Kasetsart J. (Nat. Sci.) 31 : 381 - 384 (1997)

Infectivity Titrations of *Pseudomonas solanacearum* on Tomato

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ABSTRACT

The dose of *Pseudomonas solanacearum* was controlled by injecting various amounts of bacteria directly into the plant. Ten varieties of tomato (30 days old) were injected with 6 levels of *Pseudomonas solanacearum* (10^3 - 10^8 cfu/ml) by a micropipette containing 0.1 ml (100 µl). Each inoculum was inserted diagonally into stem at the third leaf axil from the top. Finally the total number of bacteria in each plant was 10^2 - 10^7 cfu/ml. Disease index rating were recorded daily for 3 weeks. The results indicated that the varieties 245, 373, 390 and TK-70 were susceptible (S). Most of them had ED_{50} value around 10^2 - 10^4 cfu/plant. On the opposite variety 285 was resistant (R). The varities 8, 15 and 366 were moderately resistant (MR) to moderately susceptible (MS). The degree of resistance of variety 95 is correlated to the concentration of the inoculum. At low concentrations of bacteria, this variety showed resistant but became more susceptible rapidly when the concentration of bacteria increased. On the other hand, the variety 96 had little or no correlation between the concentration of bacteria and % wilt. This experiment showed that Micropipette technique was highly accurate in evaluating bacterial wilt resistance since it could determine the effect of dose on the degree of wilt in each tomato variety. This technique was recommended for intensive work.

Key words : *Pseudomonas solanacearum*, screening, wilt resistant, tomato, micropipette technique, infectivity titration

INTRODUCTION

Bacterial wilt of tomato caused by the soil borne pathogen *Pseudomonas solanacearum* limits tomato production in both tropical and subtropical regions. A wide host range has been described for the pathogen, but the strains attacking solanaceous crops are a serious pathogen specifically in Southeast Asia. Chemical control of the disease has been attempted with little success (Kelman, 1953). Resistant varieties, thus, offer an effective means to control this disease. Studies on screening technique is very important to evaluate resistant varieties. There are many inoculation procedures such as clipping technique [clipping of the leaf with a pair of scissors dipped previously in bacterial suspension (McCarter, 1973)], stem inoculation method (forcing a sharp needle into the stem through a drop of bacterial suspension which was placed in the axil of the second or third expanded leaf below the stem apex.), root inoculation technique [cutting the lateral roots with a scalpel along one side of the plant to a depth of approximately 4 cm and pouring 10 ml. of bacterial suspension over the lateral roots, or dipping the roots in bacterial suspension (Winstead and

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Kelman, 1952)], *etc.* In each procedure as described above the exact number of bacterial cells which invade into plant cannot be calculated. Some susceptible plant may not show wilt symptom because no or not enough bacteria invades the plant to develop wilt symptom.

This experiment was attempted to control the dose of *Pseudomonas solanacearum* by injecting various amounts of bacteria directly into the plant and determine the effect of dose through the degree of wiltness in the plant.

MATERIALS AND METHODS

Ten varieties of 30 days old tomato seedling and 6 levels (10^3 - 10^8 cfu/ml) of bacterial concentrations were used in this experiment.

Isolate of *Pseudomonas solanacearum* TBW_1 (from tomato) were maintained in sterile distilled water and kept in a cooler at 13°C. The inoculum was prepared from virulent isolate selected in TZC medium (Kelman, 1954) and increased on TZC medium without 2,3,5 triphenyl

Table 1 Tomato varieties and probit analysis.

AVRDC acc. no.	Varietal name	Intercept	Slope	Chi-square (4 dif.)	LD_{50}	Max. prop.	Min. prop.	Disease reaction
L8	VC 91 VG_1	3.011346283	2.691540969	3.422159109	7.388532	0.4	0.05	MR-MS
L15	8-1-2-9	3.568728726	1.327434043 E-01	3.49701602	10.78224	0.3	0.05	MR-MS
L95	Venus	1.091512321	4.475172642 E-01	1.76886722	8.733713	0.2	0	R-S
L96	Saturn	3.962015854	3.656063846 E-02	5.232832061	28.39075	0.3	0.1	MR
L245	KL-1	2.945701294	4.801566469 E-01	21.36444754	4.278392	0.8	0	S
L285	Changs	1.490323379	2.631328616 E-01	2.637306853	13.33803	0.05	0	R
L366	Ohio MR-13	3.879196103	0.144863298	6.896335399	7.736976	0.5	0.15	MR-MS
L373	East-North	4.432539449	2.344850267 E-01	2.831802119	2.420028	0.8	0.35	S
L390	Nan-Tzu (local var.)	3.374312864	0.44425453	18.24912896	3.659359	1	0.15	S
L4077	TK-70	2.820441317	0.923550789 E-01	13.5416676	4.426802	0.85	0.05	S

R = resistant
MR = moderately resistant
MS = moderately susceptible
S = susceptible

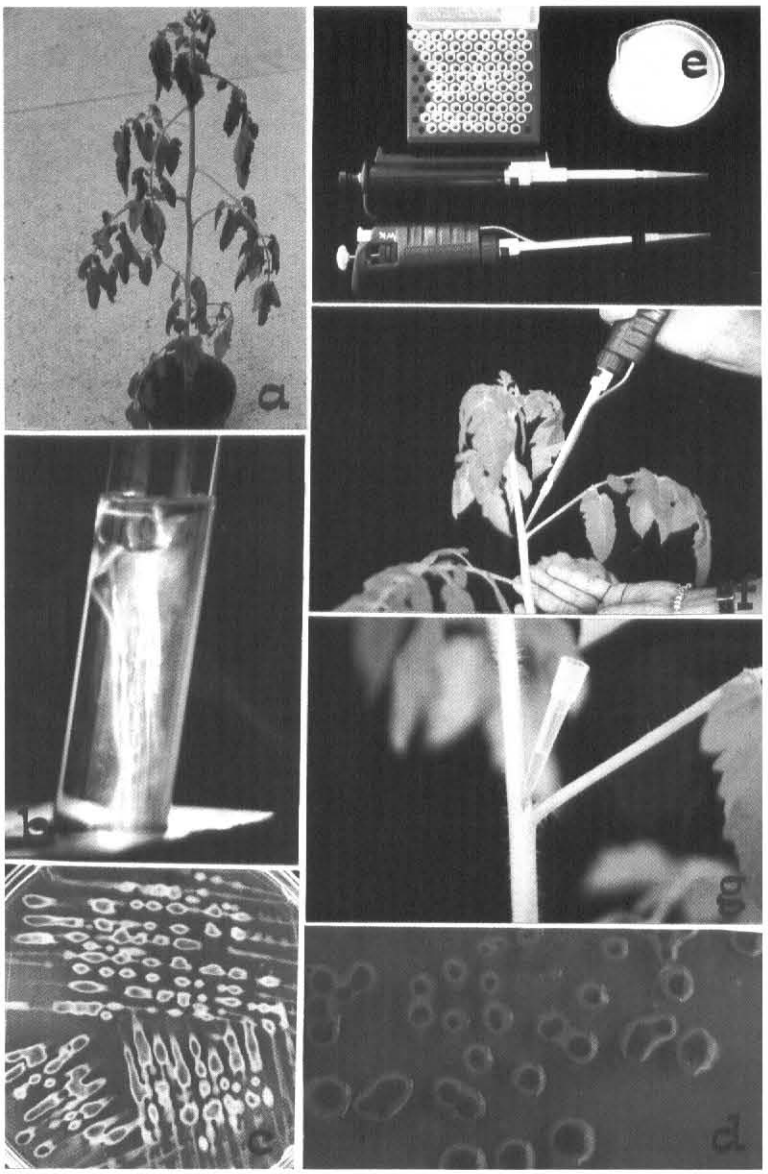

Figure 1 a. Diseased tomato shows leaf epinasty and wilting symptom while leave still green
b. Bacterial exudate from diseased plant
c. Virulent colony (wild type) of *P. solanacearum* on TZC medium formed an irregularly-round, fluidal, white colony with a pink center
d. Non-virulent colony (mutant type) of *P. solanacearum* on TZC medium formed a round, butyrous, deep red colony with a narrow bluish border
e. Micropipette, plastic tip and bacterial suspension for use in Micropipette technique
f. Bacterial suspension was inserted diagonally into stem at the third leaf axil from the top
g. Uptake of the inoculum by the stem normally was completed in 3-4 hrs.

tetrazolium chloride for 48 hrs. at 30°C and then suspending them in sterile distilled water (SDW) to reach $OD_{600\ nm}$ = 0.2 (2.17 x 10^8 cfu/ml). Serial dilutions were made to provide 6 grade inoculum levels ranging from 10^3 - 10^8 cfu/ml. The concentration of bacteria at each inoculum level was determined by standard dilution plating. A micropipette containing 0.1 ml (100 µl) of inoculum was inserted diagonally into stem at the third leaf axil from top. Finally the total number of bacteria in each plant was 10^2 - 10^7 cfu/ml. To facilitate insertion of the micropipette, a sterile wire with the diameter slightly wider than that of the pipette was inserted and withdrawn from the stem at the same site. The uptake of the inoculum by the stem normally was completed in 3-4 hrs., after that the pipette was removed. Twenty plants of each variety were tested at each of the 6 inoculum levels. After inoculation, the plants were transferred to growth room of 28-38°C (night and day). Disease index ratings were recorded daily and the final record were made 21 days after the inoculation. L 390 variety was as a susceptible check. The data was analyzed using probit analysis and a probit line was fitted (probit of incidence of wilt as a function of log concentration) using a BASIC computer program.

RESULTS AND DISCUSSION

When 10 varieties of tomato were evaluated by injection 6 levels of *Pseudomonas solanacearum* (10^3 -10^8 cfu/ml) directly into the plant, the results from probit analysis in Table 1 indicated that the varieties 245, 373, 390 and TK-70 were susceptible. All of them had a bad fit (large chi-square) and high slope (about 0.4) except variety 373 had a good fit and low slope. Most of them had ED_{50} value around 10^2 - 10^4 cfu/plant. On the opposite variety 285 was resistant. It had low slope and low maximum wilt (0 - 0.05). ED_{50} was outside of the range of concentrations used. The varieties 8, 15 and 366 were intermediate between the moderately resistant and the moderately susceptible. They had low slope, intercept greater than 3, chi square indicated a reasonable fit and maximum to minimum wilt around 0.5 to 0.05. The variety L-95 had a low intercept, high slope (around 4.4) and maximum wilt at 0.2. This means that the degree of resistant of this variety was correlated to the concentration of the inoculum. At low concentrations of bacteria, this variety was resistant but became more susceptible rapidly when the concentration of bacteria increased.The variety L-96 had a high intercept (nearly that of the susceptibles) but very low slope (only 0.036) and maximum wilt only 0.2 which suggested that there was little or no correlation between concentration of bacteria and % wilt.

ACKNOWLEDGEMENT

The author sincerely thanks Dr. Jonathan E. Yuen for his excellent advice for this experiment. This work was financially supported by AVRDC.

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