Pak.J.Bot., 27(2): 447-450, 1995.

PATHOGENICITY SPECTRUM OF *XANTHOMONAS CAMPESTRIS* PV. *CITRI STRAINS* IN PAKISTAN

M.A. AKHTAR, M.H. RAHBER-BHATTI* AND M. ASLAM

Crop Diseases Research Institute,
National Agricultural Research Centre, Islamabad, Pakistan.

Abstract

Relative aggressiveness of eight strains of *Xanthomonas campestris* pv. *citri* was tested on detached citrus leaves at 22°C and of another seven at 24°C. All the strains showed variability in their relative aggressiveness as measured through development of lesions. Number of lesions produced by any strains was significantly affected by the strain: cultivar interaction thus indicating host parasite specificity for this character. Three levels of aggressiveness i.e. high, intermediate and low aggressiveness were noticed among the strains.

Introduction

Citrus bacterial canker disease (CBCD) caused by strains of *Xanthomonas campestris* pv. *citri* (Hasse) Dye, is a destructive disease that seriously affects most commercially important citrus cultivars (Wakimato, 1967; Civerol & Fan, 1982; Bach *et al.*, 1983; Stall & Seymor, 1983; Civerolo, 1985; Hartung & Civerolo, 1989). In Pakistan, CBCD is prevalent throughout the country and is causing considerable quantitative and qualitative damages to citrus fruits (Akhtar *et al.*, 1993). Variablity in aggressiveness of Asian strains of the pathogen has been reported from other citrus growing countries (Civerolo, 1984, 1985). Host parasite interaction in CBCD with respect to process of infection, lesion development and lesions extension has been described by Koizumi (1976). Pathogenic variation within *X. campestris* pv. *citri* has been evaluated by reactions of artificially inoculated leaves of various citrus species, including hybrids and their relatives (Khan & Hingorani, 1970; Serizawa, 1971). Pathogenicity among 15 strains of *X. campestris* pv. *citri* were characterized as severe, mild and low based on the reaction of *Murraya exotica*. Among the four forms of CBCD, CBCD-A strains were found more aggressive than CBCD-B, CBCD-C and CBCD-D forms (Alcaraz, 1982, 1986). The present paper deals with spectrum of pathogenicity in *X. campestris* pv. *citri* isolates from Pakistan.

Materials and Methods

A detached leaf assay was performed to determine relative aggressiveness in strains of *X. campestris* pv. *citri*. Bacterial strains CDRI,NARC XC-93, XC-97, XC-98, XC-101, XC-124, XC-126, and XC-129 were grown on yeast dextrose calcium carbonate agar (YDCA) for 72h and a loopful of growth suspended in phosphate buffered saline (PBS) and adjusted to A590= 0.1. The suspension contained 10^8 cfu\ml

*Department of Plant Pathology, Sindh Agricultural University, Tandojam, Pakistan.

Table 1. The number of lesions on detached leaves of different citrus cultivars inoculated with various isolates of *Xanthomonas campestris* pv. *citri* following pin prick method incubated at 22°C.

Cultivers	Bacterial Strains XC-49	XC-51	XC-53	XC-57	XC-58	XC-60	XC-61	XC-62	Mean
Musambi/sour orange	6.00	6.00	1.67	4.33	4.67	6.00	4.67	6.00	6.16
Kinow/Rough lemon	6.00	5.00	4.67	7.33	8.00	5.67	9.00	6.00	5.25
Blood Red/Sour orange	7.33	6.33	6.67	6.00	7.33	5.33	9.00	5.33	6.54
Kinow/Sour orange	8.33	5.00	4.67	9.00	5.00	4.33	5.33	4.67	5.75
Fentrell's early sour orange	7.00	3.67	5.00	5.00	5.00	5.33	3.33	6.33	5.41
Fentrill's early/ rough lemon	4.33	5.00	5.67	5.00	5.67	3.33	8.00	5.33	5.29
Musambi/rough lemon	3.33	3.67	4.00	3.67	3.001	3.67	5.67	3.67	6.04
Blood Red/rough lemon	6.00	7.67	7.00	9.00	7.67	8.33	7.33	8.67	5.79
Mean·	3.83	5.29	5.08	5.41	7.71	6.45	6.66	5.79	---

LSD value for citrus cultivar = 0.665

LSD for bacterial isolates = 0.665

LSD value for interaction = 1.882

as determined by dilution plating on YDCA. This inoculum was serially diluted 100 fold in sterile PBS and used to inoculate leaves collected from Citrus cultivars (CVS) Kinnow\Rough lemon, Hamlin, Pixie, Frost Marsh and *M. exotica*. The detached leaves were placed with abaxial side upward on four layers of paper towel and inoculated with a multiple pin mount charged with inoculum of bacterial suspension. On each of five leaves 20 sites were inoculated to determine the number of cfu infiltrated at each site. The inoculated leaves were dried in a laminar air flow hood till water-soaking disappeared. The leaves were then surface sterilized with 1% sodium hypochlorite containing 0.2% sodium dodecyl sulfate for 3m. This treatment removes bacteria from leaf surfaces without toxicity to bacteria present within leaf tissue. After five rinses with sterile water, the leaves were ground in 10 ml of water in a mortar with a pestle. The number of cfu in the homogenate was determined on nutrient glucose agar. Approximately 70 cfu were infiltrated at each site. In another set, leaves were inoculated with the bacterial strains as mentioned earlier and kept in a growth room at 24°C under 12h illumination. There were three leaves/plate with three replicates of each treatment. Number of lesion developed on the inoculated sites were counted after 14 days incubation. The experiment was repeated twice.

In another experiment conducted in a growth chamber at 22°C under 12h illumination, young fully expanded leaves of citrus cvs. Musambi/Sour orange, Kinnow/Rough lemon, Blood red/Sour orange, Kinnow/Sour orange. Feutrell's Early /Sour orange, Feutrell's early/Rough lemon, Musambi/Rough lemon and Blood red/Rough lemon were inoculated with bacterial strains XC-49, XC-51, XC-53, XC-57, XC-58, XC-60, XC-61 and XC-62 containing 10^8 cfu/ml in PBS with multiple pin mount. Three leaves/ plate were kept on two glass slides on four layers of paper towel saturated with PBS. Each treatment was replicated three times. Data on lesion development was recorded 14 days after inoculation and analyzed statistically.

Results and Discussion

All strains used in the present study exhibited differences in their relative aggressiveness against different test cvs. On the basis of mean number of lesions produced on all test cvs, strain XC-58 appeared to be most aggressive, followed by strains XC-60 and XC-61, while XC-49 appeared to be least aggressive (Table 1). Strains XC-58 and XC-61 were most aggressive on cv. Kinnow /Rough lemon as they produced the highest number of lesions per leaf. Similarly the behavior of XC-61 was identical against kinnow/rough lemon and Blood red/Sour orange. Of the strains tested at 24°C, XC-98 was more aggressive than other strains (Table 2). More number of lesions were formed on frost marsh than *M. exotica* and Hamlin. The symptom produced by XC-98 on frost marsh became visible within 5-7 days, but developed more slowly on *M. exotica*. Average number of lesions on kinnow/rough lemon produced by various strains were different significantly ($P < 0.01$). XC-101 appeared more aggressive as it formed more lesions as compared to XC-97 on kinnow/rough lemon (Table 2). Strain XC-124 and XC-129 showed identical reaction on frost marsh, but their response differed to pixie, Hamlin, kinnow/rough lemon and *M. exotica*.XC-93

Table 2. The number of lesions on detached leaves of different citrus cultivars inoculated with different strains of *Xanthomonas campestris* pv *citri* by pin prick method and inoculated at 24°C

	Bacterial Strains							
Cultivars	XC-93	XC-126	XC-97	XC-124	XC-98	XC-129	XC-101	Mean
Kinow/rough lemon	18.67	12.00	9.33	16.67	15.67	17.00	19.00	15.47
Hamlin	13.67	7.67	16.00	8.67	19.00	14.00	15.67	13.52
Pixie	14.33	7.67	12.00	15.00	17.33	17.67	18.67	14.66
Frost Marsh	13.33	9.00	13.33	18.00	19.33	18.00	9.67	14.38
Murraya exotica	8.33	3.33	8.67	6.00	8.00	7.00	7.00	6.90
Mean	13.66	7.33	11.86	12.86	15.86	14.73	14.00	--

LSD for citrus cultivar = 1.23

LSD for bacterial isolates = 1.459

LSD for interaction = 3.263

and XC-97 were not significantly different with respect to mean number of lesion on frost marsh and *M. exotica*, both these strains were significantly different in response to other cultivars. XC-126 appeared to be less aggressive on all the cultivars. *M. exotica* was found more resistant to XC-126. Variability in aggressiveness has also been reported from other citrus growing countries (Civerolo, 1984, 1985).

The differential behavior of strains against host cultivars indicates presence of genetic specificity between factors controlling aggressiveness in these strains and the corresponding resistance in the cvs. It is generally believed that species used as stock contributes towards making the scion resistant to CBCD (Khan & Hingorani, 1970; Serizawa, 1971). The data in Tables 1 and 2 not only support this observation but also indicate that phenomenon of stock's contributing towards scion's resistance to the disease is also dependent on the stock scion combination. Where all strains were less aggressive against Kinnow/sour orange as compared to Kinnow/rough lemon, they exhibited similar aggressiveness against red blood irrespective of whether it was budded on sour orange or rough lemon.

References

Akhtar, M.A., M.I.Haque and M.H.Rahber-Bhatti. 1993. Bacterial diseases a menace to crop productivity in Pakistan. *Sci. Technol. & Devel.*, 12:22-24.

Alcaraz, F.L. 1982. Inoculation with *Xanthomonas citri* by low pressure spraying. *Fitopatologia*, 17: 15-20.

Alcaraz, F.L. 1986. Multiplication of *Xanthomonas campestris* pv. *citri* in the leaf tissue of different citrus species. *Fitopatologia* 21: 52-60.

Bach, E.E., A.P.C. Alba and J.R. Neto. 1983. Detection of strains of *Xanthomonas campestris* pv. *citri* (Hasse) Dowson by enzyme-linked immunosorbent assay. *Fitopatol. Brasileiva*, 7: 407-415.

Cliverolo. E.L. and F. Fan. 1982. *Xanthomonas campestris* pv. *citri* detection and identification by enzyme-linked immunosorbent assay. *Plant Dis.*, 66: 231-236.

Civerolo, E.L. 1984. Bacterial canker disease of citrus. *J. Rio. Grande Vol. Hortic Soc.*, 37: 127-146.

Civerolo, E.L. 1985. Citrus bacterial canker disease. The bacterium *Xanthomonas campestris* pv. *citri*. *An. Int. Perspective*, 11-17.

Hartung, J.S. and E.L. Civerolo. 1989 . Restriction fragment length polymorphism distinguish *Xanthomonas campestris* strains isolated from Florida citrus nurseries from *X.c.* pv. *citri*. *Phytopathology*, 79: 793-799.

Khan, I.D. and M.D. Hingorani. 1970. Strain studies on *Xanthomonas citri* (Hasse) Dowson. *J.Hort. Sci.*, 45: 15-17.

Koizumi, M. 1976. Behaviour of *Xanthomonas citri* (Hasse) Dowson in the infection process. 1. Multiplication of the bacteria and histological changes fallowing needle-prick inoculation. *Ann. Phytopath. Soc. Japan*, 42: 407-416.

Stall, R.E. and C.P. Seymor. 1983. Canker, a threat to citrus in the Gulf coast States. *Plant Dis.*, 67: 581-585.

Serizawa, S. 1971. Studies on citrus canker. II. Pathogenicity of the *Xanthomonas citri* strains. *Bull. Shizuoka Pref. Citrus Exp. Stn.*, 9=89-96.

Wakimoto, S. 1967. Some characteristics of citrus canker bacteria. *Xanthomonas citri* (Hasse) Dowson and the related phages isolated from Japan. *Ann. Soc. Japan*, 33: 301-310.

(Received for Publication 2 February, 1995)