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Relation of Plant Age to Bacterial Multiplication in Pepper and Tomato Leaves Inoculated with *Xanthomonas campestris* pv. *vesicatoria*

Jong Tag Lee and Byung Kook Hwang*
**Department of Agricultural Biology, College of Natural Resources, Korea University, Seoul 136-701, Korea*

Xanthomonas campestris pv. *vesicatoria*에 감염된 고추와 토마토잎에서의 세균증식과 식물나이와의 관계

이종탁 · 황병국*
고려대학교 자연자원대학 농생물학과

ABSTRACT : Multiplications and pathogenic reactions of different pepper and tomato strains of *Xanthomonas campestris* pv. *vesicatoria* were evaluated in the most upper leaves of pepper and tomato plants at different growth stages. Hypersensitive reactions were induced in mature pepper plants by inoculation with only the tomato strains but not with the pepper strains, suggesting the expression of age-related resistance in pepper plants. The age-related resistance also seems to be correlated with an apparent inability of the bacteria to multiply as extensively in mature as in young plants. No significant differences among the Korean and U. S. pepper cultivars tested were found in bacterial multiplication, irrespective of bacterial strain or plant growth stage. Korean tomato cultivars tested also were highly susceptible to either tomato or pepper strains during the development of tomato plants.
***Key words* : *Xanthomonas campestris* pv. *vesicatoria*, pepper, tomato, age-related resistance.**

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is a destructive disease of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.). The disease which affects leaves, stems, and fruit has a wide geographic distribution occurring whenever pepper and tomato are grown under overhead irrigation or during warm, rainy weather. The most serious losses are due to leaf infections that cause defoliation, thereby reducing fruit yield. The pathogen known to be seed-borne is often difficult to manage by manipulating cultural practices. Disease control using bactericides often is not efficacious because of antibiotic resistance (16, 20), or copper resistance (2, 14). Bactericides are also less effective when environmental conditions are optimal for disease development. However, practical use of genetic resistance in breeding of pepper and tomato cultivars seems most useful for controlling bacterial spot in the fields (1, 5, 8, 18).

After invasion of the pepper plant via stomata or wounds, *X. c.* pv. *vesicatoria* multiplies in the intercellular spaces of the leaf tissue, giving rise to disease symptoms (19). Depending on the susceptibility of the particular plant cultivar, two different types of disease reactions can be observed. In the susceptible plant, circular, water-soaked lesions occur and later become necrotic with brown centers and thin chlorotic borders. In the resistant plant, avirulent strains induce the hypersensitive reaction (HR) with necrosis or papery white color. The HR is a local defense reaction accompanied by a rapid necrosis of the infected tissue, thus preventing multiplication of the bacteria in the infected region.

Three groups of *X. c.* pv. *vesicatoria* have been differentiated based on the virulence for either pepper or tomato or both plant species (17). The groups include the pepper group which is virulent only on pepper, the tomato group which is virulent only on tomato, and the pepper-tomato group which is

*Corresponding author.

virulent on both pepper and tomato. Within the pepper and pepper-tomato groups, races 1, 2, and 3 can be distinguished on the basis of virulence on differential pepper lines (6, 7, 11). Single dominant resistance genes, *Bs*1, *Bs*2, and *Bs*3 were first reported in pepper lines PI 163192, PI 260435, and PI 271322, respectively (4, 5, 10). The genes induce hypersensitive reactions (HR) to particular races of *X. c.* pv. *vesicatoria*. The *Bs*3 and *Bs*1 confer resistance in pepper plants to race 1 and race 2, respectively, whereas the *Bs*2 restricts races 1, 2, and 3. Varying levels of resistance to bacterial spot have also been reported in the tomato cultivars (18). In particular, the tomato genotype Hawaii 7998 exhibited a high level of resistance when infiltrated with strains of *X. c.* pv. *vesicatoria* (9).

Seedling pepper plants at eight-leaf stage are usually susceptible to *X. c.* pv. *vesicatoria*, regardless of host genotype. However, age-related resistance, which is distinctly expressed as pepper plants mature, may be very effective in controlling bacterial spot. The objectives of the present work was to compare populations of *X. c.* pv. vesicatoria in different pepper and tomato cultivars. Age-related resistance to bacterial spot was also evaluated by inoculating different pepper and tomato strains to pepper and tomato plants at seedling and mature plant stages.

MATERIALS AND METHODS

Bacterial strains. The four strains of *Xanthomonas campestris* pv. *vesicatoria* originating from pepper and tomato were used in this study (Table 1). Strain Ds1 was isolated in 1991 from pepper (*Capsicum annuum* L.) in Korea. The other strains (81-23, 87-77, and Bv5-4a) were provided by R. E. Stall, Department of Plant Pathology, University of Florida, Gainsville.

Cultures of bacteria were grown in yeast-nutrient broth (5 g yeast extract and 8 g nutrient broth per liter). The bacterial strains were preserved at −70℃ in the same medium containing 15% glycerol.

Host materials. Pepper cultivars Kumkang, Jinpum, Kingkun, Taeyang, Hanbyul, Hongilpum, Cajun 1, and Capritto, and tomato cultivars Kangyuk, Kwangmyung, Kwangsu, Kwangyang, Yeongsu, and Weolkwang were used in this study. All Korean pepper and tomato cultivars were provided by Jungang Seed Company and Heungnong Seed Company. The pepper cultivars Cajun I and Capritto were obtained from L. Black, Department of Plant Pathology, Louisiana State University, Baton Rouge, LA.

Table 1. Strains of *Xanthomonas campestris* pv. *vesicatoria* used in this study

Strain	Host of isolation	Country of isolation	Reference or source
Ds1	pepper	Korea	This study
81-23	pepper	Florida	Minsavage *et al.*, 1990
87-77	tomato	Ohio	Minsavage *et al.*, 1990
Bv5-4a	tomato	Argentina	Minsavage *et al.*, 1990

Seeds of the pepper and tomato cultivars tested were sown in a plastic tray (55×35×15 cm) containing steam-sterilized, peat-soil mix. Six seedlings at the six-leaf stage were transplanted in each plastic pot (5×15×10 cm) containing peat-soil mix. Pepper and tomato plants were raised in a growth chamber with temperatures ranging from 23 to 27℃ and 16 hr-photo period.

Bacterial inoculation. Inoculum was prepared from 24 hr-old cultures of *X. c.* pv. *vesicatoria* in yeast-nutrient broth by centrifuging at 3,000 g for 15 min. The harvested bacterial cells were then suspended in sterile tap water and diluted to $A_{660}=0.06$ (10^8 cfu/ml) prior to inoculation.

Pepper plants of eight-leaf and second-branch stages, and tomato plants of four-leaf and eight-leaf stages were inoculated by infiltrating the inoculum (10^8 cfu/ml) into the abaxial side of the fully expanded leaves with an atomizer connected to a compressor, until an area of at least 4 cm^2 in a leaf appeared water-soaked. After inoculation, plants were incubated in a moist chamber at 25℃ for 24 hr and returned to a growth chamber with temperatures ranging from 23 to 27℃, and 16 hr-photo period.

Evaluation of bacterial population in leaves. Bacterial populations in either pepper or tomato leaves were determined at time intervals after inoculation of *X. c.* pv. *vesicatoria*. Two segments of inoculated leaves (4 cm^2 each) of the most upper leaves of each plant were triturated in 10 ml sterile tap water. The resulting suspensions were serially diluted with sterile water. The dilutions of 0.1 ml was spread onto Tween media (10 g peptone, 10 g potassium

bromide, 0.25 g calcium chloride, 0.3 g boric acid, 10 ml Tween 80, 50 mg cycloheximide, 65 mg cephalexin, 12 mg 5-fluorouracil, and 0.4 mg tobramycin per liter) (15). Inoculated plates were incubated at 28℃ for 3~4 days. The numbers of colonies appearing were transformed into $\log_{10}$ values. The experiment was repeated twice and each experiment had three replications.

RESULTS

Multiplication of *X. c.* pv. *vesicatoria* in pepper (cv. Hanbyul) leaves at different time intervals after inoculation was distinctly different between pepper and tomato strains of the bacteria or between plant growth stages (Fig. 1). Bacterial populations of pepper strains Ds1 and 81-23 in pepper leaves were larger than those of tomato strains 87-77 and Bv5-4a. In particular, strain Ds1 isolated from Korean peppers was highly virulent to the pepper cultivar Hanbyul among all the four strains tested. Multiplication of the strains 81-23, 87-77, and Bv5-4a was apparently more pronounced in the eight-leaf stage than second-branch stage of plants. However, reduction of bacterial multiplication at the later plant growth stage was not observed when inoculated with the strain Ds1.

Typical bacterial spot lesions were produced on pepper leaves inoculated with each of pepper and tomato strains of *X. c.* pv. *vesicatoria* (Fig. 2). Four days after inoculation, pepper plants of the eight-leaf stage developed susceptible lesions, which appeared water-soaked and turned yellow. After 10 days, large chlorotic and necrotic areas were developed by all the strains tested. Generally, the lesions were slightly sunken on the upper leaf surface and slightly raised on the lower surface. Severely spotted leaves turned yellow and dropped. However, plants of the second-branch stage showed a hypersensitive, light tan and papery white lesions incited by the tomato strains 87-77 and Bv5-4a. In contrast, the pepper strains Ds1 and 81-23 developed typically

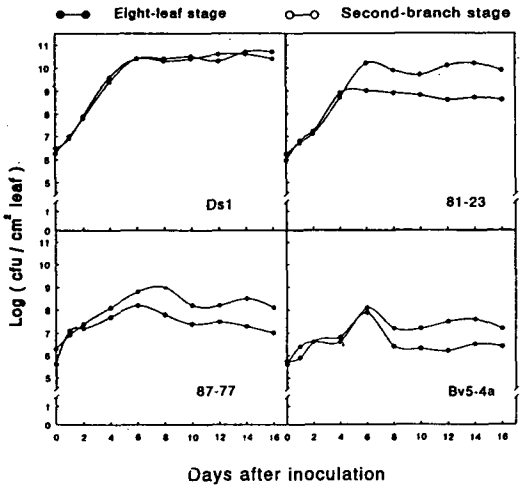

Fig. 1. Multiplication of Ds1, 81-23 (pepper strains), 87-77 and Bv5-4a (tomato strains) of *Xanthomonas campestris* pv. *vesicatoria* in leaves of pepper cultivar Hanbyul at the two developmental stages. Values are the means for three replications of one experiment. Similar results were obtained from the second experiment.

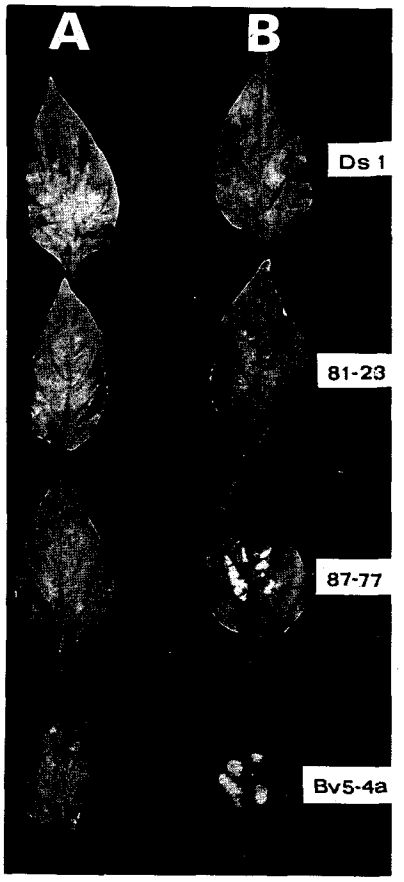

Fig. 2. Differential reactions of pepper cultivar Hanbyul to Ds1, 81-23 (pepper strains), 87-77 and Bv5-4a (tomato strains) of *Xanthomonas campestris* pv. *vesicatoria* at the eight-leaf (A) and second-branch (B) stages.

susceptible lesions on the pepper leaves even at the second-branch stage.

When inoculated with each of the strains 81-23, 87-77, and Bv5-4a, the eight pepper cultivars tested multiplied about 10~100 times more bacteria in leaves of the eight-leaf stage than in those of the second-branch stage (Fig. 3). However, in multiplication of Korean strain Ds1 in pepper leaves, there were no differences between the two plant growth stages in all cultivars tested except the cultivar Jinpum. In general, no significant differences among the pepper cultivars tested also were found in bacterial multiplication, irrespective of bacterial strain or plant growth stage.

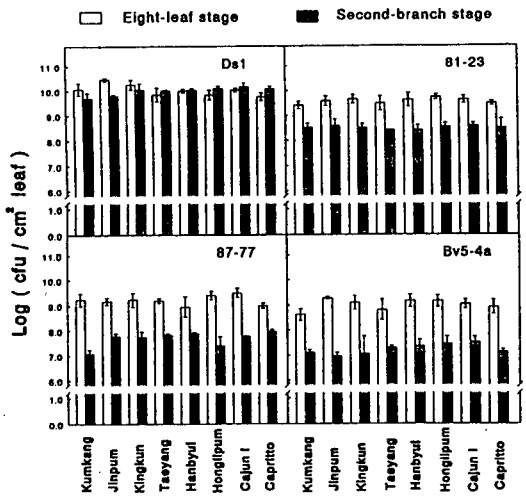

Fig. 3. Bacterial populations in leaves of eight pepper cultivars at 7 days after inoculation with Ds1, 81-23 (pepper strains), 87-77 and Bv5-4a (tomato strains) of *Xanthomonas campestris* pv. *vesicatoria* at the two plant growth stages. Values are means± standard errors for three replications of one experiment. Similar results were obtained from the second experiment.

In tomato leaves (cv. Kwangyang) inoculated with the tomato strains 87-77 and Bv5-4a, the bacterial populations were 10-fold more than in those inoculated with the pepper strains Ds1 and 81-23 (Fig. 4). At 4 days after inoculation with the tomato strains 87-77 and Bv5-4a, the increases in the number of bacterial cells was more pronounced in the tomato leaves of four-leaf stage than in those of eight-leaf stage. However, until 10 days after inoculation with the pepper strains Ds1 and 81-23, there were no differences between the two plant growth stages in bacterial multiplication. Thereafter, the reduction in bacterial population occurred in tomato leaves of eight-leaf stage.

Inoculation with either pepper or tomato strains

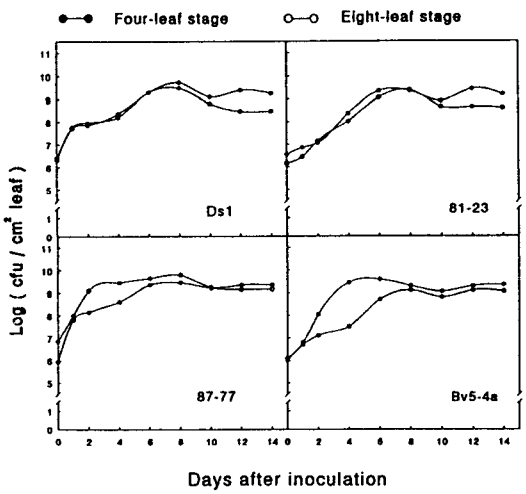

Fig. 4. Multiplication of Ds1, 81-23 (pepper strains), 87-77 and Bv5-4a (tomato strains) of *Xanthomonas campestris* pv. *vesicatoria* in leaves of tomato cultivar Kwangyang at the two developmental stages. Values are the means for three replications of one experiment. Similar results were obtained from the second experiment.

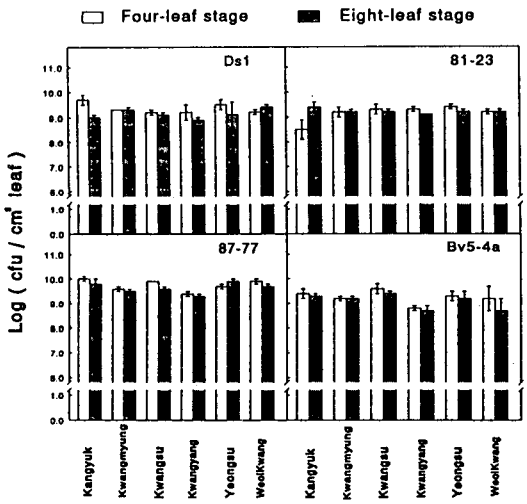

Fig. 5. Bacterial populations in leaves of six tomato cultivars at 7 days after inoculation with Ds1, 81-23 (pepper strains), 87-77 and Bv5-4a (tomato strains) of *Xanthomonas campestris* pv. *vesicatoria* at the two plant growth stages. Values are means± standard errors for three replications of one experiment. Similar results were obtained from the second experiment.

could not easily distinguish among the six Korean tomato cultivars in the level of resistance to *X. campestris* pv. *vesicatoria* in terms of bacterial multiplication in tomato leaves (Fig. 5). Bacterial populations in tomato leaves at 7 days after inoculation with each of the four strains were similar one another in the Korean tomato cultivars tested and at the two plant growth stages, except for the cultivar Kangyuk inoculated with the pepper strains Ds1 and 81-23. Lesions of all cultivars tested appeared water-soaked, and then turned yellow. At 10 days after inoculation, some hypersensitive lesions were observed in pepper leaves inoculated with the strains Ds1 and 81-23 at eight-leaf stage.

DISCUSSION

Inoculation with each of the pepper and the tomato strains of *X. c.* pv. *vesicatoria* resulted in typically susceptible lesions on the leaves of all pepper plants at the eight-leaf stage. However, the hypersensitive reactions were induced in mature pepper plants of second-branch stage by inoculation with only the tomato strains (Fig. 1). The development of hypersensitive responses to tomato strains in mature pepper plants rather than seedling plants may be due to the expression of age-related resistance in pepper plants. In earlier studies, it has been demonstrated that the tomato strain of *X. c.* pv. *vesicatoria* consistently induced a visible different kind of hypersensitive response on pepper plants than that caused by the pepper strain (3, 6).

The expression of age-related resistance to *X. c.* pv. *vesicatoria* in pepper plants could well be explained by the comparison of bacterial multiplication in the seedling and mature pepper plants. The bacterial populations in all pepper cultivars at the second-branch stage were strikingly reduced, when compared to that at the eight-leaf stage. The hypersensitive symptoms also were observed in most of the Korean and U. S. pepper cultivars inoculated with the tomato strains, but not with the pepper strains of *X. c.* pv. vesicatoria at the second-branch stage. These results would imply that age-related resistance of pepper plants to the bacterial spot disease may not be expressed only in particular pepper genotypes, but may be a generalized result of physiological changes in leaf tissues during aging (12). In general, the age-related resistance also seems to be correlected with an apparent inability of the bacteria to multiply as extensively in mature as in young plants. The lower bacterial population in mature plants may be due to lack of favorable nutrients, or to presence of a preformed inhibitor, or to an inhibitor induced in the host cells as a result of bacterial action. However, our findings of the drastic reduction of bacterial multiplication of the tomato strains in mature pepper plants accompanied by the development of hypersensitivity suggest that mature pepper plants may be non-host plants not susceptible to the tomato strains in fields. Klement and Goodman (13) indicated earlier that many phytopathogenic bacteria induced a hypersensitive reaction when inoculated into plants not susceptible to infection in nature.

Interestingly, the Korean pepper strain Ds1 of *X. c.* pv. *vesicatoria* multiplied well in all pepper cultivars during the development of plants, irrespective of plant age. In contrast, the multiplication of the U. S. pepper strain 81-23 was greatly retarded in all pepper cultivars at the mature, second-branch stage, although it did not induce a hypersensitive reaction. These results indicated that pepper strains of *X. c.* pv. *vesicatoria* may also multiply slowly at mature pepper plants depending on the pathogen genotypes, but may not induce a non-host, hypersensitive reaction in mature pepper leaves. In particular, the tomato strains would not cause greatly the bacterial spot disease in pepper plants, because they do not produce susceptible symptoms. In conclusion, mature plants of pepper may express resistance to the bacterial spot disease, thereby inhibiting the bacterial multiplication and further inducing hypersensitivity by the tomato strains, but not by the pepper strains. However, the molecular genetical and biochemical mechanisms to the induction of hypersensitivity in mature pepper plants should be elucidated in detail.

Korean tomato cultivars did not show any level of resistance to either tomato strains or pepper strains at the two growth stages of tomato plants. No significant differences in the disease development and the bacterial multiplication were found between the plant growth stages of tomato or between the tomato strains and the pepper strains. These data demonstrated that any of either the tomato or pepper strains did not induce hypersensitivity or age-related resistance in Korean tomato cul-

tivars during the development of tomato plants and also that the Korean tomato cultivars do not carry any level of resistance to the bacterial spot.

요 약

더뎅이병균(*Xanthomonas campestris* pv. *vesicatoria*)의 고추균주와 토마토균주의 증식과 병반응을 생육시기가 다른 고추와 토마토식물의 상위잎에서 평가하였다. 성숙한 고추식물체에서는 토마토균주가 접종되었을 때 과민반응이 나타났으나 고추균주가 접종되었을 때는 과민반응이 나타나지 않았으며 어린 식물에서는 모두 감수성 병반이 형성되었다. 이것은 고추에서 성체식물저항성이 발현됨을 뜻하며, 성숙한 식물에서는 어린 식물에서 처럼 세균이 왕성하게 증식하지 못함을 나타낸다. 한국산 고추품종과 미국산 고추품종을 비교 시험한 결과 세균의 균주나 식물생육시기에 관계없이 세균증식에서 품종간 차이가 없었다. 한국산 토마토품종은 토마토균주와 고추균주에 대해 고도의 감수성을 보였으며 생육시기에 따른 차이도 보이지 않았다.

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