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MicroTom – A Model Plant System to Study Bacterial Wilt by *Ralstonia solanacearum*

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MicroTom is a miniature tomato plants with various properties that make it as a model system for experiments in plant molecular biology. To extend its utility as a model plant to study a plant - bacterial wilt system, we investigated the potential of the MicroTom as a host plant of bacterial wilt caused by *Ralstonia solanacearum*. We compared the disease progress on standard tomato and MicroTom by two inoculation methods, root dipping and soil drenching, using a race 1 strain GMI1000. Both methods caused the severe wilting on MicroTom comparable to commercial tomato plant, although initial disease development was faster in root dipping. From the diseased MicroTom plants, the same bacteria were successfully reisolated using semiselective media to fulfill Koch's postulates. Race specific and isolate specific virulence were investigated by root dipping with 10 isolates of *R. solanacearum* isolated from tomato and potato plants. All of the tested isolates caused the typical wilt symptom on MicroTom. Disease severities by isolates of race 3 was below 50% until 15 days after inoculation, while those by isolates of race 1 reached over 50% to death until 15 days. This result suggested that MicroTom can be a model host plant to study *R. solanacearum* - plant interaction.

Keywords **:** bacterial wilt, MicroTom, *Ralstonia solanacearum*

Model plants, such as *Arabidopsis*, rice, and tomato plants, have contributed to the advanced in plant molecular biology. The major reasons for the model plants are small plant size, small genome size, short life cycle and the availability of a transformation system (Izawa and Shimamoto, 1996; Leutwiler et al., 1984). Tomato (*Solanum lycopersicum*) is a good model plant and an economically important plant. *Agrobacterium*-mediated transformation of tomato plants has been reported but in relatively low transformation efficiency (McCormick et al., 1986). The low transformation efficiency could be an obstacle to advance tomato genetics and functional genomics as a model plant. However, a high-throughput transformation system has recently been developed in a dwarf tomato cultivar MicroTom (Dan et al., 2006; Sun et al., 2006). The *Agrobacterium*-mediated transformation system in MicroTom resulted in an average of 56% transformation frequency, which will be adequate for functional genomics by DNA tagging analysis and also for industrial production of transgenic plants (Dan et al., 2006). Thus, MicroTom could be served as an excellent model system for plant functional genomics.

MicroTom is a dwarf tomato cultivar bred for home gardening purpose (Scott and Harbaugh, 1989). The cultivar is different from commercial tomato cultivars by two recessive genes conferring the dwarf phenotype (Meissner et al., 1997). Because of dwarf feature, the plants can grow at a high density (1,357 plant/m^2) and short life cycle for fruit harvest 70-90 days of after sowing. The life cycle is almost equivalent to that of Arabidopsis. The dwarf features, short life cycle and high transformation frequency of MicroTom would be advantageous to serve as a model system to study plant-pathogen interaction.

Bacterial wilt caused by the soilborne bacterium *Ralstonia solancearum* is a serious plant disease in the tropics and in warm climate regions in the world and causes severe losses to many agricultural crops (Hayward, 1991). The pathogen causes lethal wilt of more than 200 plant species in 50 families including most of *Solanacea* plants. The host plants include potato, tomato, pepper, tobacco, eggplant, banana, cowpea, ginger and peanut (Hayward, 1991, 1994). Thus, it is feasible to test if MicroTom, one of the tomato cultivar, could serve as a host plant for various plant diseases. Takahashi et al. (2005) indicated the suitability of MicroTom plants to study plant-pathogen interaction because of its general susceptibility to most of tomato diseases caused by fungi, bacteria and viruses. This previous study showed that MicroTom is weakly susceptible to the limited number of *R. solanacearum* isolates. In this

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study, we have evaluated the virulence of various isolates of *R. solanacearum* using race 1 isolated from tomato plants and race 3 isolated from potato plants under the purpose of investigation of race specific susceptibility of MicroTom.

Materials and Methods

Bacterial strains and culture conditions. *Ralstonia solanacearum* isolates used for this study were indicated in Table 1. They were previously collected from wilted tomato plants and potato plants between 1997 and 2005 (Jeong et al., 2007). All the isolates were routinely grown on 2,3,5-triphenyl tetrazolium chloride (TZC) agar medium (Kelman, 1954) or CPG medium (Schaad et al., 2001) at 30°C. An isolate SL740 was isolated from wilted tomato plant at Eumseong, Chungcheongbuk-do using semiselective medium SM-1 and identified by biochemical tests as previously described (Schaad et al., 2001). The GMI1000 is a well characterized race 1 strain of *R. solanacearum* and used as a control bacteria to evaluate bacterial virulence.

Biovar determination. Biovar determination for the isolate SL740 was conducted based on utilization and oxidation of single alcohols and carbohydrates as previously described (Schaad et al., 2001). Briefly, the mineral medium supplemented with peptone and bromothymol blue (1 g of $NH_4H_2PO_4$, 0.2 g of KCl, 0.2 g of $MgSO_4 \cdot 7H_2O$, 1 g of peptone, 80 mg of bromothymol blue, 3 g of agar, 1 liter of water) was adjusted to pH 7.0 and the autoclave medium was supplemented with filter sterilized 10% carbohydrates solution to have final 1% carbohydrate concentration. Finally, about 3 ml of the molten medium was dispensed into sterile test tubes. Bacterial cells grown on TZC agar medium for 48 h at 30°C were suspended in sterile distilled water to adjust bacterial cell density to approximately 10^8 CFU/ml. Twenty microliter of the bacterial suspension was inoculated in the prepared medium in test tubes. Each treatment included three replications with uninoculated tubes as control. Yellow color change have been recorded daily by incubating the tubes until 14 days at 30°C. Strains GMI1000 (biovar 3), SL0341 (biovar 4), SL535 (biovar 4), and SL2064 (biovar 2) were used as references.

Plant growth and inoculum preparation. MicroTom seeds were kindly provided by Dr. Doil Choi at Seoul National University. Tomato seeds (cv. Seogun) were purchased from Kayayookmyo Corp., Korea. Plant seeds were surface sterilized in 1.0% sodium hypochlorite solution for 5 min and then rinsed in strerile distilled water for 5 times until all the traces of the disinfectant was removed. The surface sterilized seeds were germinated in moistened filter paper. The surface sterilized sprouted seeds were transferred to the pot containing the commercial horticulture nursery-media soil (Punong Co., Ltd, Korea) and maintained in the plastic house for 6 weeks at 20 to 30°C. Before inoculation, the plant were transferred into a growth chamber and grown at 30°C for 12 hr dark and 25°C for 12 hr light cycle with 70% relative humidity. The commercial Tomato and MicroTom plants were inoculated with *R. solanacearum* isolates on six week old plants and 5 or 6 weeks old plants, respectively.

Bacterial inoculums were prepared by growing the indicated isolates in mannitol-glutamate broth medium (Keane et al., 1970) for 24 h at 30°C with 150 rpm. The bacterial cells were harvested by centrifugation and resuspended in sterile distilled water and adjusted to absorbance of 0.3 at 600 nm with a population of 2×10^8 CFU/ml.

Inoculation methods and disease rating. Two experiments with two different inoculation methods were carried out to study the virulence of *R. solanacearum* isolates on MicroTom plants. In the first experiment, the reference strain GMI1000 alone was tested to select effective inoculation method inducing the disease. Based on the results, the second experiment was conducted with other 9 isolates of *R. solanacearum* to determine the virulence.

Table 1. *Ralstonia solanacearum* isolates and their origins used in this study

Isolate	Origin	Host	Race	Biovar	Source
SL312	Gwangju, Gyeonggi-do	Tomato	1	3	Jeong et al., (2007)
SL341	Gimcheon, Gyeongsangbuk-do	Tomato	1	3	Jeong et al., (2007)
SL535	Jincheon, Chungcheongbuk-do	Tomato	1	4	Jeong et al., (2007)
SL740	Eumseong, Chungcheongbuk-do	Tomato	1	4	This study
SL776	Goseong, Gyeongsangnam-do	Tomato	1	3	Jeong et al., (2007)
SL1020	Suwon, Gyeonggi-do	Tomato	1	4	Jeong et al., (2007)
SL2064	Namjeju, Jeju-do	Potato	3	2	Jeong et al., (2007)
SL2268	Namjeju, Jeju-do	Potato	3	2	Jeong et al., (2007)
SL2313	Namhae, Gyeongsangnam-do	Potato	3	2	Jeong et al., (2007)
GMI1000	Guyana	Tomato	1	3	Boucher et al., (1985)

Root dipping method: This method was done by immersing 6 weeks old plant roots in 100 ml of the bacterial suspension for 30 min (Park et al., 2007). Control Plant roots treated with sterile distilled water. The inoculated plants were planted in plastic pots containing the commercial horticulture nurserymedia soil and incubated in a growth chamber at 30°C for 12 hr light and 25°C for 12 hr dark cycle with 70% relative humidity.

Soil drenching method: The well grownup healthy plants of 6 weeks old were selected. The bacterial inoculum was prepared as mentioned earlier. Inoculation was carried out by applying 10^7 CFU of bacterial suspension per gram of soil near the rhizosphere region of each plant as previously described (Schaad et al., 2001). All inoculations included three replications and uninoculated controls. Plants were monitored for disease progress over a 15 day period after inoculation and disease was rated using the following scale: 0, no wilting; 1, 1-25% wilt symptom; 2, 26-50% wilt symptom; 3, 51-75% wilt symptom; 4, 76-100% wilt symptom or dead (Roberts et al., 1988).

Results and Discussion

Bacterial wilt on tomato and MicroTom. To compare the disease progress between tomato plants and MicroTom plants, *R. solanacearum* GMI1000 was inoculated using root dipping method. The strain GMI1000 showed the similar pattern of disease progress of plant wilting on tomato plants and MicroTom plants with less severe symptoms in MicroTom plants (Fig. 1). Since disease progress in tomato and MicroTom were similar against the strain GMI1000, the MicroTom plant may also have the same susceptibility to other *R. solanacearum* isolates. In fact, MicroTom was susceptible to the crown gall caused by *Agrobacterium tumefaciens* (Takahashi et al., 2005). Interestingly, *Pseudomonas syringe* pv. *tomato* DC3000 could not aggressively infect the MicroTom (Takahashi et al., 2005). There may be cultivar specific resistance mechanism present in the cultivar MicroTom against some plant pathogenic bacteria. Therefore, we further tested for bacterial virulence with more *R. solanacearum* isolates in this study.

When two different inoculation methods were compared in MicroTom plants using GMI1000, initial disease development was the faster by root dipping method than that of soil drenching method. However, the disease severity was not significantly different between two methods to 15 days after 11 of inoculation (Fig. 2). The wilt symptoms on the MicroTom plants by two methods were not different, except the speed for the disease development (Fig. 3). Although the speed for initial disease development was different between two inoculation methods, it was not possible to conclude that root dipping is the more effective

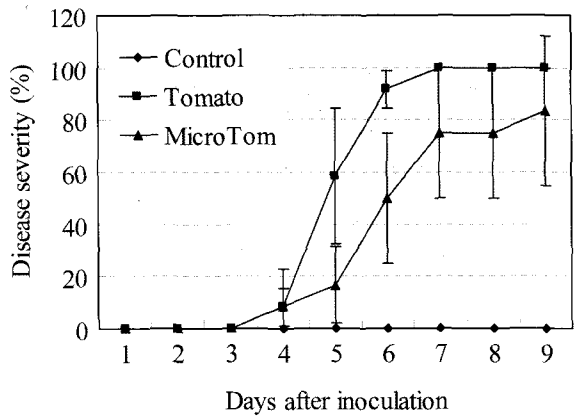

Fig. 1. Pathogenicity tests of GMI1000 on tomato and MicroTom plants performed by root dipping. Values are the average of three replications and vertical bars represent the standard deviation.

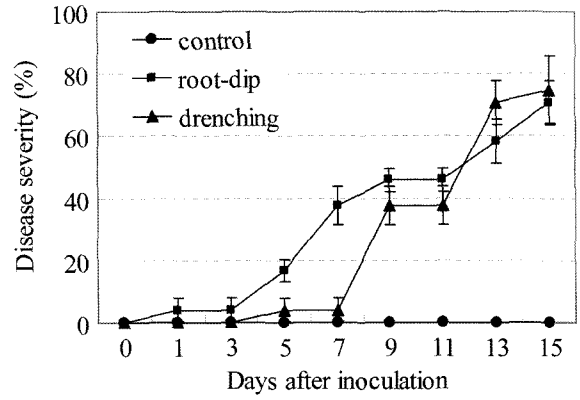

Fig. 2. Progression of wilting symptom in MicroTom plants over a period of 15 days by two inoculations methods with GMI1000. Values are the average of three replications and vertical bars represent the standard deviation.

way to cause the disease on MicroTom plant. This is because two methods were compared with different bacterial inoculum potential. Nevertheless, we have chosen the root dipping method to evaluate bacterial virulence of *R. solanacearum* isolates in MicroTom. This is because the root dipping is relatively easy and exhibited the more consistent result (data not shown). When the wilt plant stem cutting of MicroTom was immersed in sterile water, white bacterial streams could be detected. *R. solanacearum* GMI1000 could be easily reisolated from the diseased MicroTom plant as the diseased tomato plants (data not shown).

It is likely that the fast development of wilting by root dipping method is probably due to easy entrance of pathogenic bacteria into plant root system by higher cell density, which allow for faster colonization of plant xylem vessel. Since the wilt symptom developed in the disease is due to bacterial colonization of xylem vessel and produc-

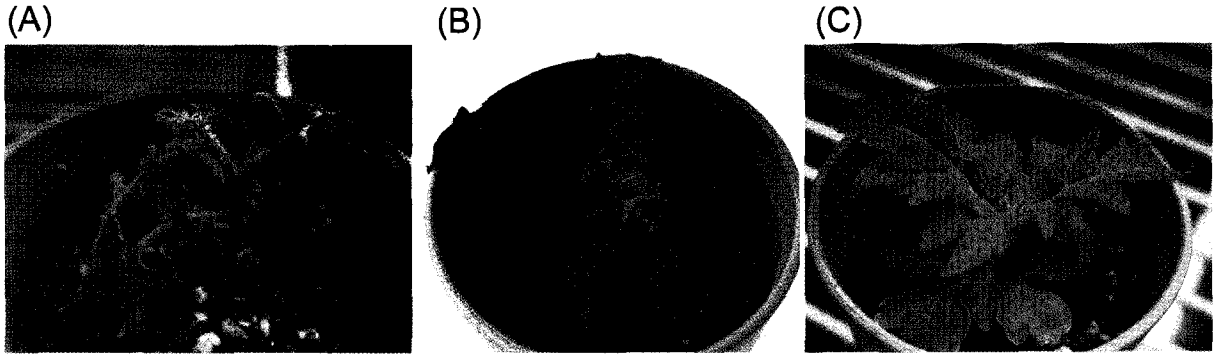

Fig. 3. Disease sympotom showing typical wilt on MicroTom by GMI1000. (A) Wilt of MicroTom 7 days after root dipping, (B) Wilt of MicroTom 15 days after soil drenching, (C) Uninoculated control.

tion of extracellular polysaccharides (Saile et al., 1997; Schell, 2000), the earlier entrance and rapid colonization by more bacterial cells may be linked with fast symptom development. Successful root invasion by bacterial cells are important for the disease development by *R. solanacearum* (Vasse et al., 1995; Wallis et al., 1978). Uprooting the plant from soil for root dipping may result in some unexpected wounding in the root system, which will facilitate the bacterial invasion of plant root system. The number of *R. solanacearum* cells which penetrate root and proceed for infection was probably less in soil drenching method compared to root dipping. However natural incidence of bacterial wilt is similar to soil drenching method, since bacterial cells are wide spread in agricultural fields in Korea (Kang et al., 2004).

Bacterial wilt on Micro-Tom by other isolates of *R. solanacearum*. Ten isolates of *R. solanacearum* were subject bacterial pathogenicity analysis on MicroTom plant using root dipping method. Those included *R. solanacearum* race 1 (biovar 3 or biovar 4) isolated from tomato plants and race 3 (biovar 2) isolated from potato plants (Table 1). Our biovar determination of SL740 revealed that this isolate belonged to biovar 4 of race 1. Throughout two independent experiments, all bacterial isolates exhibited remarkable bacterial wilt symptom ranging from 25% to 100% after 15 days of inoculation (Fig. 4 and Fig. 5). Most of isolates of race 1 showed the higher disease severity compared to those of race 3, such as SL2064, SL2268, and SL2313. The disease severities by all race 3 isolates tested were not above 50% in average until 15 days after inoculation, while most of race 1 isolates caused almost complete wilt of MicroTom. No clear difference in disease severity between biovar 3 and biovar 4 of race 1 isolates was observed. Our result was somewhat different from the similar work by Takahashi et al. (2005). By inoculation of

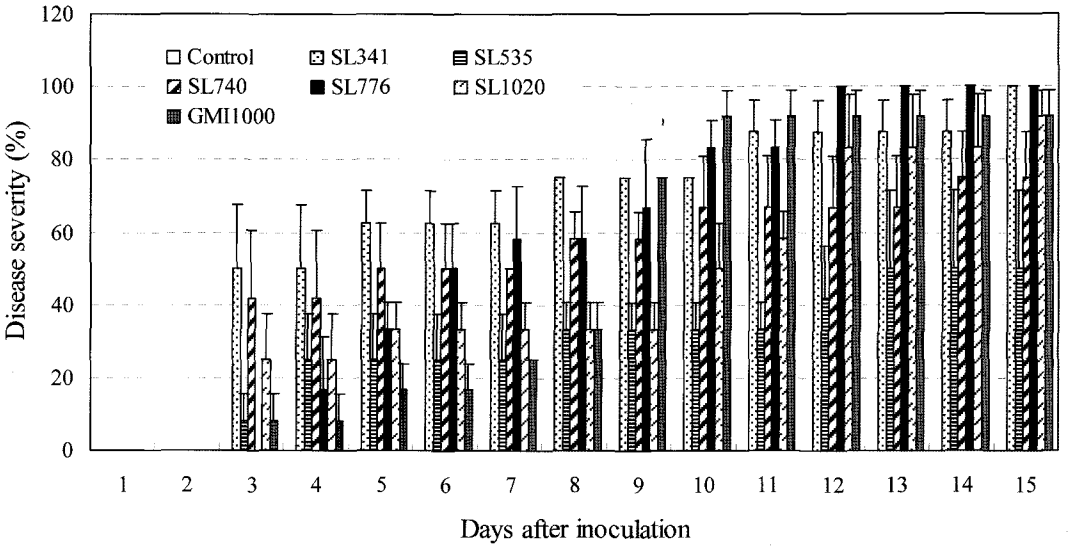

Fig. 4. Pathogenicity test using 6 isolates of *R. solanacearum* race 1 by root dipping on MicroTom. Values are the average of three replications and vertical bars represent the standard deviation.

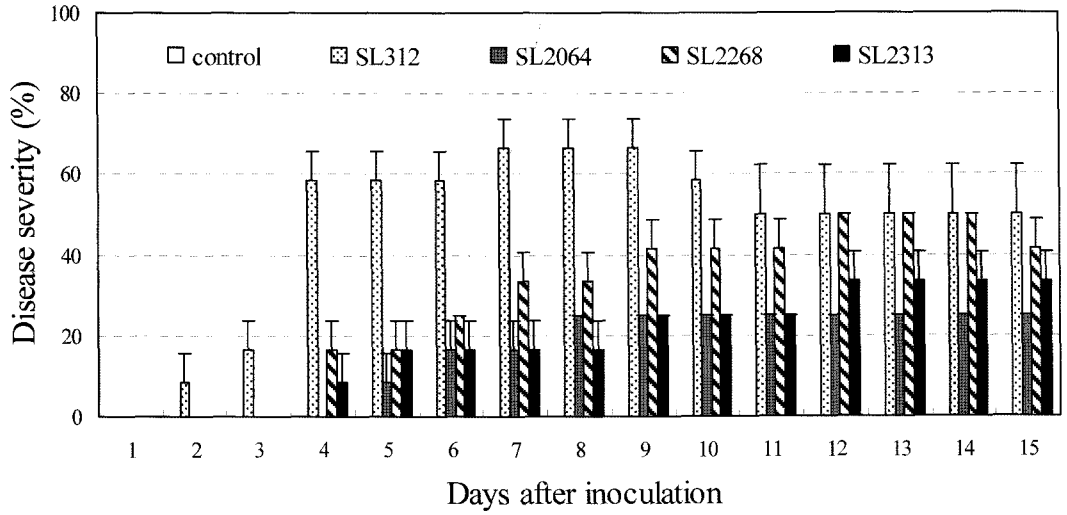

Fig. 5. Pathogenicity test using 3 isolates of *R. solanacearum* race 3 and 1 isolate of race 1 by root dipping on MicroTom. Values are the average of three replications and vertical bars represent the standard deviation.

17 isolates of race 1 of *R. solanacearum* to MicroTom, only five of them showed the apparent disease symptom which resulted in 20% disease severity (Takahashi et al., 2005). The similar root dipping method with the same bacterial density was carried out on MicroTom, while Takahashi et al (2005) washed the plant roots with tap water 30 min after root dipping, which potentially removed the attached *R. solanacearum* cells. At this moment, it is not clear for the contradictory results.

R. solanacearum are considered as species complex and highly competent for genetic exchange *in planta* (Bertolla et al., 1999). The *R. solanacearum* strains show remarkable pathogenic variability in host range (Sequeira and Averre, 1961). Bacterial race system and biovar differentiation were widely used for classification of *R. solanacearum* isolates although there is no correlation between biovar and race except biovar 2 and race 3 (Smith et al., 1995). The race 1 (biovar 3 or biovar 4) infects most of *Solanacea* plants in mostly tropical climates. The race 3 (biovar 2) naturally infects potato plants to cause brown rot and they also cause the disease on potato by artificial inoculation at cool temperature (Ciampi and Sequeira, 1980). The race 3 (biovar 2) generally cause weak virulence on some *Solanacea* plants by artificial inoculation except potato. Therefore, our result showing the less disease severity on MicroTom by three isolates of race 3 may reflect the weak virulence of race 3 on tomato plants.

In conclusion, we demonstrated that most of *R. solanacearum* isolates can infect MicroTom and develop the typical wilt symptoms using different races of *R. solanacearum*. The disease caused by *R. solanacearum* was confirmed by reisolating the inoculated bacteria and consistent disease development. Although relatively weak virulence was recognized on MicroTom by inoculating race 3 (biovar 2) isolates of *R. solanacearum*. We could not confirm the race-specific virulence of *R. solanacearum* on MicroTom. Therefore, this result suggested that MicroTom could be used as a model host plant to study *R. solanacearum* – plant interaction.

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