units (cfu) per milliliter (cfu/ml)) of the strains, with three replicates. After inoculation, plants were covered with polyethylene bags for 24 h at 28°C. The bags were removed 1 day later and the plants maintained in a controlled climate room (28°C, 70% relative humidity and 16/8 hours day/night). Disease development was evaluated 7 to 14 days after inoculation and re-isolations were made from the diseased plants. Sterile distilled water and a reference strain of *Xav* (GSPB 224) obtained from Dr. K. Rudolph (Göttingen University, Germany) were used as negative and positive controls.

Identification of the strains was initially confirmed by morphological, biochemical and physiological tests including potassium hydroxide solubility for Gram reaction, catalase reaction, oxidative/fermentative metabolism, starch and esculin hydrolysis, acid from arabinose, and HR on tobacco leaves (Lelliott and Stead, 1987; Schaad *et al.*, 2001). All tests were replicated three times. For serological tests, das-ELISA with a polyclonal antibody (Loewe, Biochemica GmbH, Sauerlach, Germany) was used to confirm identification of the strains

according as described (McLaughlin and Chen, 1990). PCRs were performed in a thermal cycler using *Xav* specific primers RST9 and RST10 for amplification of the 355 bp *hrp* gene as reported by Leite *et al.* (1995).

Strains were screened for sensitivity to copper and streptomycin sulfate as described by Ritchie and Dittapongpich (1991). Sensitivity was tested on SPA medium (20 g of sucrose, 5 g peptone, 0.5 g of dibasic potassium phosphate, and 15 g agar in 1 l of distilled water) amended with appropriate chemicals. Fresh stock solutions of copper (cupric sulfate, Merck) and streptomycin (streptomycin sulfate, Sigma) were prepared in sterile distilled water, filter-sterilized (0.22 µm pore size), and appropriate concentrations were added to SPA after autoclaving and cooling to 45°C before pouring into Petri plates. Bacterial cultures were grown on YDC for 36-72 h, suspended in sterile distilled water, and the concentration adjusted to 10<sup>8</sup> cfu ml<sup>-1</sup>. One to three microliters of the suspension were spotted on SPA, and SPA amended with different concentrations of cupric sulfate (30-150 µg ml<sup>-1</sup>) and streptomycin sulfate (20-100 µg ml<sup>-1</sup>). Plates were incubated for 36-48 h at 28°C, and presence or absence of growth was recorded.

A total of 67 bacterial strains were isolated and purified. All strains grew on YDC as round, convex, mucoid, yellow colonies typical of *Xav*. In pathogenicity tests, pepper plants inoculated with bacterial suspensions of the 67 strains and reference strains (GSPB 224) gave characteristic bacterial spot symptoms on pepper leaves in 7 to 14 days. No symptoms appeared on negative control plants. All strains were pathogenic on pepper plants cv. Bursa yaglik. Re-isolations made from artificially infected plants yielded the bacterium originally inoculated. All strains were Gram-negative and oxidative, and were positive for catalase reaction, starch and esculin hydrolysis, acid from arabinose, HR on tobacco leaves, and negative for oxidase reaction. The strains strongly reacted in das-ELISA. The mean absorbance values of three replications were between 2.592 and 2.796 at A405 wavelength. In PCR assays, a specific band at 355 bp was observed for all strains. Thus all tests were in accordance with identification of the strains as *Xav*.

In sensitivity to copper and streptomycin assays, all strains grew on SPA amended with 100 µg ml<sup>-1</sup> cupric sulfate but not on SPA amended with 150 µg ml<sup>-1</sup>. Copper resistance was found in all strains. Our data were similar to results reported by Ritchie and Dittapongpich (1991), Buonauro *et al.* (1994), and Sahin and Miller (1996) on *Xav* copper sensitivity. Only five strains of *Xav* grew on SPA amended with 20 µg ml<sup>-1</sup> of streptomycin sulfate but none grew on other concentrations of streptomycin sulfate (100 µg ml<sup>-1</sup> and 150 µg ml<sup>-1</sup>). Thus streptomycin-resistant strains were not detected in our study. Streptomycin sulfate is not allowed for agricultural use in Turkey. However, copper compounds such as Bordeaux

mixture, cupric hydroxide, various formulations of basic cupric sulfate, ammoniac copper, and copper salts have been routinely used for control of bacterial spot disease of pepper in Turkey. Standard applications of copper compounds in the region now give poor control of the disease, and the large numbers of copper-tolerant strains of *Xav* in pepper fields are probably responsible.

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