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Relationship between the Population of *Ralstonia solanacearum* in Soil and the Incidence of Bacterial Wilt in the Naturally Infested Tobacco Fields

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The population of *Ralstonia solanacearum* (*Rs*) in soil is very important as a primary inoculum source of bacterial wilt in tobacco fields. To investigate the population of *Rs*, physical properties and chemical components during the tobacco growing season, soil samples were taken from the fifteen fields which were located in the flue-cured tobacco growing area, Ansung, Kyunggi province and Wonju, Kangwon province. Two fields of the fifteen were bacterial wilt free. Six fields had less than 10% plants being diseased and seven over 10%. The *Rs* population level determined by using SMSA medium generally showed an up-and-down pattern being low in May, high in Jun and July and low in August. The soil population in May and June showed a positive correlation with the incidence of bacterial wilt (r=0.571*, r=0.688), but P_2O_5 content of soil was negatively correlated with the disease incidence (r= -0.539*). These results suggest that *Rs* population in soil examined in May or in June, and the P_2O_5 content in soil should be key factors to determine the bacterial wilt potential of tobacco fields.**

Keywords : Bacterial wilt, *Ralstonia solanacearum*, Tobacco

Bacterial wilt which is one of the most devastating tobacco diseases is widely spread in tobacco growing regions of the world. The causal agent, *Ralstonia solanacearum*, is a soil-borne plant pathogen. With over 450 host species belong to more than 50 botanical families, this bacterium has an unusually wide host range (Hayward, 1991; Lucas, 1975). There have been the numerous control measures developed, but no single practice provides the complete control of bacterial wilt (Melton et al., 2004). Therefore an effective management program, which includes crop rotation, nematodes management, chemical application, resistant cultivars, and proper cultivation, stalk and root destruction after harvest is needed for efficient management of bacterial wilt (Lucas, 1975; Shew and Lucas, 1991; Fortnum and Martin, 1998).

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In Korea, the first case of bacterial wilt was reported in 1904 and thereafter the disease has been spread nation-wide. In the recent years, it has been increased 5 to 10% every year, especially in the flue-cured tobacco growing area (Kang et al., 2002). So it is important for the Korean tobacco growers to control the disease efficiently.

The aim of this study was to evaluate the influence of naturally infested soil population of *R. solanacearum* on the severity of bacterial wilt in relation to physical properties and chemical components of the tobacco field soil.

Materials and Methods

Selection of fields surveyed and sampling field soil. For the research, fifteen commercial tobacco fields in the flue-cured tobacco growing area of Korea, 6 fields in Samjuckmyon Ansung of Kyunggi province and 9 fields in Buronmyeon Wonju of Kangwon province, were selected. We classified them on the basis of the rate of wilt disease incidence: 5 fields below 5% of incidence, 5 fields' 5 to 20% and 5 fields over 21%, respectively. Soil samples were taken about 1 kg at 5 m interval to the depth of 5-15 cm at 5 randomly chosen sites within 2-4 lows located at the center of the tobacco planted fields. The collected soil samples from same field were mixed well and each vinyl bag (25 × 30 cm) contained 2 kg mixed soil was made. They were moved into a laboratory and were dried for 24 hrs at the room temperature in the shade. Then they were used as a sample for assessing bacterial population, and analyzing soil physical and chemical properties.

Media preparation. To determine the population of *R. solanacearum* and total bacteria, a semi-selected medium, SMSA (Englebrecht, 1994; French, et al., 1995) and nutrient agar were used. The SMSA was prepared as follows: 10.0 g Bacto Peptone (Difco), 5.0 ml Glycerol, 1.0 g Casamino acid, 15.0 g Bacto agar added to 1000 ml distilled water. After autoclaved for 15 min. at 121°C, and then added 25 mg (about 1250 U) Bacitracin (Sigma B-0125), 100 mg (about 600,000 U) Polymyxin B sulfate (Sigma P-1004), 5 mg Chloramphenicol (Sigma C-3175), 0.5 mg Penicillin-G (Sigma P-3032), 5 mg Crystal Violet, 50 mg 2,3,5,-triphenyltetrazolium chloride to the melted medium at a temperature of 50°C.

Determination of bacterial population in soil. The population of *R. solanacearum* and total bacteria were monitored monthly with SMSA and Nutrient agar (Difco). For this purpose, each 10 g soil was removed from soil samples described above. It was

suspended in 90 ml of sterilized water and then the suspension was shaken in rotary shaker (120 rpm) for 30 min. It was diluted serially in sterile distilled water, and then aliquots (100 μl) from 10-fold dilutions were spread with a glass rod on the surface of each plate of SMSA and nutrient agar. The plates seeded were incubated at 28 for 72 hrs and then colonies were counted.

Measurement of soil properties. Collected soil samples sieved (2 mm) for determination of pH, total N, and extractable P, Ca, and Mg. Soil pH were measured in a 1:2 soil/ water slurry. N was measured with a LECO C&N 2000 Analyzer (LECO Corp., St. Joseph, MI). Samples were combusted at 1350°C. Extractable P, Ca and Mg were extracted with Mehlich-3 solution, and concentrations were determined with a thermo Jarrell-Ash inductively coupled plasma spectrometer (Thermo Jarrell-Ash, Grand Junction Co.).

Results

The incidence of bacterial wilt was surveyed in early August. Bacterial wilt was not observed in the two fields. The incidence of bacterial wilt was below 10% in seven fields, and over 10% in six fields (Table 1).

Before the detection of the population of *R. solanacearum* in field soils, sensitivity of SMSA medium for detection of *R. solanacearum* was estimated. After bacterial suspension, 1.7×10^9 cfu/ml was added one ml/g of soil to three different soil samples, respectively. By the same bacterial suspension inoculation test, 1.7×10^9 cfu/ml of the bacterium was detected on nutrient agar plates, while 2.2×10^8 cfu/ml of it was detected on SMSA agar plates (Table 2).

The level of average *Rs* population was high in June and July, and low in August. In September, when tobacco was harvested, it reached to the highest level. The range of *Rs* population detected from soil samples with SMSA medium was varied from undetectable level (below $\times10^1$ cfu/g) to 5.0×10^4 cfu/g of oven dried soil. Bacterial wilt in surveyed fields had developed from mid June when average temperature was over 21°C and the disease incidence had sharply increased in August when tobacco was in harvest (Fig. 2).

Table 1. Information of tobacco fields surveyed in this study

Field code	Site	Soil type	Disease incidence (%)
AS-1	Samjuk-myon, Ansung, Kyunggi	Sandy loam	4.5
AS-2	〃	Sandy loam	5.0
AS-3	〃	Sandy loam	6.5
AS-4	〃	Sandy loam	0.0
AS-5	〃	Sandy loam	62.5
AS-6	〃	Sandy loam	2.0
WB-1	Buron-myon, Wonju, Kangwon	Sandy loam	6.0
WB-2	〃	Sandy loam	12.5
WB-3	〃	Loamy sand	20.0.
WB-4	〃	Loamy sand	2.0
WB-5	〃	Sandy loam	26.5
WB-6	〃	Sandy loam	1.5
WB-7	〃	Sandy loam	0.0
WB-8	〃	Loamy sand	18.5
WB-9	〃	Sandy loam	62.5

Table 2. Bacterial population detected on the media used in this study

Media	Colony forming unit/g[a]			
	Rs	*Rs*+Soil I[b]	*Rs*+Soil II	*Rs*+Soil III
Nutrient agar	1.7×10^9	2.0×10^8	1.0×10^8	3.0×10^8
SMSA	2.2×10^8	1.2×10^8	3.3×10^7	5.0×10^6

[a] 10 g of soil diluted to 100 ml of sterile distilled water and smeared 0.1 ml aliquots of each diluent on the agar plates.
[b] 10 ml of *Ralstonia solanacearum* (*Rs*) suspension (1.7×10^9 cfu/ml) put into the diluents of sterilized water and field soil.

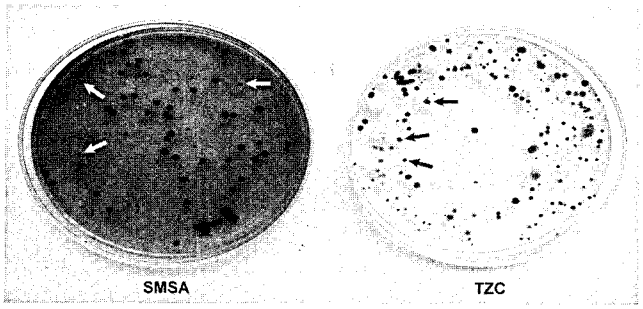

Fig. 1. Comparison of the SMSA (Semi-selective agar) medium for the growth of *Ralstonia solanacearum* (colonies indicated by the arrow) and the inhibition of soil microbes with the TZC (2, 3, 5, triphenyltetrazolium chloride) agar medium from Kyunggi sandy loam soil.

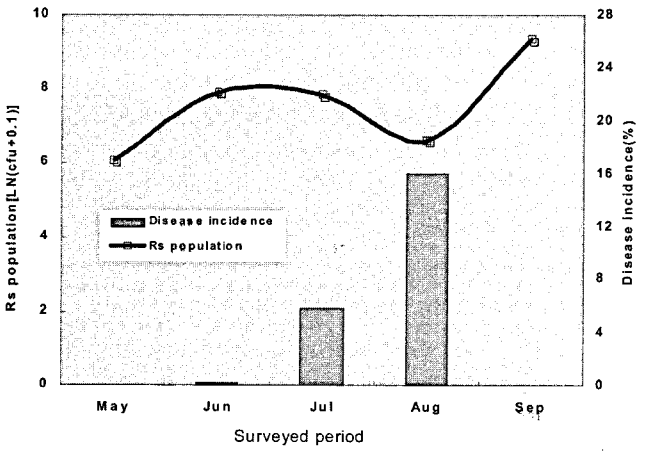

Fig. 2. Seasonal changes of *Ralstonia solanacearum* population in tobacco fields and bacterial wilt incidence.

Table 3. Correlation between the soil population of *Ralstonia solanacearum* and the incidence of bacterial wilt in tobacco fields

Surveyed period	Correlation coefficient
May	r = 0.571*
June	r = 0.688**
July	r = 0.331
August	r = 0.413

Table 4. Relationships of the soil population of *Ralstonia solanacearum* and the bacterial wilt incidence with the soil chemical components

Chemical components	Correlation coefficient	
	R. solanacearum population*	Disease incidence
pH	-0.450	-0.075
EC	0.060	-0.067
N	-0.069	-0.170
P_2O_5	-0.303	-0.539*
K	0.429	0.248
Ca	-0.219	-0.098
Mg	-0.142	0.042
OM	-0.285	-0.493

*Soil Rs population determined in mid May.

There was a positive correlation between soil *Rs* population and the incidence of bacterial wilt in May (r=0.571*) and in June (r=0.688**) (Table 3).

Relationship between soil properties and bacterial wilt incidence was shown that soil phosphate (P_2O_5) content was negatively correlated with the disease incidence (r=-539*) (Table 4).

Discussion

It is important to understand the survival mechanisms of *Ralstonia solanacearum* (*Rs*) in soil and how it affects the occurrence of bacterial wilt for working out a good control strategy. We examined monthly the soil *Rs* population changes of the tobacco fields with SMSA medium having been developed by Elphinstone (1997).

Rs population was grown about ten fold less in colony forming unit on the SMSA medium than in that on nutrient agar. This suggests that chemicals and antibiotics added to the medium for assisting selectivity. The selective media can be a helpful tool in studying the ecology of plant pathogens (Chen and Echandi, 1982; Granada and Sequeira, 1983; Elphinstone et al., 1997; Ito et al., 1998). But the greatest limitation in the study of soil bacteria is the difficulty in detecting low populations (DeBoer, 1982). However, several selective media have been reported which may assist in the differentiations of *R. solanacearum* (Schroth et al., 1979). Several limitations of selective media used for determination of soil Rs population still remain, even if it has been recently developed. Therefore more specific and sensitive detection and quantification technique such as introducing molecular biological methods is required to develop for better understanding the ecology of *Rs* in soil. *R. solanacearum* race-specifically vary in capacity to survive in soil (Schroth et al., 1979). Survival of the pathogen is the greatest in wet but well-drained soils, whereas being adversely effected by soil desiccation and by flooding (Hayward, 1991). In this study, low level of *Rs* population was generally recovered from soil samples collected in May. This may be the result that the viable cells of the pathogen converted to the viable-but-nonculturable (VBNC) state or that they went to dead by resisting low temperature, starvation and dehydration through winter and spring (van Elsas et al., 2000; Grey and Steck, 2001; van Overbeek et al., 2004). The *Rs* population level was high in June and July, low in August and the highest in September. This is relevant to the rainfall (soil moisture content) during the period before sampling (data not presented). The soil *Rs* population in May and June was positively correlated with the disease incidence, but it was difficult to explain clearly.

Nitrate-N at high concentration depressed growth of pathogen and wilt development in tobacco (Lucas, 1975). But in this study, soil phosphate content negatively affects on bacterial wilt incidence. More detailed study is required for explaining clearly the reason. Improving selective media and a method for measuring *Rs* population present low in soil, *Rs* population from soil will give us key information to estimate the bacterial wilt potential of the fields.

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