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Responses of cucumber cultivars to induction of systemic resistance against anthracnose by plant growth promoting fungi

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Abstract

Initial experiment on the reactions of five Japanese cultivars of cucumber to *Colletotrichum orbiculare* infection in the greenhouse revealed that cv Suyo and Gibai were susceptible and moderately susceptible, respectively, while cv Shogoin fushinari and Sagami hanjiro were resistant to infection by *C. orbiculare*; cv Ochiai fushinari was moderately resistant. The ability of 16 plant growth promoting fungi (some isolates belonged to species of *Phoma* and some non-sporulating isolates) isolated from zoysiagrass rhizospheres to induce systemic resistance in the above five cucumber cultivars was tested by growing plants in potting medium infested with barley grain inocula of PGPF in the greenhouse. The second true leaves of 21-day-old plants were challenge inoculated with *C. orbiculare* and disease assessed. Nine, out of 16 isolates, caused significant reduction of disease caused by *C. orbiculare* in at least two cultivars. *Phoma* isolates (GS8-1 and GS8-2) and non-sporulating isolates (GU21-2, GU23-3, and GU24-3) significantly reduced the disease in all the five cultivars. The disease suppression in cucumber was due to the induction of systemic resistance, since the inducer(s) and the pathogen were separated spatially and that the inducer did not colonize aerial portions. The resistance induced by certain isolates in a susceptible cultivar was less than that in a resistant cultivar. Disease suppression caused by isolate GU21-2 was similar to the *C. orbiculare* induced control in certain cultivars. The average rate of expansion of lesion diameter on leaves due to *C. orbiculare* was slower due to induction with the selected plant growth promoting fungi compared to the uninduced control plants. Roots of four cultivars were colonized by only three isolates, however, roots of one cultivar (Suyo) was colonized by five isolates suggesting the cultivar-specific root colonization ability.

Abbreviations: cv = cultivar(s), PGPF = plant growth promoting fungal isolates, PGPR = plant growth promoting rhizobacteria.

Introduction

Systemic resistance in cucumber (*Cucumis sativus* L.) can be induced by several pathogenic as well as non-pathogenic microbes against pathogenic microbes. Among the pathogens, *Colletotrichum orbiculare* (Berk. & Mont.) Arx (= *C. lagenarium* (Pass.) Ellis & Halst.) was extensively studied as an inducer of systemic resistance in cucumber [Kuc *et al.*, 1975; Kuc and Richmond, 1977; Kuc and Strobel, 1992; Kuc, 1990]. Apart from *C. orbiculare, Cladosporium cucumerinum* Ell. & Arth. and hypovirulent isolates of *Fusarium* were also used to induce resistance in cucumber

[Hammerschmidt *et al.*, 1976; Ishiba *et al.*, 1981]. Pathogenic bacteria and viruses have also been employed to induce systemic resistance in cucumber [Jenns and Kuc, 1980; Caruso and Kuc, 1979]. Among the non-pathogenic bacteria, plant growth promoting rhizobacteria (PGPR) were shown to induce systemic resistance in cucumber [Wei *et al.*, 1991]. Recently, we have shown that certain non-pathogenic fungi isolated from zoysiagrass (*Zoysia tenuifolia* Willd. ex Trin.) rhizospheres could induce systemic resistance in cucumber and afford 90% protection against a low inoculum pressure of 10^4 spores ml^{-1} of *C. orbiculare* [Meera *et al.*, 1993, 1994a, 1994b].

A survey of literature reveal that cultivars of cucumber have not been studied for their reaction to anthracnose disease. However, Kuc and Richmond [1977] showed that certain American cultivars are resistant to some extent against anthracnose. So far, in Japan, very few high yielding cultivars have been studied for their reaction to anthracnose. From our previous study, cv Gibai was found susceptible to anthracnose in the greenhouse [Meera *et al.*, 1995]. *Colletotrichum orbiculare* is an economically important disease causing pathogen on cucumber in Japan, particularly in greenhouses. Studies conducted in this laboratory showed that certain sporulating and non-sporulating fungal isolates from zoysiagrass rhizosphere promoted plant growth and suppressed the soilborne diseases of a number of crops [Hyakumachi *et al.*, 1992, 1993a, 1993b; Shivanna *et al.*, 1994]. Some of the non-sporulating fungal isolates that were formerly thought to be sterile, sporulated less frequently after 30 days of incubation under rear ultraviolet light and they were identified recently as species of *Phoma*, while the rest could not be identified, since they failed to sporulate (Unpublished information). In the present investigation, some isolates of *Phoma* and certain non-sporulating fungal isolates were tested for their ability to induce systemic resistance against *C. orbiculare* in five selected Japanese cultivars of cucumber. Further, root colonization ability of PGPF isolates in these cultivars was also studied.

Materials and methods

Reactions of cucumber cultivars to infection by C. orbiculare. The pathogen *C. orbiculare* isolate 104T was used throughout the study. Seeds of five near isogenic cultivars – Gibai, Sagami hanjiro, Ochiai fushinari, Shogoin fushinari, and Suyo (Asahi Seed Co. Ltd., Tokyo, Japan) were surface disinfected (1% NaOCl solution for 2 min) and sown in 100 g of autoclaved (for three consecutive days, each time for 60 min at 121 °C and 1.5 kg/cm^2) potting medium ('Star bed', Kyodohiryo Co. Ltd., Aichi, Japan) contained in sterile plastic pots (6 cm dia × 6 cm height). The N, P, and K contents of potting medium were 200, 1500, and 200 mg L^{-1}, respectively, of potting medium. The plants were grown in the greenhouse (average of 28 °C and 80% RH) for 21 days. The plants received natural solar illumination of winter, spring, and summer seasons (1993 and 1994). The solar illumination during winter and spring was supplemented with light (12/12 h light darkness cycle) from artificial day light, cool florescent tubes (20000 to 25000 lux). The spore suspension of the pathogen (10^5 spores ml^{-1}) was prepared in sterile distilled water and 20 10 µl drops of the inoculum were placed on laminae of true leaves. The inoculated plants were incubated in dark, humid chamber (100% RH) for 30 h. The incubated plants were placed back to the greenhouse benches for the disease development for another six days. The experiment was conducted four times with four replicates in each treatment. In each replicate there were four plants.

Assessment of disease. The total number of lesions and the total lesion diameter (mm) on leaves of plants that were inoculated with the pathogen were measured after six days. The disease severity (DS) was calculated (Meera *et al.*, 1995) by using the formula:

$$DS = \frac{\text{Summation disease index}}{\text{Number of inoculum drops applied (20)}}$$

Disease index was based on the lesion diameter that ranged from 0 to 5 scale (0 = no lesions, 1 = chlorotic lesions measuring 1–2 mm, 2 = brown lesions measuring 2–3 mm, 3 = dark-brown lesions measuring 3–4 mm, 4 = lesions measuring 4–5 mm and 5 = lesions measuring 5 mm and above).

Induction of resistance with PGPF isolates and C. orbiculare *in different cultivars of cucumber.* Cultures of 16 different PGPF isolates (Isolates of *Phoma* spp.: GS6-1, GS6-2, GS6-4, GS7-3, GS7-4, GS8-1, GS8-2, GS8-3, GS8-6, GS10-1, GS10-2, and GS12-2, and non-sporulating isolates: GU21-2, GU23-3, GU24-3, and GU26-1) collected from zoysiagrass rhizospheres were used in the experiment. These PGPF isolates were mass cultured on autoclaved barley (*Hordeum vulgare* L.) grains under axenic conditions [Shivanna *et al.*, 1994] and powdered to 1–2-mm size inoculum particles. The barley grain inocula of PGPF isolates were amended (2%, w/w) to potting medium separately. A 100-g of potting medium-inoculum mixture was placed in sterile pots (6 cm dia × 6 cm height) and sown with an individual surface disinfected cucumber seed. Seeds were also sown in unamended potting medium. The protected and unprotected plants of five cultivars were grown for 21 days in the greenhouse. The growing conditions were as described earlier. The second true leaves were inoculated with 20 10 µl drops of 10^5 spores ml^{-1} of *C. orbiculare* and incubated as described in the first experiment.

Plants of all five cultivars were also systemically induced with *C. orbiculare* to test the level of protection afforded by the pathogen. Plants were grown in unamended autoclaved potting medium for 15 days in the greenhouse. The first true leaves of plants were inoculated with 20 10 µl drops of 10^5 spores ml^{-1} of the pathogen and the inoculated plants were incubated by following the procedures described earlier. The disease was assessed. Six days later, the second true leaves of the pathogen-induced plant were inoculated with the same density of the pathogen inoculum. The plants were incubated for the expression of disease. Each treatment had four replications, with three plants in each replicate. Experiments were conducted three times during winter, spring, and summer seasons, 1993 and 1994. The total number of lesions and total lesion diameter per leaf of unprotected and protected plants that were challenge inoculated with the pathogen were measured.

Rate of expansion of lesion diameter. The total diameter of necrotic lesions in unprotected and protected plants of four selected cultivars (cv Shogoin fushinari, Sagami hanjiro, Suyo, and Gibai) was measured every alternate day starting from the sixth day after challenge inoculation until the fourteenth day. The rate of expansion of lesion diameter was calculated by measuring the difference of the lesion diameter occupied during the sixth to the fourteenth day period and dividing it by the number of intervals (4).

Colonization ability of PGPF isolates in different cultivars of cucumber. All 16 PGPF isolates were tested for their colonization ability of five cucumber cultivars. The barley grain inocula (2%, w/w) of PGPF isolates were amended to the autoclaved potting medium (100 g) and sown with a single surface disinfected seed. After 21 days of growth, in the greenhouse, plants were uprooted and the shoot and root portions were separated and washed thoroughly in water. The surface disinfected shoot and root portions were cut into 200 1-cm long bits and placed on the chloramphenicol amended (200 mg L^{-1}) potato dextrose agar (PDA) [Meera *et al.*, 1994b]. The fungal colonies emerging from the bits were compared with the original cultures of PGPF on PDA and their identity was confirmed. The number of shoot or root bits that produced mycelial colonies out of a total of 200 bits were enumerated and the percentage of re-isolation of the particular PGPF was calculated. There were four replications in each treatment with three plants in each replicate. The experiment was conducted two times.

Statistical analysis. The data from repeated trials were combined after the analysis for homogeneity of variances using Bartlett's test [Gomez and Gomez, 1984]. Experiment of the reaction of cucumber cultivars to *C. orbiculare* infection, was laid out in the completely randomized design involving five cultivars as variables. The second experiment was conducted in the split-plot design containing 16 PGPF isolates along with controls as main plot factors and five cultivars as sub-plot factors. Only those isolates which induced resistance compared to the untreated control in at least two cultivars were selected for the analysis of variance. The data of the rate of lesion diameter expansion due to the pathogen on leaves of different cultivars protected or unprotected with PGPF isolates was also analyzed by the split-plot

method. The data of root colonization ability of PGPF isolates in five cultivars were analyzed as above. The treatment means were separated by employing either Fisher's least significant difference test (LSD, $P = 0.01$ and 0.001) or Duncan's new multiple range test (DMRT, $P = 0.05$).

Results

Reactions of cucumber cultivars to infection by C. orbiculare. Reactions of cucumber cultivars were based on the disease severity; 0 = highly resistant, up to 2 = resistant, up to 3 = moderately resistant, up to 4 = moderately susceptible, and up to 5 = susceptible. The reactions of cucumber cultivars to anthracnose varied. Cultivar Suyo was the most susceptible with a DS of 5. While cv. Gibai was moderately susceptible, cv Ochiai fushinari was moderately resistant. The remaining two, cv Sagami hanjiro and Shogoin fushinari, were resistant to *C. orbiculare* infection with a DS of less than 2.

Induction of resistance with PGPF isolates and C. orbiculare *in different cultivars of cucumber.* Among 16 PGPF isolates tested, nine isolates that reduced disease at least in two cultivars were selected for comparison (Table 1). The remainder of seven isolates were not effective.

Isolates GS8-1 and GU21-2 followed by GS8-2, GU23-3, GU24-3, and GS10-1 reduced ($P \leq 0.05$) the lesion number in all the five cultivars compared to the respective uninduced controls. Isolate GS6-4 reduced ($P = 0.05$) lesion numbers in the moderately resistant cv Ochiai fushinari and resistant cv Sagami hanjiro and Shogoin fushinari, while GS8-6 reduced the lesion number only in the resistant cultivars. On the other hand, isolate GS8-3 reduced the lesion number in cv Suyo, Gibai, and Ochiai fushinari that were susceptible, moderately susceptible, and moderately resistant, respectively; but was unable to reduce the lesion number on leaves of resistant cv Sagami hanjiro and Shogoin fushinari (Table 1).

All the isolates (GS8-1 and GU21-2 followed by GS8-2, GU23-3, GU24-3, and GS10-1) that reduced ($P \leq 0.05$) the lesion number in five cultivars also significantly reduced the lesion diameter. Isolates GU23-3 and GU24-3 signifi-

Table 1. Effect of induction of different cultivars of cucumber with plant growth promoting fungal isolates on the reduction of total number of lesions[1] due to leaf infection by *Colletotrichum orbiculare* relative to uninduced control[2]. A spore density of 10^5 spores ml^{-1} (20 10 µl drops) was challenge inoculated on the laminae of second true leaves of 21-day-old plants grown in the greenhouse

Treatments	Cultivars / Total number of lesions (%)									
	Suyo		Gibai		Ochiai fushinari		Sagami hanjiro		Shogoin fushinari	
GS6-4	100.0 a[3]	A[3]	100.0 a	A	67.9 d	B	63.3 b	C	69.7 cd	B
GS8-1	72.7 e	A	60.9 e	B	57.7 e	B	43.0 e	C	56.1 f	B
GS8-2	75.3 e	A	74.4 c	A	61.5 e	C	59.2 c	C	68.1 cd	B
GS8-3	84.8 d	B	65.6 de	D	71.1 d	C	96.3 a	A	100.0 a	A
GS8-6	100.0 a	A	100.0 a	A	99.7 a	A	55.8 cd	C	67.4 de	B
GS10-1	93.7 bc	A	87.9 b	B	58.6 e	D	60.0 bc	D	75.1 bc	C
GU21-2	76.8 e	A	46.8 f	B	51.9 f	B	50.9 d	B	40.0 g	C
GU23-3	90.8 c	A	68.3 d	C	83.3 c	B	45.2 e	D	78.4 b	B
GU24-3	94.9 b	A	60.2 e	C	88.7 b	B	52.2 d	D	60.9 ef	C
C. orbiculare[4]	58.5 f	A	40.5 g	B	33.7 g	C	37.7 f	BC	34.1 g	C

[1] Values are the mean of three trials, each with four replicates.
[2] Values are given in percentage of the values of uninduced control. Total lesion number per leaf: 100% = 19.7 (Suyo), 19.5 (Gibai), 16.8 (Ochiai fushinari), 10.8 (Sagami hanjiro), and 8.0 (Shogoin fushinari).
[3] Values followed by same letter(s) (lower case letters for columns and upper case letters for rows) are not significantly different according to DMRT ($P = 0.05$).
[4] Induced with *C. orbiculare* by placing 10^5 spores ml^{-1} (20 10 µl drops) on the lamina of first true leaf of cucumber that were grown for 15 days in unamended potting medium.

cantly ($P = 0.05$) reduced the lesion diameter on leaves of all cultivars tested, although they were slightly less effective in reducing the lesion number. Isolate GS6-4 reduced the lesion diameter on leaves of cv Suyo (susceptible), Ochiai fushinari (moderately resistant), Sagami hanjiro (resistant), and Shogoin fushinari (resistant), however, it failed to reduce the lesion diameter on laminae of cv Gibai (moderately susceptible). On the other hand, isolate GS8-3 reduced the leaf lesion diameter of the susceptible, moderately susceptible, and moderately resistant cultivars but not in the resistant cultivars (Table 2).

The positive, *C. orbiculare* induced control reduced the lesion number and lesion diameter in all the cultivars irrespective of their susceptibility or resistance. Only isolate GU21-2 reduced ($P = 0.05$) the lesion number to the same extent as in the positive induced control in the resistant cv Shogoin fushinari and lesion diameter in cv Ochiai fushinari, Sagami hanjiro, and Shogoin fushinari (Tables 1 and 2). The effectiveness of other PGPF isolates was always less than the pathogen-induced control.

Significant interactions ($P = 0.05$) obtained between cultivars and PGPF treatments during analysis of variance indicated that the efficiency of PGPF isolates depended on the cultivar indicating the cultivar-specific reactions (Table 1 and 2).

Rate of expansion of lesion diameter. The diameter of leaf lesions of plants protected and unprotected with selected PGPF isolates (GS8-1, GS8-2, GS8-3, and GU21-2) increased gradually with an increase in the incubation period after challenge with the pathogen. However, depending on the type of cultivar and the type of inducer isolate treatment, the rate of expansion of lesion diameter in unprotected and protected plants differed significantly ($P = 0.05$) compared to uninduced control (Fig. 1).

The diameter of lesions in uninduced plants of susceptible cv Suyo increased at the rate of 11.2 mm, while in plants induced with GS8-3, the lesion diameter expanded at the rate of 1.9 mm every alternate day and in plants induced with isolates GS8-1, GS8-2, and GU21-2 increased at a rate that ranged from 5.3 mm to 7.7 mm. In the moderately susceptible cv Gibai, the total lesion

Table 2. Effect of induction of different cultivars of cucumber with plant growth promoting fungal isolates on the reduction of total diameter of lesions[1] due to leaf infection by *Colletotrichum orbiculare* relative to uninduced control[2]. A spore density of 10^5 spores ml^{-1} (20 10 µl drops) was challenge inoculated on the laminae of second true leaves of 21-day-old plants grown in the greenhouse

Treatments	Cultivars / Total lesion diameter (%)									
	Suyo		Gibai		Ochiai fushinari		Sagami hanjiro		Shogoin fushinari	
GS6-4	88.3 b[3]	B[3]	100.0 a	A	63.2 d	D	38.0 e	E	68.8 c	C
GS8-1	44.5 h	B	47.2 e	AB	50.3 f	A	43.0 d	B	46.3 e	B
GS8-2	63.3 f	A	65.5 c	A	57.5 e	B	56.8 c	B	67.3 c	A
GS8-3	65.2 f	B	67.4 c	B	67.6 c	B	93.8 a	A	90.6 a	A
GS8-6	97.4 a	A	100.0 a	A	81.3 a	B	59.0 c	D	69.5 bc	C
GS10-1	78.2 c	B	91.6 b	A	47.8 f	E	68.9 b	C	62.7 cd	D
GU21-2	48.7 g	A	41.0 f	B	27.1 g	D	33.8 fg	C	24.8 f	D
GU23-3	69.6 e	B	66.3 c	C	74.4 b	A	34.8 ef	D	76.3 b	A
GU24-3	74.0 d	A	60.0 d	C	67.4 c	B	35.5 ef	E	55.7 d	D
C. orbiculare[4]	39.9 i	A	22.0 g	D	26.4 g	C	31.2 g	B	18.2 f	D

[1] Values are the mean of three trials, each with four replicates.

[2] Values are given in percentage of the values of uninduced control. Total lesion diameter per leaf: 100% = 117 mm (Suyo), 96 mm (Gibai), 78 mm (Ochiai fushinari), 46 mm (Sagami hanjiro), and 36 mm (Shogoin fushinari).

[3] Values followed by same letter(s) (lower case letters for columns and upper case letters for rows) are not significantly different according to DMRT ($P = 0.05$).

[4] Induced with *C. orbiculare* by placing 10^5 spores ml^{-1} (20 10 µl drops) on the lamina of first true leaf of cucumber grown for 15 days in unamended potting medium.

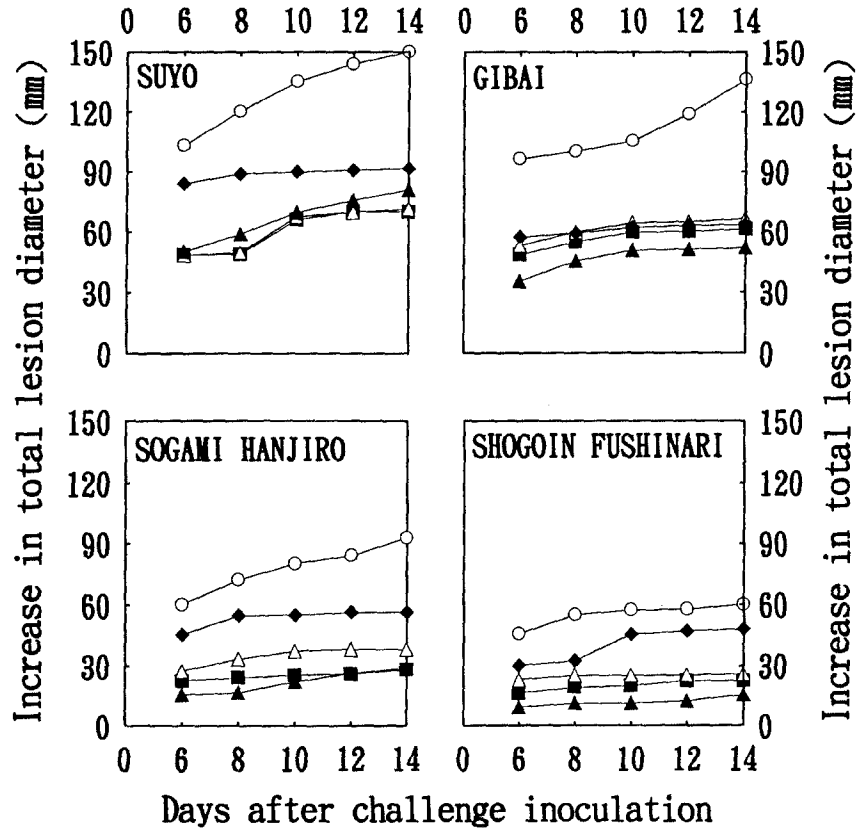

Fig. 1. Increase in the total lesion diameter (mm per leaf) from the sixth day after challenge inoculation with 20 10 µl drops of 10^5 spores ml^{-1} of *Colletotrichum orbiculare* up to the fourteenth day on leaves of four cucumber cultivars (Suyo, Gibai, Sagami hanjiro, and Shogoin fushinari) induced with plant growth promoting fungal isolates GS8-1 (■), GS8-2 (△), GS8-3 (◆), and GU21-2 (▲) in comparison to uninduced control (○). LSD(s) to compare means of two isolates in a cultivar are 0.88 mm and 1.17 mm at $P = 0.01$ and 0.001, respectively. LSD(s) to compare means of two isolates in any two cultivars are 0.83 mm and 1.12 mm at $P = 0.01$ and 0.001, respectively.

diameter expanded at the rate of 10.0 mm in uninduced control plants, while in plants induced with all four selected PGPF isolates, it ranged from 1.6 mm to 4.2 mm. A maximum reduction in the lesion diameter was caused when plants were induced with isolate GS8-3. The expansion rate of the lesion diameter slowed down significantly ($P = 0.001$) due to all four isolates in cv Sagami hanjiro and Shogoin fushinari except GS8-3 that failed to restrict the lesion diameter expansion in cv Shogoin fushinari.

Colonization ability of PGPF isolates in different cultivars of cucumber. Amongst 16 PGPF isolates tested, GS8-1, GS8-2, and GS8-3 colonized roots of all cultivars selected. However, in addition to the above isolates, GU23-3 and GU24-3 colonized roots of cv Suyo. These isolates, GU23-3 and GU24-3, failed to colonize roots of other cultivars. Isolate GS8-3 was re-isolated from 50% of root bits of cv Suyo (susceptible), while isolates GS8-1 and GU24-3 were re-isolated from 37% and 30% of root bits, respectively. Isolate GS8-3 showed a high colonization ability of root bits (59%) followed by GS8-1 (35%) in cv Gibai (susceptible) and Ochiai fushinari (moderately resistant). However, in roots of cv Shogoin fushinari and Sagami hanjiro (resistant), the re-isolation of GS8-3 was greatly limited to 10–12%. On the other hand, isolate GS8-1 was re-isolated from 25% of root bits in different cultivars, while GS8-2 was re-isolated from fairly less root bits except in case

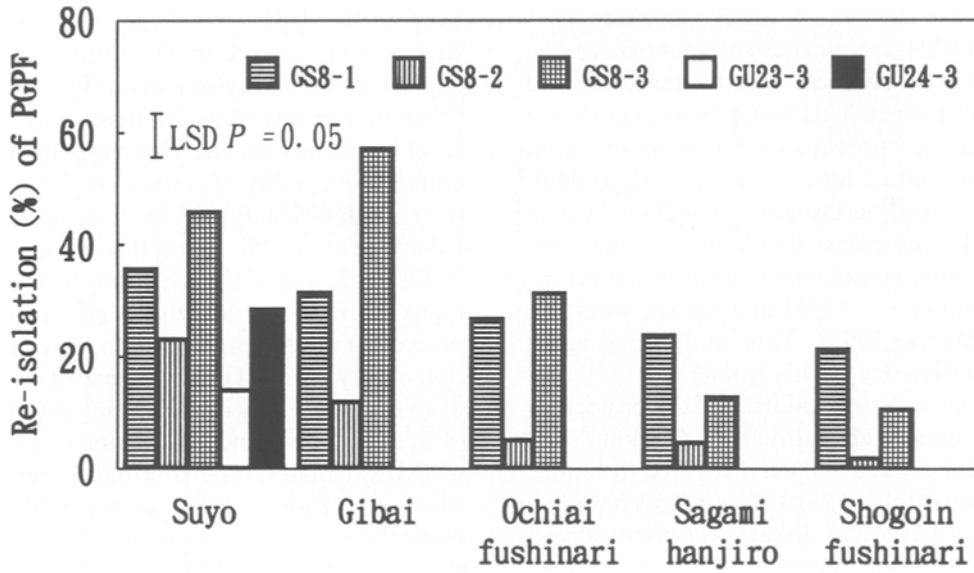

Fig. 2. Percentage of re-isolation of plant growth promoting fungal (PGPF) isolates (GS8-1, GS8-2, GS8-3, GU21-2, GU23-3, and GU24-3) from roots of different cucumber cultivars, Suyo, Gibai, Ochiai fushinari, Sagami hanjiro, and Shogoin fushinari following supplementation of potting medium with barley grain inocula (2%, w/w) of PGPF isolates and grown for 21 days in the greenhouse. LSD to compare means of re-isolation of two isolates in roots of any two cultivars is 8.25% at $P = 0.05$.

of roots of cv Suyo (Fig. 2). None of the PGPF isolates could be recovered from the aerial shoot portions.

Discussion

Among the five cultivars tested in the present study, cv Shogoin fushinari and Sagami hanjiro were resistant while, cv Ochiai fushinari was moderately resistant and the remaining cv Gibai and Suyo were moderately susceptible and susceptible, respectively, to infection by *C. orbiculare*. The cv Suyo and Shogoin fushinari tested in the present study, were also used by Ishiba *et al.* [1981] to study the cultivar reaction to induction of resistance by species of *Fusarium*. However, they did not classify the reactions of these cultivars to *C. orbiculare* infection. Their results showed that *C. orbiculare* produced many more spots on leaves of cv Suyo than on leaves of cv Shogoin fushinari, however, lesion area due to pathogen in these two cultivars varied only slightly. The present study grees with the previous one [Meera *et al.*, 1995] hat cv Gibai is susceptible to anthracnose and when compared to the cultivars tested, it is moderately susceptible.

The present study supports our previous view that PGPF isolates could be used to induce systemic resistance in cucumber [Meera *et al.*, 1994b; Meera *et al.*, 1995]. We demonstrated that the aerial portions of cv Gibai [Meera *et al.*, 1994b] and also of other cultivars used in this study, induced with PGPF isolates were free from PGPF colonization. We also showed that PGPF isolates were not antagonistic and did not produce antibiotic substances *in vitro* against *C. orbiculare* [Meera *et al.*, 1994b]. Out of 16 PGPF isolates used to induce systemic resistance in five cultivars of cucumber, nine isolates reduced the number of lesions in at least two cultivars. However, only a few isolates could reduce the total number of lesions and lesion diameter in all cultivars. The efficiency of some of the PGPF isolates was less in a susceptible cultivar than in a resistant cultivar. Kuc and Richmond [1977] obtained resistance in both susceptible and resistant cultivars and they showed that induced resistance further enhanced in a resistant cultivar due to induction with *C. orbiculare*. Ishiba *et al.* [1981] also obtained vari-

ations in the reactions of cucumber cultivars induced with *Fusarium* sp. against anthracnose.

Isolate GU21-2 reduced anthracnose to the lower level in all cultivars tested when compared to the other isolates. This isolate was also shown to reduce anthracnose in cv Gibai as mycelial inoculum and culture filtrate [Meera *et al.*, 1994b], and as autoclaved barley grain inoculum [Meera *et al.*, 1995]. This isolate also protected cucumber plants until nine weeks when tested in the greenhouse [Meera *et al.*, 1995] and for six weeks in the field [Meera, 1994]. This study once again proves the efficiency of this isolate.

In unprotected-susceptible and unprotected-moderately susceptible cultivars, the lesion diameter expanded continuously and covered the entire leaf and the petiole, 13–14 days after challenge inoculation. Such severe disease was not observed in the susceptible and moderately susceptible cultivars protected with PGPF isolates. The average rate of expansion of lesion diameter was faster in the susceptible than in the resistant cultivar. The above observation corroborated with that of Dean and Kuc [1986] who found significant differences between the size of lesions in protected and unprotected plants 4–10 days after challenge. The slow expansion rate of lesion diameter in the protected plants compared to unprotected plants could be due to the rapid lignification of host tissue due to induction as suggested by Dean and Kuc [1986]. Our observation with cucumber seedlings induced with PGPF isolates GS8-1 and GU21-2 and later challenge inoculated with *C. orbiculare* increased the amount of lignin which might explain this reduced rate of lesion expansion [M. B. Shivanna, M. S. Meera and M. Hyakumachi, unpublished].

In the susceptible cv Suyo, five isolates could colonize roots while, only three isolates could colonize roots of the remainder of cultivars, suggesting the cultivar-specific root colonization ability. Ishiba *et al.* [1981] also obtained a similar response of cultivars to colonization by a hypovirulent isolate of *Fusarium*. Isolate GS8-3 colonized roots of the susceptible, moderately susceptible, and moderately resistant cultivars but not the resistant cultivars. The induction of resistance by this isolate was high in the susceptible to moderately resistant cultivars while it was low in the resistant cultivars. This unusual behavior of GS8-3, although not understood, could be explained by its cultivar-specific root colonization ability. However, isolate GS8-1 colonized roots more or less to the same extent in all cultivars, but had a slightly better ability in a susceptible cultivar. The protection in rest of the cultivars was at a similar level due to this isolate. This suggests that the root colonization ability of certain PGPF isolates might play a significant role in inducing protection [Meera *et al.*, 1995]. The ability of isolates, GU21-2, GU23-3, and GU24-3, to protect most of the cultivars without colonizing roots suggest that the protection might not depend on the root colonization ability alone. Thus we think of the existence of two or more possible mechanisms of induction of systemic resistance in cucumber cultivars due to PGPF isolates. The first one is related to the root colonization ability of the PGPF isolates, while the second one relates to factor(s) other than root colonization ability of certain isolates.

In the present study, the pathogen-induced resistance control showed a better protection than most of the PGPF isolates. Similar results were also obtained by Wei *et al.* [1991] in pathogen-induced cucumber plants compared to PGPR induced plants. However, isolate GU21-2 induced protection to the same extent as that of the pathogen-induced control. This similarity, however, varied among cultivars. Maurhofer *et al.* [1994] also obtained good protection with PGPR which was similar to that caused by tobacco necrosis virus induced control in tobacco (*Nicotiana tabaccum* L.). Kuc [1990] has shown that the necrosis caused by *C. orbiculare* triggers the host response resulting in the recognition of the pathogen. However, the resistance response of cucumber cultivars induced by PGPF isolates could be related to the root colonization ability or due to the triggering of the host responses by mechanism(s) not clear at this stage. Compared to the pathogen-induced resistance, which requires necrosis to initiate resistance, induction by PGPF does not appear to require necrosis. Plants induced with the pathogen have been shown to exhibit higher level of protection even against the high pathogen inoculum pressure [Dean and Kuc, 1986], and the protection remained effective until flowering once a booster inoculum was provided [Kuc and Richmond, 1977]. My observation on cucumber immunization with *C. orbiculare* showed a weak protection in the susceptible cv

Suyo, when the pathogen was challenge inoculated at a high spore pressure of 10^6 ml^{-1} or more [Meera, unpublished]. Induction with *C. orbiculare* has been observed to cause disease spread to the non-immunized plants in its vicinity in a greenhouse [Decaslzo *et al.*, 1990]. On the other hand, with PGPF isolates, no apparent damage is caused to any of the plant part. Cucumber plants, once induced by certain PGPF isolates, stay in the state of immunization up to nine weeks in the greenhouse [Meera *et al.*, 1995]. However, at high pathogen inoculum pressure, the protection level was lowered. The PGPF treated plants were healthy and showed enhanced growth both in the controlled conditions as well as in the greenhouse [Meera *et al.*, 1995]. In fact, PGPF treated plants grew better and produced more yield in the field [M. B. Shivanna, M. S. Meera and M. Hyakumachi, unpublished]. Since certain PGPF can control soilborne diseases (caused by species of *Fusarium, Pythium,* and *Rhizoctonia*) of cucumber plants [Hyakumachi *et al.*, 1993b], a single soil amendment with efficient PGPF isolate could be sufficient to control both soilborne and foliar diseases apart from obtaining increased plant growth and yield.

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