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Studies on Cross Protection of Fusarium Wilt of Cucumber IV. Protective Effect by a Nonpathogenic Isolate of *Fusarium oxysporum* in a Greenhouse and Fields

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오이 덩굴쪼김병의 교차보호에 관한 연구 IV. 비병원성 *Fusarium oxysporum* 접종에 의한 온실과 포장에서의 방제효과

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ABSTRACT : A nonpathogenic *Fusarium oxysporum* isolate 4-1 obtained from a cucumber plant showed a consistently high protection against fusarium wilt of cucumber caused by *F. oxysporum* f. sp *cucumerinum* with an efficacy of 67~100% in three separate greenhouse experiments. Isolate 4-1 was frequently reisolated from cucumber roots 90 days after inoculation, and incited discoloration of cucumber roots at the high inoculum level as 10^5 conidia/g soil or higher. Fusarium wilt incidence in the plots treated with isolate 4-1 was reduced from 56% to 18%, from 11% to 1%, and from 35% to 8%, compared with the untreated control plots, in 1993, 1994 and 1995 field experiments, respectively.

***Key words* : cucumber, *Fusarium oxysporum* f. sp. *cucumerinum*, cross protection, fusarium wilt, nonpathogenic strain.**

Fusarium wilt of cucumber, incited by *Fusarium oxysporum* f. sp. *cucumerinum* is one of the most devastating soilborne diseases of cucumber in Korea. Fusarium wilt depends on soil as a source of initial inoculum. Because the disease is difficult to control by fungicide applications, current control measures depend heavily on grafting with resistant root stocks such as bottle gourd. However, grafting with other plants requires much time and effort, and also results in lowering fruit quality particularly in taste.

Fusarium wilt protection by counter-infection of a nonpathogenic fusarium has been reported in several vegetables (2, 6~9) and in some cases such as sweet potato (4) it is being used practically in the fields. Antibiotic production and competition in infection court or in nutrition were suggested as mechanisms of the protection (5, 8).

This study is a part of researches to develop a practical method of cross protection by counter-infection with a nonpathogenic strain for the control of fusarium wilt of cucumber. Some of the results on this research have been published elsewhere (10~12). The purpose of the present study is to evaluate protection efficacy of few selected nonpathogenic isolates against fusarium wilt of cucumber in a greenhouse and fields.

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MATERIALS AND METHODS

Isolates used. Nine nonpathogenic isolates of *Fusarium oxysporum* obtained from various plant stems and soil were used in this study. Origin of the isolates has been described in other studies (12). Nit-mutants of the isolates were developed when necessary to monitor the behavior of a particular isolate using the method described by Correll *et al.* (1). Only the nit-mutants having identical properties to their wild types were selected.

Inoculum preparation and inoculation. *F. oxy-*

sporum isolates were grown in a soil-corn meal mixed medium (soil 480 g, corn meal 20 g, water 80 ml) at 26°C for 15 days, and were used as inocula. The soil medium was prepared by mixing a commercial seedbed soil and the soil inoculum at the rate of 2:1, that gives an approximate inoculum density as 1.7×10^7 conidia/g soil. By raising the plants in the prepared soil medium, cucumber plants were inoculated with *F. oxysporum* for testing its pathogenicity and cross protection effect. Inoculum density was adjusted when necessary by changing a mixing rate of the soil and the inoculum.

Greenhouse evaluation. Cucumber cultivar Eunsung was seeded in 8-cm-diameter pots containing the soil medium that was infested with each nonpathogenic isolate, and raised in the greenhouse for 30 days. The seedlings were then transplanted into a plot, 2 m wide 20 m long, artificially infested with *F. oxysporum* f. sp. *cucumerinum* in a polyethylene filmhouse. A total of 6, 4 and 8 seedlings per nonpathogenic isolate were planted at a 25 cm space in the plot in the 1st, 2nd, and 3rd experiments, respectively. Wilt incidence was examined 90 days after transplanting. The experiments were done during March to May, May to July, and July to September in the same plot.

Measurement of colonization ability. To compare the colonization ability of the nonpathogenic isolate 4-1 with the pathogen, nit-mutants of both isolates that are identical in other properties to their wild types were developed to monitor their behavior. Cucumber seeds were inoculated by planting in plastic boxes (40×60 cm) containing the soil medium infested with each isolate at six different density levels of 10^1 to 10^6 conidia/g soil. Treatment consisted of 12 to 16 plants by isolate. The colonization ability were examined 90 days after seeding. Root discoloration was examined all plants with the naked eye after washing. Root colonization ability was determined based on detection frequency of the inoculated isolate from main root portions of the cucumber plants. For this purpose cucumber roots were cut into 1-cm-long pieces after washing under tap water to remove any adhering soil, soaked in a 10% NaClO solution for 5 min for surface disinfection, and plated on Komada medium (3) that is selective for *F. oxysporum*. Colonies developed on the media after a 4-day incubation at 26°C were ascertained to confirm *F. oxysporum*. In order to estimate population density of the isolate in plant stems, 1-cm-long piece of vascular portions from basal stems after peeling the cuticle layer off was ground in sterile water, and then plated on the selective medium after proper dilutions with three replications. Colony numbers developed on the medium were counted 4 days after incubation at 26°C.

Field experiment. Thirty-day-old cucumber seedlings of cultivar Eunsung grown in the soil medium infested with the isolate 4-1 were transplanted in the farmer's fields under a structure near Suwon, where fusarium wilt of cucumber occurs severely every year. Isolate 4-1 was selected for the field experiment, because it showed best protection in the greenhouse tests. Cultural practices including plant spacing, fertilization, irrigation and insect control followed the standard method practiced in Suwon area. Two treatments (isolate 4-1 treated and untreated) were randomly assigned with three replications in a field plot 5 m wide and 99 m long. The number of wilted plants was recorded based on whole plants at the end of growing season. The experiments have been done in the same plot in 1993, 1994 and 1995, respectively.

RESULTS

Wilt protection in the greenhouse. In the three separate experiments, protective effects differed greatly with isolate and experiments (Table 1). Nine isolates varied in their control values from 18% to 89% on the average. However, isolate 4-1 obtained from a cucumber plant showed a consistently high protection against fusarium wilt, which accounted for 100%, 67% and 100% of the control effects in the 1st, 2nd and 3rd experiments, respectively. This isolate was selected for further studies.

Colonization ability of the nonpathogenic isolate 4-1. Both the pathogen and the nonpathogenic isolate 4-1 were frequently reisolated from cucumber roots 90 days after inoculation regardless of inoculum level applied in the soil (Table 2). However, unlike the case of roots, the population of the nonpathogenic isolate 4-1 in the vascular portions from basal stems of the cucumber plants was far less than that of the pathogen, and was scarcely found at lower inoculum levels as 10^5 conidia/g soil or below. However, isolate 4-1 incited discoloration of the cucumber roots at inoculum levels of 10^5 conidia/g soil or higher.

Field performance of the isolate 4-1. Fusarium wilt incidence in the plots treated with isolate 4-1 was reduced from 58% to 18%, from 11% to 1% and from

Table 1. Protection of cucumber plants by nine selected nonpathogenic isolates of *Fusarium oxysporum* from fusarium wilt infections in the greenhouse

Nonpathogenic isolate	% wilt incidence[a]			Average
	1st exp.	2nd exp.	3rd exp.	
3-1	33 (60)[b]	75 (0)	62 (0)	57 (22)
13-2	50 (40)	50 (33)	38 (39)	46 (37)
14-2	67 (19)	50 (33)	62 (0)	60 (18)
15-3	50 (60)	75 (0)	25 (60)	50 (32)
SII-1	0 (100)	75 (0)	38 (39)	38 (48)
2-3	17 (80)	75 (0)	50 (19)	47 (36)
15-4	17 (80)	25 (67)	25 (60)	22 (70)
Ch-n	17 (80)	75 (0)	0 (100)	31 (58)
4-1	0 (100)	25 (67)	0 (100)	8 (89)
Uninoculated	83 (0)	75 (0)	62 (0)	73 (0)

[a] Six, four, and eight seedlings were transplanted into a pathogen-infested plot in the 1st (March~May), 2nd (May~July) and 3rd (July~September) experiments, respectively.
[b] Control value (%).

Table 2. The effect of the inoculum level of *F. oxysporum* f. sp. *cucumerinum* and a nonpathogenic *F. oxysporum* isolate 4-1 on their colonization ability in roots and stems of cucumber plants

Inoculum level (conidia/g soil)	% plants wilted		% root discolored		% detection from root		No. cfu/basal stem[b]	
	P[a]	NP	P	NP	P	NP	P	NP
10^6	81	0	100	62	100	100	655	17.9
10^5	38	0	89	19	100	100	320	0.2
10^4	19	0	100	0	100	100	450	0.2
10^3	25	0	100	0	100	100	350	0.2
10^2	0	0	56	0	100	69	129	0.2
10^1	0	0	25	0	69	88	148	0.4
0	0	0	0	0	44	0	0	0.2

[a] P : Pathogen (*F. oxysporum* f. sp. *cucumerinum*), NP : nonpathogen (*F. oxysporum* 4-1).
[b] One cm piece of vascular portion from a basal stem was ground in sterile water to estimate population of *F. oxysporum* using a dilution plate method. Nit mutants were used for detection.

Table 3. Inhibition of fusarium wilt in cucumber by pre-inoculation of the nonpathogenic *Fusarium oxysporum* isolate 4-1 in the commercial fields under structure

Treatment[a]	1993		1994		1995	
	No. plants tested	No. plants wilted	No. plants tested	No. plants wilted	No. plants tested	No. plants wilted
Isolate 4-1	55	10 (18%)[b]	1200	16 (1%)	1200	96 (8%)
Untreated	45	25 (56%)	650	70 (11%)	1200	420 (35%)

[a] Cucumber plants were pre-inoculated by planting seeds in a soil-corn meal mixed medium infested with isolate 4-1. Plants in the untreated control were raised in the same medium without inoculation. Thirty-day-old seedlings were transplanted into a field plot naturally infested with *F. oxysporum* f. sp. *cucumerium*. Wilt incidence was examined at the end of the season.
[b] % infection.

35% to 8%, compared to that of the untreated control plots, in 1993, 1994 and 1995 field experiments, respectively (Table 3). The control effect was greater when the level of wilt incidence in the untreated plots was low.

DISCUSSION

Protection efficacy shown in the greenhouse study varied greatly with experiment. For instance, the isolate 3-1 showed 60% control value in the 1st experiment, but showed no control effect in the 2nd and 3rd experiments. The reason for this variation is not clear, however, it might be due partly to the level of soil population of the pathogen and colonization ability of the nonpathogenic isolate on cucumber plants that might interact with particular environments during the experiment period. In contrast, isolate 4-1 showed a consistently high protection effect throughout the experiments. The attribute for this stable protection is not yet known, but it could be partly associated with high root colonization ability of this isolate shown in Table 2.

Isolate 4-1 caused root discoloration, as always observed in the pathogenic isolate, only at the high inoculum density as 10^5 conidia/g soil or higher. Ogawa (5) observed that a nonpathogenic isolate pre-inoculated to sweet potato caused minor discoloration on the inoculated stem portions. He reported that only the plants with discoloration were protected by the nonpathogenic isolate and concluded that the observed discoloration is closely associated with an induction of resistance in the plant. Whether the root discoloration observed in this study involves protection efficacy by the nonpathogenic isolate remains to be determined.

Unlike root colonization, the ability of stem colonization differed considerably between the pathogen and isolate 4-1. Stem population of isolate 4-1 was approximately 1/3 of that of the pathogen at the high inoculum level as 10^6 conidia/g soil, but it was extremely low at lower inoculum density. If the level of the nonpathogenic isolate population in the stem operates as one of the key factors for determining cross protection efficacy, the observed low ability of stem colonization shown by isolate 4-1 should be improved further to obtain better protection.

Population level of the pathogen in cucumber plants, particularly in stems, is apparently related to the development of wilt symptom. In this study cucumber plants were wilted when pathogen population in the stem reached a certain level. Based on this observation, the level of stem population of *F. oxysporum* can be used as a factor for predicting the disease outbreak after their relationships were determined.

In the field experiments, the control value by the isolate 4-1 ranged 67% to 88%. The control effect was increased apparently as the level of disease incidence in the untreated plots was lower. This indicates that the protection efficacy by a nonpathogenic isolate increases at lower pathogen density in fields. Consequently, in order to increase the protection efficacy, it would be important either to increase the stem colonization ability of a nonpathogenic isolate through maintaining its soil population level high, or to reduce the pathogen population in the field by sanitation.

요 약

오이에서 분리한 비병원성 *Fusarium oxysporum* 균주 4-1은 온실에서 오이에 선접종하였을 때 덩굴쪼김병에 안정적인 방제효과를 보여 세 번의 시험에서 그 방제가가 67~100%에 달하였다. 이 균주는 접종후 90일에도 오이 뿌리에서 높은 빈도로 재분리 되었으며 g 토양당 분생포자 농도가 10^5개 이상의 높은 농도로 접종하였을 때는 뿌리의 갈변현상을 초래하였다. 1993년부터 1995년에 걸친 세번의 포장시험에서 이 균주는 오이덩굴쪼김병의 발생을 무처리 발병율 56%, 11%, 35%에 비해 18%, 1%, 8%로 각각 억제하였다.

REFERENCES

1. Correll, J. C., Klittich, C. H. R. and Leslie, J. F. 1987. Nitrate nonutilizing mutants of *Fusarium oxysporum* and their use in vegetative compatibility tests. *Phytopathology* 77 : 1640-1646.
2. Davis, D. 1968. Partial control of fusarium wilt in tomato by formae of *Fusarium oxysporum*. *Phytopathology* 58 : 121-122.
3. Komada, H. 1975. Development of a selective medium for quantitative isolation of *Fusarium oxysporum* from natural soil. *Rev. Plant Prot. Res.* 8 : 114-124.
4. Ogawa, K. 1989. Studies on biological control of fusarium wilt of sweet potato with cross protection. *Ann. Phytopath. Soc. Japan* 55 : 385-387.
5. Ogawa, K. 1984. Biological control of fusarium wilt of sweet potato by nonpathogenic *Fusarium oxysporum*. *Plant Prot.* 38 : 535- 538.
6. Paulitz, T. C., Park, C. S. and Baker, R. 1988. Biological control of fusarium wilt of cucumber with nonpathogenic isolates of *Fusarium oxysporum*. *Can. J. Microbiol.* 33 : 349-353.
7. Tezuka, N. and Makino, T. 1991. Biological control of fusarium wilt of strawberry by non-pathogenic *Fusarium oxysporum* isolated from strawberry. *Ann. Phytopath. Soc. Japan* 57 : 506- 511.
8. Wymore, L.A. and Baker, R. 1982. Factors affecting

cross protection in control of fusarium wilt of tomato. *Plant Dis.* 66 : 908-910.

9. Yamaguchi, K., Sano, T., Arita, M. and Takahashi, M. 1992. Biocontrol of fusarium wilt of tomato and verticillium wilt of eggplant by non-pathogenic *Fusarium oxysporum* MT0062. *Ann. Phytopath. Soc. Japan* 58 : 188-194.
10. Yang, S. S. and Kim, C. H. 1993. Studies on cross protection of fusarium wilt of cucumber I. Distribution and pathogenicity of *Fusarium oxysporum* in soil. *Korean J. Plant Pathol.* 9 : 19-25.
11. Yang, S. S. and Kim, C. H. 1993. Studies on cross protection of fusarium wilt of cucumber II. Relationships between inoculum density, temperature, and wilt development. *Korean J. Plant Pathol.* 9 : 92-97.
12. Yang, S. S. and Kim, C. H. 1993. Studies on cross protection of fusarium wilt of cucumber III. Selection of nonpathogenic isolates and their protective effect in the greenhouse. *Korean J. Plant Pathol.* 9 : 92-97.