Bull. Fac. Life Env. Sci. Shimane Univ., 4 : 1 − 6, December 20, 1999

# Response of the Rice Sekiguchi Lesion (*sl*) Mutant, cv. Sekiguchi-himenomochi, to *Magnaporthe grisea* *

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**Abstract** Pathological response of the rice Sekiguchi lesion (*sl*) mutant (cv. Sekiguchi-himenomochi) originated from cv. Himenomochi (resistance gene *Pi-k*) to *M. grisea* was investigated. When the rice *sl* mutant was inoculated with *M. grisea* spores, large differences in hyphal growth were seen in rice cells and lesions formed on the leaves among *M. grisea* strains. When wild (cv. Himenomochi) and *sl* mutant (cv. Sekiguchi-himenmochi) rice plants were inoculated with incompatible *M. grisea* races, hyphal growth was very poor in rice sheath cells of both mutant and wild rice plants. On the other hand, only Sekiguchi lesions were formed on leaves of the mutant, although only necrotic spots appeared on those of the wild rice. In *M. grisea* races compatible with a wild rice (cv. Himenomochi), hyphal growth was not inhibited in leaf-sheath cells of both mutant and wild rice plants. On the other hand, both Sekiguchi and blast lesions formed on the leaves of the *sl* mutant, although necrotic and blast lesions formed on the leaves of the wild rice plant. Many spores were produced on blast lesions. However, no sporulation was observed on the Sekiguchi lesions, even though the size of the Sekiguchi lesions was larger than that of blast lesions. These results indicated that the Sekiguchi lesion formation is a manifestation of a resistant response of the rice *sl* mutant to *M. grisea*, and that the rice *sl* mutant, cv. Sekiguchi-himenomochi, has a blast resistance gene *Pi-k*.

Key Words: Rice Sekiguchi lesion mutant, *Magnaporthe grisea*, blast resistance gene

## Introduction

Sekiguchi and Furuta (1965) reported the spontaneous occurrence of the rice Sekiguchi lesion (*sl*) mutant, which was named cv. Sekiguchi-asahi, from cv. Asahi. A unique lesion, called Sekiguchi lesion, formed on leaves of this mutant. Such rice *sl* mutants have also been discovered in two American rice lines (Marchetti *et al.*, 1983). Sekiguchi lesion formation in these *sl* mutants was regulated by a single recessive gene, *sl* (Kiyosawa, 1970; Marchetti *et al.*, 1983). The lesions were first visible as water soaked gray spots which enlarged rapidly to finally become orange to orange-brown in color. This unique lesion was induced by infection with *Cochliobolus miyabeanus* (Ito et Kuribayashi) Drechsler ex Dastur or *Magnaporthe grisea* (Hebert) Barr (imperfect stage, *Pyri-*

* This study was supported in part by Grant-in-Aid (Nos. 07660058 and 09660050) from the Ministry of Education, Science, and Culture of Japan.
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*cularia oryzae* Cavara). Some chemicals or fungal metabolites induced leaf necrosis similar to the Sekiguchi lesion (Kiyosawa, 1970; Arase *et al.*, 1990). Previous reports (Arase *et al.*, 1988; Kiyosawa, 1970) have demonstrated that the *sl* mutant, cv. Sekiguchi-asahi, has a blast resistance gene, *Pi-a*, besides a single recessive gene, *sl*.

In1991, a new rice *sl* mutant was discovered in cv. Himenomochi and was named cv. Sekiguchi-himenomochi (Isota *et al.*, 1996). In this paper, we report the response of the blast resistance gene of cv. Sekiguchi-himenomochi to *M. grisea*.

## Materials and Methods

### Plant and fungus

Rice cvs. Asahi (resistance gene *Pi-a*), Himenomochi (resistance gene *Pi-k*), Mohkoto (resistance gene +), Sekiguchi-asahi (resistance gene *Pi-a*), and Sekiguchi-himenomochi (resistance gene is unknown) were used in this study. All rice plants were grown in the greenhouse as reported previously (Arase *et al.*, 1990). Strains Ken 54-04 (race 003), Ken 60-19 (race 037), Naga 64-8, Naga 69-150 (race 007), Pa1-41A (race 031), SH 85-125 (race 001), and 0528-2 (race 333) of *M. grisea* were used. Spores of *M. grisea* were synchronously formed using the method reported previously (Arase *et al.*, 1990). Compatibility between rice cultivar and fungal race is shown in Table 1.

### Inoculation

To investigate hyphal growth of *M. grisea*, the inner surfaces of detached rice leaf-sheaths were inoculated with a spore suspension ($5 \times 10^5$ spores/ml) of *M. grisea*. Inoculated sheaths were kept in a moist chamber for 36 hr at 26 C. The hyphal growth index of *M. grisea* was determined under a light microscope using the method of Takahasihi (1950).

A spore suspension ($5 \times 10^4$ spores/ml) from each strain was sprayed onto intact rice plants at 5-6 leaf-stage. Inoculated rice plants were kept in a moist chamber for 24 hr at 26 C in darkness, and then in the laboratory under diffused light conditions. Lesion formation and sporulation were investigated 7 days

**Table 1** Compatibility between rice plant and *M. grisea*

| Rice cultivar (resistance gene) | *M. grisea* strain (race) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Ken 54-04 (003) | Ken 60-19 (037) | Naga 64-8 (033) | Naga 69-150 (007) | Pa1-41A (031) | SH 85-125 (001) | 0258-2 (333) |
| Himenomochi (*Pi-k*) | I[a)] | C[b)] | C | I | C | I | C |
| Sekiguchi-himenomochi (?) | ? | ? | ? | ? | ? | ? | ? |
| Asahi (*Pi-a*) | C | C | C | C | I | I | C |
| Sekiguchi-asahi (*Pi-a*) | C | C | C | C | I | I | C |
| Mohkoto (+) | C | C | C | C | C | C | C |

a) Incompatible. b) Compatible.

**Table 2** Hyphal growth of *M. grisea* in rice leaf-sheath cells

| Rice cultivar | Strain of *M. grisea* (race)[x)] | |
|---|---|---|
| | Naga 69-150 (007) (Mean±SE)[y)] | Ken 60-19 (037) (Mean±SE) |
| Himenomochi | 0.8±0.0a | 4.3±0.8b |
| Sekiguchi-himenomochi | 0.7±0.1a | 3.8±0.4b |
| Asahi | 4.1±0.4b | 4.4±0.7b |
| Sekiguchi-asahi | 4.2±0.4b | 3.7±0.6b |
| Mohkoto | 4.1±0.6b | 4.2±0.6b |

x) A spore suspension of *M. grisea* was inoculated to the inner surface of detached rice leaf-sheaths. After 36 hr, hyphal growth of *M. grisea* in leaf-sheath cells was determined using Takahashi's method.

y) Values with a column followed by the same letter do not differ significantly ($P<0.05$) according to Duncan's new multiple range test.

after inoculation.

## Results

### Response of rice sl mutant plants to *M. grisea* at the cellular level

When the detached rice leaf-sheaths of rice plants were inoculated with strain Naga 69-150 (race 007) of *M. grisea*, hyphal growth was very poor in leaf-sheath cells of both cvs. Himenomochi and Sekiguchi-himenomochi. In these cultivars, all invading hyphae were located in the first invaded cells. In rice cvs. Asahi, Mohkoto and Sekiguchi-asahi, however, infection hyphae grew out of the first invaded cells into the neighboring cells 36 hr after inoculation (Table 2). On the other hand, in strain Ken 60-19 (race 037), all cultivars used showed susceptibility as demonstrated by significant hyphal growth in the sheath cells.

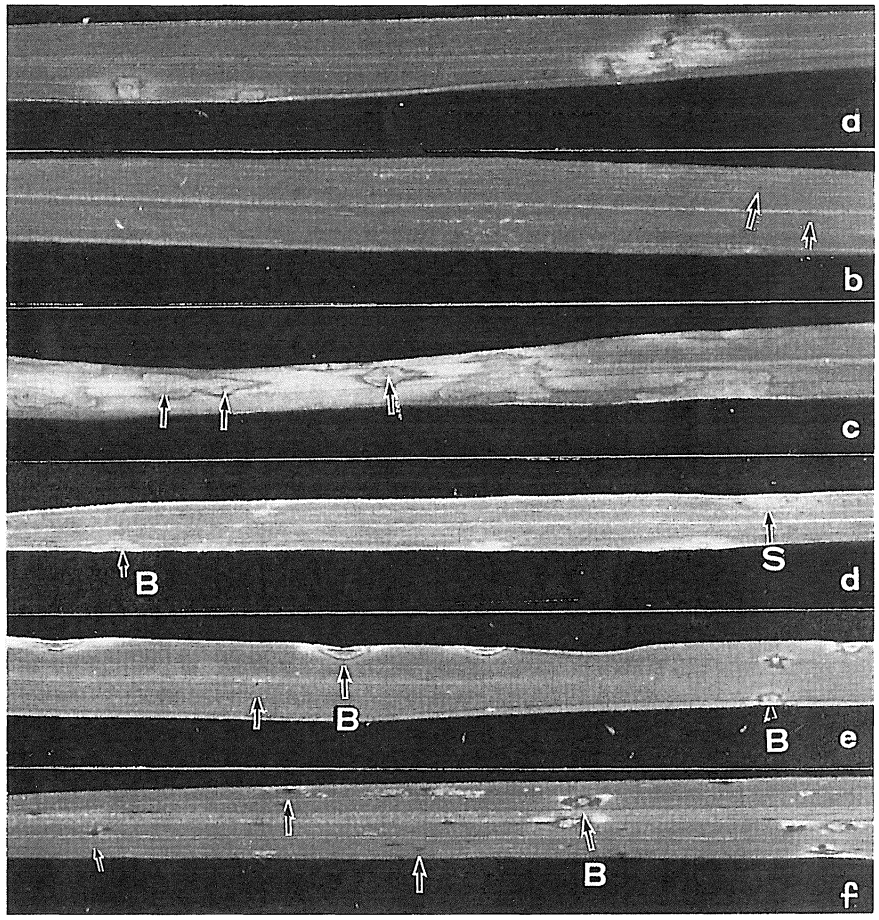


**Fig. 1** Lesion formation by *M. grisea* on the rice *sl* mutant and wild plants.
a: cv. Sekiguchi-himenomochi x strain Naga 60-150 (incompatible interaction)
Only Sekiguchi lesions were formed.
b: cv. Himenomochi x strain Naga 69-150 (incompatible interaction)
Few necrotic lesions (arrows) were formed.
c: cv. Sekiguchi-asahi x strain Naga 60-150 (compatible interaction)
Both Sekiguchi and blast (arrows) lesions were formed.
d: cv. Sekiguchi-himenomochi x strain Ken 60-19 (compatible interaction)
Both Sekiguchi (S) and blast (B) lesions were formed.
e: cv. Himenomochi x strain Ken 60-19 (compatible interaction)
Both necrotic (arrow) and blast (B) lesions were formed.
f: cv. Mohkoto x strain Ken 60-19 (compatible interaction)
Both necrotic (arrows) and blast (B) lesions were formed.

**Sekiguchi lesion formations induced by *M. grisea* on rice *sl* mutants**

When four rice cultivars (Asahi, Himenomochi, Mohkoto, Sekiguchi-asahi and Sekiguchi-himenomochi) were inoculated with *M. grisea* spores, there was a large difference in lesion formation between both wild (Asahi, Himenomochi, and Mohkoto) and mutant (Sekiguchi-asahi and Sekiguchi-himenomochi) rice cultivars. In rice cv. Sekiguchi-himenomochi, only Sekiguchi lesions were formed by inoculation with strains Ken 54-04, Naga 69-150, and SH85-125 of *M. grisea*. In strains Ken 60-19, 0852-8, Pa1-41A, and Naga 64-8, however, both Sekiguchi and blast lesions were formed on the leaves. On the other hand, in cv. Himenomochi, although only necrotic lesions were formed by the former strains, both necrotic and blast lesions were formed by the latter strains (Table 3).

In cv. Sekiguchii-asahi, strains Pa1-41A and SH85-125 of *M. grisea* induced only Sekiguchi lesions, although other strains induced both Sekiguchi and blast lesions. On the other hand, in cv. Asahi, the former strains induced necrotic lesions, although the latter strains induced both necrotic and blast lesions on leaves. In cv. Mohokoto, all strains induced both necrotic and blast lesions (Fig. 1).

**Sporulation on Sekiguchi, blast and necrotic lesions**

Many spores were produced on blast lesions regardless of culivars (Table 4). However, no sporulation was observed on the Sekiguchi lesions, even though the size of the Sekiguchi lesions was larger than that of the blast lesions.

**Table 3** Difference in lesion formation between wild type (cv. Himenomochi) and *sl* mutant (cv. Sekiguchi-himenomochi) rice to *M. grisea*

| Strain of *M. grisea* (race) | Wild type | | | *Sl* mutant | | |
|---|---|---|---|---|---|---|
| | N | B | S | N | B | S |
| Ken 54-04 (003) | +[a] | -[b] | - | - | - | + |
| Ken 60-19 (037) | + | + | - | - | + | + |
| Naga 64-8 (033) | + | + | - | - | + | + |
| Naga 69-150 (007) | + | - | - | - | - | + |
| Pa1-41A (031) | + | + | - | - | + | + |
| SH 85-125 (001) | + | - | - | - | - | + |
| 0528-2 (333) | + | + | - | - | + | + |

A spore suspension of each strain of *M. grisea* was inoculated to cvs. Himenomochi and Sekiguchi-himenomochi. Necrotic (N), blast (B), and the Sekiguchi (S) lesion formations were investigated 7 days after inoculation.

a) Lesion formation was observed. b) No lesion formation was observed.

**Table 4** Sporulation in necrotic (N), blast (B) or Sekiguchi (S) lesion on wild and sl mutant rice plants induced by *M. grisea*

| Rice cultivar | Strain Naga 60-150 | | | Strain Naga 64-8 | | |
|---|---|---|---|---|---|---|
| | N | B | S | N | B | S |
| Himenomochi | 0/20[a] | 0/2 | no[b] | -[c] | - | - |
| Sekiguchi-himenomochi | no | 0/1 | 0/40 | no | 13/16 | 0/11 |
| Asahi | 0/40 | 40/40 | no | - | - | - |
| Sekiguchi-asahi | no | 37/0 | 0/40 | - | - | - |
| Mohokoto | 0/40 | no | 39/40 | | | |

a) Number of sporulated lesions / Number of total lesions checked. b) No lesion formation.

c) Not observed.

## Discussion

When the leaves of both the wild (cv. Himenomochi) and mutant (cv. Sekiguchi-himenomochi) rice plants were inoculated with Naga 69-150 (race 007) and Ken 54-04 (race 003) strains of *M. grisea*, only Sekiguchi lesions were formed on the mutant. On the wild rice cultivar, typical necrotic lesions were formed. Under the inoculation with Naga 64-8 (race 033), Ken 60-19 (race 037), and 0582-2 (race 333) strains, Sekiguchi and blast lesions were formed on the mutant, while blast lesions were formed on wild rice cultivar. Further, such differences in lesion formation among fungal strains were observed in hyphal growth in leaf-sheath cells. Hyphal growth of Naga 69-150 and Ken 54-04 strains was significantly inhibited in the rice cells of both cultivars but that of strains Naga 64-8, Ken 60-19, and 0582-2 was not. In previous reports (Arase *et al.*, 1988; Kiyosawa, 1970), it was demonstrated that when the leaves of cv. Sekiguchi-asahi, which has a blast resistance gene *Pi-a*, were inoculated with an incompatible race of *M. grisea*, only the Sekiguchi lesions were formed, and this lesion formation was regarded as a manifestation of resistant response of the rice *sl* mutant. Our observations in this study indicated that cv. Sekiguchi-himenomochi has also the blast resistance gene(s) to Ken 54-04, Naga 69-150, and SH 85-125 strains of *M. grisea*. The response of the *sl* mutant, cv. Sekiguchi-himenomochi to *M. grisea* races is summarized in Table 5. The Sekiguchi lesion formation of the rice *sl* mutant, cv. Sekiguchi-himenomochi, induced by the incompatible race of *M. grisea* corresponded well to necrotic spot formation as the resistant response (hypersensitive response) in a wild type rice, cv. Himenomochi. The above-described evidence indicates the presence of the blast resistance gene *Pi-k* in rice cv. Himenomochi.

Our studies indicate that the Sekiguchi lesion formation is a manifestation of a race-specific resistance response of *sl* mutants to *M. grisea*. However, when *sl* mutants inoculated with *M. grisea* spores were immediately placed under continuous irradiation, only Sekiguchi lesions were induced on leaves regardless of the interaction type between fungal races and *sl* mutants. This indicates that Sekiguchi

**Table 5** Comparison of response of rice cultivars, which have different resistance genes, to M. grisea

| Differential Cultivar (Resistance gene) | Race of *M. grisea* (Strain used in this study) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 333 (0852-8) | 037 (Ken 60-19) | 031 (Pal-41A) | 033 (Naga 64-8) | 007 (Naga 69-150) | 003 (Ken 54-04) | 001 (SH 85-125) |
| Shin 2 (*Pi-k*$^s$) | S[a)] | S | S | S | S | S | S |
| Aichi-asahi (*Pi-a*) | S | S | R | S | S | S | R |
| Ishikari-shiroke (*Pi-i*) | R[b)] | S | R | R | S | R | R |
| Shinsetsu (*Pi-a, Pi-i*) | R | S | R | R | S | R | R |
| Kanto 51 (*Pi-k*) | S | S | S | S | R | R | R |
| Toto (*Pi-a, Pi-k*) | S | S | R | S | R | R | R |
| Toto (*Pi-i, Pi-k*) | R | S | R | R | R | R | R |
| Toto (*Pi-a, Pi-i, Pi-k*) | R | S | R | R | R | R | R |
| Sekiguchi-asahi (*Pi-a*) | s[c)] | s | r | s | s | s | r |
| Sekiguchi-himenomochi (*Pi-k*) | s | s | s | s | r[d)] | r | r |

a) Blast lesion formation.
b) No lesion or necrotic spot formation.
c) Sekiguchi and blast lesion formations.
d) Sekiguchi lesion formation.

lesion formation under continuous irradiation is a manifestation of a race-nonspecific response in *sl* mutants. At present we have no definitive explanation for the difference in the responses induced by different light treatments of *sl* mutants. However, our results indicate the following possibilities: 1) the induction of Sekiguchi lesions is a marker for defense system activation in *sl* mutants; 2) *M. grisea* induces the Sekiguchi lesions, which are markers of disease resistance under light conditions, regardless of race; and 3) compatible races of *M. grisea* have an ability to suppress resistance as well as the ability mentioned above. Elucidation of the factors related to the above two abilities will provide the possibility of detecting disease determinants such as fungal elicitors or suppressors in rice blast disease.

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