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Prevalence and Incidence of Bacterial Spot Disease Caused by *Xanthomonas campestris* pv. *vesicatoria* on Pepper in the Eastern Mediterranean Region of Turkey

Mustafa Mirik, Yesim Aysan and Ozden Cinar
Department of Plant Protection, Faculty of Agriculture, University of Cukurova, 01330 Adana, Turkey

Abstract: Bacterial spot of pepper, caused by *Xanthomonas campestris* pv. *vesicatoria,* has been epidemic in the last few years in Adana and Osmaniye, located in the eastern Mediterranean region of Turkey. Pepper plants in fields were surveyed in 2002, 2003 and 2004 for bacterial spot disease. The surveys indicated that disease prevalence was recorded as 52, 91 and 100% in 2002, 2003 and 2004. Disease incidence was higher and more severe on plants. Isolated strains during the 3-year survey in the region were identified by morphological, physiological, biochemical tests, pathogenicity on pepper plants, das-ELISA and amplification of 355 bp *hrp* genes by PCR.

Key words: Pepper, bacterial spot, survey, ELISA, PCR

INTRODUCTION

Pepper is an important vegetable with a production of 275,000 tons 2003 in Turkey[1]. Several fungal and bacterial diseases of pepper have resulted in reduced yield in the eastern Mediterranean region of Turkey. Bacterial spot disease, caused by *Xanthomonas campestris* (syn *axonopodis*) pv. *vesicatoria* (Doidge) Dye, is a major problem in the region during the recent years[2]. The disease is most severe in Adana and Osmaniye cities in the region because of the high temperatures and rainfall in summer of the last years. A local cultivar, Karaisali Salcalik, is generally grown in the field for processing pepper. The disease symptoms in the region usually appear in June on pepper plants when the conditions are favorable.

The symptoms of the disease on pepper are characterized by small, angular spots with yellow halo on leaves. The centers of the spots are dry and tear. The margins of the affected leaves are rimmed with a narrow band of necrotic tissue. Finally heavily infected leaves are dropped while still green. The lesions on fruit are blister-like, irregular, dark, raised spots, frequently surrounded by a water soaked border. When the fruit lesions enlarge, they become brown and rough and have a cracked or warty appearance. Affected fruits may not be marketable. Bacterial spot organism may survive between seasons on pepper seeds[3], volunteer plants, plant debris and weeds as epiphytes[4].

The objectives of the research were to determine the prevalence and incidence of the bacterial spot disease in the eastern Mediterranean region of Turkey in 2002, 2003 and 2004. The collected plant samples in the survey were examined and the pathogenic bacterium isolated. The isolated strains were identified by pathogenicity on pepper plants, morphological, physiological, biochemical tests, das-ELISA (Louwe, 07131) and amplification of 355 bp *hrp* genes by PCR.

MATERIALS AND METHODS

Surveys with ten-day intervals were conducted in 83 fields, at least 40 da, during June and August of 2002, 2003 and 2004 for the presence and absence of bacterial spot in Adana (Ceyhan, Karaisalı, Salbas, Pirili, Guvenc, Kuyucu, Kuzgun and Orcun, villages) and Osmaniye (Kadirli, Sunbas, Mehmetli and Armaganli villages) cities of the eastern Mediterranean region of Turkey. Ten fields in a village were surveyed. Fields were divided into about equal quadrants and 100 plants of each quadrant were rated by counting pepper plants with and without bacterial spot symptoms. The numbers of healthy and infected fields were recorded for disease prevalence in the region. The percent disease incidence was recorded for each field surveyed[5].

During the inspection of the fields, infected plants with bacterial spot were collected at randomly during the 3-year surveys in the region. The collected plants were placed in paper bags and transported to the laboratory for

Corresponding Author: Mustafa Mirik, Department of Plant Protection, Faculty of Agriculture, University of Cukurova, 01330 Adana, Turkey
Tel: + 90 322 3387274 Fax: + 90 322 3386437

isolation and identification of the causal organism. Surface-sterilized small pieces of leaf spot were macerated in one milliliter of sterile distilled water. A loopful of suspension was streaked onto YDC and Tween B mediums[6] into petri plates and incubated at 25°C for 3-14 days. Single yellow colonies on YDC and light yellow colonies surrounded by zone of white area on Tween B were selected for further tests. Pepper strains were also tested for oxidase, catalase reaction, starch and esculin hydrolysis, acid from arabinose, HR on tobacco leaves described by Schaad[7]. A reference strain of *Xanthomonas campestris* pv. *vesicatoria*, GSPB 224 (Gottingen, Germany), was used as positive control in the tests. For serological tests, das-ELISA using a polyclonal antibody (Louwe, 07131), were used to confirm the identification of the strains according to the previously described das-ELISA method[8]. PCR studies were performed in a thermal cycler using primers, RST9 and RST10 spesific for *Xanthomonas campestris* by amplification of 355 bp *hrp* genes as reported by Leite[9].

A total of 67 strains isolated in the study were tested for pathogenicity on pepper plants. Foliage of 4-week-old pepper plants Cv. Bursa Yaglik was sprayed by suspension (10^8 cfu mL^{-1}) of the strains as three replicates. The inoculated plants were placed in a high humidity environment at 28°C for 7-10 days for disease development.

RESULTS AND DISCUSSION

Results (Table 1) showed disease prevalence was 52% in 2002. Disease incidence was 10% in the same year. In contrast, 91% of the fields were affected in 2003 as 20% disease incidence. Bacterial spot symptoms on pepper plants were observed in 14 of 14 fields planted in 2004. Disease incidence was 33% in 2004. Disease prevalence was 52, 91 and 100 for three years. Disease incidence was ranged between 10 and 33%. The data presented in the research that disease prevalence have risen year by year. The disease causing economic yield loses in pepper production in the region. We think that primary inoculum of the disease may contaminated seeds. The seed lots used in the region should test for bacterial spot organism for the disease management.

Pepper cultivar, Karaisali Salcalik was common cultivars in Adana and Osmaniye cities. Other cultivars used in the villages were not heavily infected. According to our observations in many fields, pepper cultivar, Karaisali Salcalik were more susceptible to the disease. The seedlings came from not commercial nursery. The farmers produce the seedlings themselves by their methods. *Xanthomonas campestris* pv. *vesicatoria* is associated with pepper seeds[3]. The disease symptoms observe in seedlings from contaminated seeds.

Table 1: Prevalence and incidence of bacterial spot disease of pepper in the fields of the eastern Mediterranean region of Turkey during 2002 to 2004

Year	Total area (da)	Surveyed area (da)	No. of fields surveyed	No. of fields with diseased plant	Incidence (%)	Prevalence (%)
2002	750	301	23	12	10	52
2003	955	421	46	42	20	91
2004	600	373	14	14	33	100

Fields with healthy peppers were only observed in Ceyhan village for three years. In the villages pepper cultivars was not Karaisali Salcalik and seedlings came from commercial nursery. In the region, commercial nurseries inspected routinely by government.

The strains isolated in the surveys were positive for catalase reaction, starch and esculin hydrolysis, acid from arabinose, HR on tobacco leaves and negative for oxidase reaction. Isolated 67 strains were pathogenic on pepper plants cv. Bursa Yaglik. The strains strongly reacted with the polyclonal antisera in das-ELISA tests. The mean absorbance values of three replications in das-ELISA tests were between 2.592 and 2.796 at A_{405} wavelength. In the PCR study, a specific band at 355 bp was observed for all the strains. According the test results, the strains were identified as *Xanthomonas campestris* pv. *vesicatoria*.

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REFERENCES

1. FAO., 2004. www.fao.org
2. Aysan, Y. and F. Sahin, 2003. Occurrence of Bacterial Spot Disease, Caused by *Xanthomonas axonopodis* pv. *vesicatoria*, on Pepper in the eastern Mediterranean region of Turkey. Plant Pathol., 52: 781.
3. Bashan, Y., Y. Okan and Y. Heins, 1982. Long-term survival of *Pseudomonas tomato* and *Xanthomonas vesicatoria* in tomato and pepper seeds. Phytopathology, 72: 1143-1144.
4. Jones, J.B., K.L. Pohronezny, R.E. Stall and J.P. Jones, 1986. Survival of *Xanthomonas campestris* pv. *vesicatoria* in Florida on tomato crop residues, weeds, seeds and volunteer tomato plants. Phytopathology, 76: 430-434.

5. Ward, H.P. and L.W. O'Garro, 1992. Bacterial spot of pepper and tomato in Barbados. Plant Dis., 76: 1046-1048.
6. McGuire, R.G., J.B. Jones and M. Sasser, 1986. Tween media for semiselective isolation of *Xanthomonas campestris* pv. *vesicatoria* from soil and plant material. Plant Disease, 70: 887-891.
7. Schaad, N.W., J.B. Jones and G.H. Lacy, 2001. Gram negative bacteria, *Xanthomonas*. In: Laboratory Guide For Identification Of Plant Pathogenic Bacteria (Eds., N.W. Schaad, J.B. Jones and W. Chun), pp: 175-193. APS Press, St. Paul Minnesota.
8. McLaughlin, R.J. and T.A. Chen, 1990. ELISA Methods for Plant Pathogenic Prokaryotes. In: Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens, A Laboratory Manual (Eds., R. Hampton, E. Ball and S. De Boer), pp: 197-204. APS Press, St. Paul Minnesota.
9. Leite, R.P. Jr., J.B. Jones, G.C. Somodi, G.V. Minsavage and R.E. Stall, 1995. Detection of *Xanthomonas campestris* pv. *vesicatoria* associated with pepper and tomato seed by DNA amplification. Plant Dis., 79: 917-922.