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# Effect of light intensity on Sekiguchi lesion development in lesion mimic mutant of rice inoculated with *Magnaporthe grisea*

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**Abstract** The effect of light intensity on Sekiguchi lesion formation and tryptamine accumulation involved in light-induced resistance of rice (cv. Sekiguchi-asahi) inoculated with *Magnaporthe grisea* was determined by using neutral density filters (ND). When the *M. grisea*-inoculated leaves were irradiated by ND-filtered lights from daylight fluorescent lamps, there was no difference in number of Sekiguchi lesions among attenuation rates 0, 20, 50, 75 and 90%. However Sekiguchi lesion development and tryptamine accumulation were enhanced with increasing the light intensity. In the dark condition (attenuation rate 100%), however, no Sekiguchi lesion formation and low level accumulation of tryptamine were observed. This result suggested that Sekiguchi lesion development and tryptamine accumulation were photosynthesis activity- and light intensity-dependently induced in Sekiguchi lesion mutant infected with *M. grisea*.

**Key words**: Sekiguchi lesion, Rice, Tryptamine, *Magnaporthe grisea*

## Introduction

Rice cultivar Sekiguchi-asahi was found as to be a propagation-type lesion mimic mutant from the cultivar Asahi in Japan (Sekiguchi and Furuta 1965). Kiyosawa (1970) has reported that this mutant forms unique lesions (called Sekiguchi lesions) on leaves inoculated with *Magnaporthe grisea* and *Cochliobolus miyabeanus*. Sekiguchi lesion formation is regulated by a single recessive gene, *sl* (also known as *spl1*) (Kinoshita 1995; Marchetti et al. 1983). We have previously demonstrated that the Sekiguchi lesion (SL) mutant has two kinds of responses to *M. grisea* infection under the different light conditions. Sekiguchi lesions were induced in leaves of the *sl* mutant inoculated with *M. grisea* spores under visible light (400-700nm), and infection hypha formation and sporulation of *M. grisea* were significantly inhibited in the Sekiguchi lesions. On the other hand, blast lesions, which indicate a susceptible response of rice, were induced in the dark or under near-UV light (290-330nm) (Arase et al. 1997). From these results, we concluded that Sekiguchi lesion formation is one of the basic elements of light-enhanced resistance in the *sl* mutant of rice and visible light is playing an important role in the expression of resistance (Arase et al. 2000). Furthermore, Iedome et al. (1995) reported that Sekiguchi lesion formation by *M. grisea* toxin was definitely dependent on the light intensity.

As a key factor in this light-enhanced resistance, the indole alkaloid compound tryptamine was isolated from the Sekiguchi lesions (Arase et al. 2001). Tryptamine is biosynthesized from tryptophan by tryptophan decarboxylase (TDC). High levels of TDC activity and tryptamine accumulation were observed in the Sekiguchi lesion induced by infection with *M. grisea* under light (Ueno et al. 2003). Tryptamine inhibited not only spore germination and appressorium formation of *M. grisea* at high concentrations (>600 μg/ml), but also the infection-hypha formation at low concentrations (150-300 μg/ml) (Arase et al. 2001).

In the present paper, we show that the effect of light intensity on Sekiguchi lesion development and tryptamine accumu-

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lation in the Sekiguchi lesion mutant inoculated with *M. grisea* was determined using neutral density filters (ND).

## Materials and Methods

### Plant and fungus

The *sl* mutant cv. Sekiguchi-asahi (resistance gene *Pi-a*) was used in this study. This cultivar was grown to the 5-6 leaf stage in a glasshouse, as described by Arase et al. (2000). Strain Naga 69-150 (race 007) of *M. grisea*, which is pathogenic to the rice cv. Sekiguchi-asahi, was used as a pathogen. *M. grisea* was grown on a rice bran agar medium at 26°C for 14 days. The grown plates were kept at 26°C for 2 days under near-ultraviolet radiation provided by fluorescent lamps (FL20s BLB; National, Osaka, Japan) after aerial hyphae on the medium were washed away by tap water. Thus, synchronously formed spores were used as inocula.

### Fungus and light condition

Detached rice leaves of the *sl* mutant at the 5-6 leaf stage were inoculated with a spore suspension ($5\times10^5$ spores $ml^{-1}$) of *M. grisea*. These inoculated rice plants were kept under the different light conditions for 72h. The spore-inoculated leaves were kept in moist plastic cases covered with Saranwrap film (SAW, light intensity; $735\pm49.6$ μW $cm^{-2}$, attenuation rate; 0%, Asahi Kasei Industries Co., Tokyo, Japan), neutral density filters (ND0.1: attenuation rate ; 20%, ND0.3: 50%, ND0.6: 75%, ND1.0: 90%, Fuji Film, Tokyo, Japan) and aluminum foil (attenuation rate; 100%, ALF) under continuous light from three 40W daylight fluorescent lamps (FLR 40SW, Mitsubishi Co., Osram, Yokohama, Japan). The leaves were irradiated by continuous light from three 40 W daylight fluorescent lamps (FLR40SW, Mitsubishi Co., Osram, Yokohama, Japan). After 72 h, the spore-inoculated leaves kept under the different light conditions were harvested for Sekiguchi lesion and tryptamine analyses.

### Sekiguchi lesion analysis

Sekiguchi lesion formation was investigated 72 h after *M. grise* inoculation. The area of each lesion was measured, and the degree of Sekiguchi lesion formation on leaves under different light intensity was shown by the ratio of lesion occupation per leaf.

### Analysis of tryptamine

The leaves from each light treatment were extracted with 80% ethanol and then its extracts were evaporated at 35°C under reduced pressure. The volume of the aqueous solution was adjusted to a weight equivalent to that of the starting weight of the extracted lesions or control leaves. To analyze tryptamine accumulation, each aqueous solution was adjusted to pH 10.7 by 0.1 N $Na_2CO_3$, and then extracted with ethyl acetate (EtOAc). The EtOAc extracts were dissolved in methanol and subjected to high-performance liquid chromatography (HPLC) (Hitachi 1,600, Wakosil-Ⅱ 5C18 AR, column size: 4.6×250 mm) and eluted with methanol : ammonium acetate (8 : 2 v/v) monitored at 281 nm. Tryptamine was detected at Rt. 2.94 min.

## Result

### Effects of light intensity on Sekiguchi lesion development in *sl*-mutant inoculated with *M. grisea*

When rice cv. Sekiguchi-asahi inoculated with *M. grisea* spores were irradiated by SAW-filtered lights from the daylight fluorescent lamps for 72 h, large orange to orange-brown lesions (Sekiguchi lesion) were formed on the leaves. In contrast, no lesion formation was observed in the dark (ALF). To investigate the effect of light intensity on the Sekiguchi lesion formation or its development, *M. grisea*-inoculated leaves covered with ND filters ND1.0, ND0.6, ND0.3 and ND0.1 were kept under the daylight fluorescent lamps. The Sekiguchi lesions were formed regardless of the attenuation rate of ND filters and significant difference was not observed in lesion num-

**Table 1** Effect of light intensity on Sekiguchi lesion formation in rice cv. Sekiguchi-asahi.

| Attenuation (%) | No. of Sekiguchi lesion per leaf |
|---|---|
| 0 (SAW) | 17.2±8.3 |
| 20 (ND0.1) | 17.6±9.1 |
| 50 (ND0.3) | 19.7±10.2 |
| 75 (ND0.6) | 16.1±5.9 |
| 90 (ND1.0) | 15.9±5.3 |
| 100 (ALF) | 0.0 |

*M. geisea*-inoculated leaves were kept in plastic cases which were covered with Saranwrap (SAW, attenuation rate; 0%), neutral density filters (ND0.1: attenuation rate; 20%, ND0.3: 50%, ND0.6: 75%, ND1.0: 90%) and aluminum foil (attenuation rate; 100%) under daylight fluorescent lamps. Number of Sekiguchi lesion formation was investigated at 72h after inoculation.

ber among ND filters used (Table 1). However, there was a large difference in development of Sekiguchi lesions among ND filters used (Fig. 1a). Under ND filters 0.1 and ND0.3, Sekiguchi lesion developed significantly like that under the SAW and occupied 43.0±14.6 and 38.4±10.1 % of the leaf area, respectively. Under ND0.6 and ND1.0 filtered lights, however, Sekiguchi lesion development were suppressed, as compared to that of ND0.1 and ND0.3, and the rates of lesion development in ND0.6 and ND1.0 were 27.8±6.1% and 10.4±6.6%, respectively (Fig. 1b).

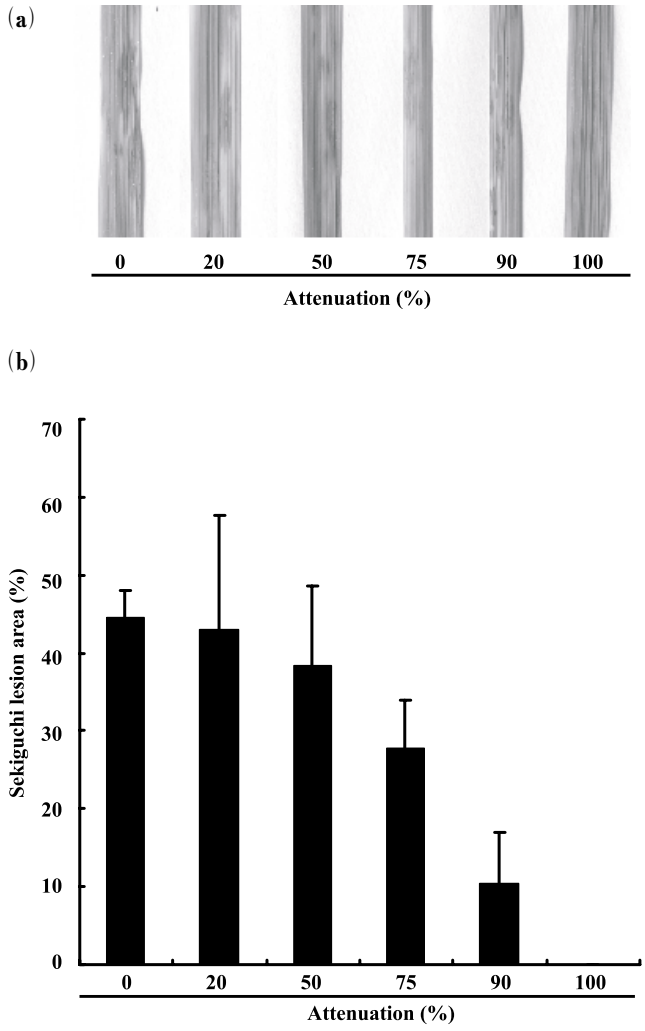


**Fig. 1** Effects of light intensity on Sekiguchi lesion formation in *sl* mutant inoculated with *M. grisea*. Inoculated leaves were kept in moist plastic cases covered with Saranwrap (SAW, attenuation rate; 0%), neutral density filters (ND 0.1: attenuation rate; 20%, ND0.3: 50%, ND0.6: 75%, ND1.0: 90%) and aluminum foil (attenuation rate; 100%) under daylight fluorescent lamps. After 72 h, the Sekiguchi lesion formation (a) and its area (b) were investigated.

**Effects of light intensity on tryptamine accumulation in *sl* mutant inoculated with *M. grisea***

The effect of light intensity on the tryptamine accumulation in leaves inoculated with *M. grisea* was investigated. The amount of tryptamine in *M. grisea*-inoculated leaves under SAW or in the dark (ALF) was 118.4±10.8 and 18.0±6.5 μg/g fresh weight, respectively. When *M. grisea*-inoculated leaves were kept under the ND filters with different attenuation rates, large differences were observed in tryptamine accumulation. Tryptamine accumulation under the ND filters 1.0, 0.6, 0.3 and 0.1 was 40.9±6.1, 71.5±17.8, 96.8±7.0 and 114.0±23.8 μg/g fresh weight, respectively. Tryptamine accumulation was light intensity dependent manner (Fig. 2).

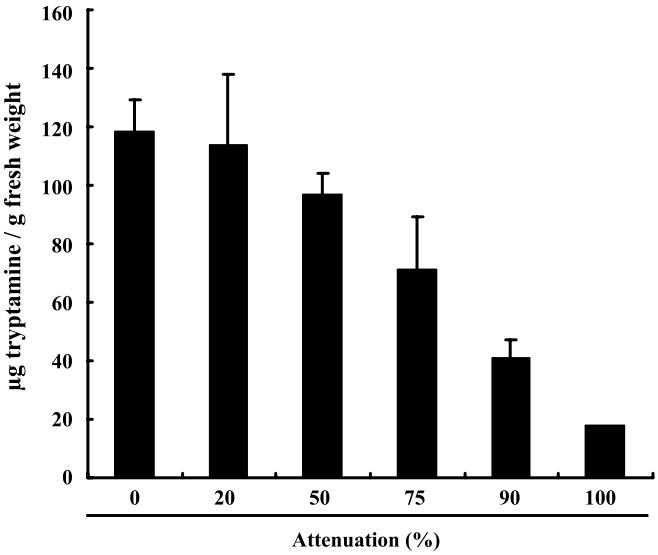


**Fig. 2** Effects of light intensity on tryptamine accumulation in *sl* mutant inoculated with *M. grisea*. Inoculated leaves were kept in moist plastic cases covered with Saranwrap (SAW, attenuation rate; 0%), neutral density filters (ND 0.1: attenuation rate; 20%, ND0.3: 50%, ND0.6: 75%, ND1.0: 90%) and aluminum foil (attenuation rate; 100%) under daylight fluorescent lamps. After 72 h, the 80% ethanol extracts from *M. grisea*-inoculated leaves were subjected to HPLC (Hitachi 1600, Divelosil ODS -5, column size: 4.6 mm×250 mm) and eluted with acetonitrile : ammonium acetate (95 : 5, v/v) monitoring at 281 nm. Tryptamine was detected at a retention time of 2.94 min. Bars represent±SD.

## Discussion

In a previous study (Arase et al. 2001), we reported the Sekiguchi lesion formation and tryptamine accumulation in *sl* mutant inoculated with *M. grisea* were significantly enhanced under visible light (400-650 nm), but not under near-UV light (300-400 nm) or in the dark. Furthermore, we demonstrated that the spore germination fluid of *M. grisea* induced Sekiguchi lesion-like necrosis in a photosynthetic activity-dependent manner (Arase et al. 1990; Iedome et al. 1995). Sekiguchi lesion formation and tryptamine accumulation were also suppressed by pretreatments with cycloheximide, heat shock and 3-(3, 4-dichlorophenyl)-1, 1-dimetylurea even under the light (Ueno et al. 2004; Imaoka et al. 2006). In this study, it was demonstrated that Sekiguchi lesion development and tryptamine accumulation were also light intensity-dependently induced in rice Sekiguchi lesion mutant inoculated with *M. grisea.* This result suggested that Sekiguchi lesion development and tryptamine accumulation were photosynthesis activity- and light intensity-dependently induced in *sl*-mutant infected with *M. grisea*.

## Reference

Arase, S., Kondo, K., Honda, Y., Nozu, M. and Nishimura, S. (1990) Studies on host-selective infection mechanism of *Pyricularia oryzar* Cavara (3) Light-dependency of leaf necrosis formation by toxin(s) from germinating spores. Ann. Phytopathol. Soc. Jpn. 56, 346-350.

Arase, S., Fukuyama, R., Tokizawa, K., Ikegami, S., Honda, Y. and Nozu, M. (1997) The effects of light and photo- and protein-synthetic inhibitors on the Sekiguchi lesion formation by *Magnaporthe grisea* in rice cv. Sekiguchi-asahi. J. Phytopathol. 145, 31-35.

Arase, S., Fujita, K., Uehara, T., Honda, Y. and Isota, J. (2000) Light-enhanced resistance to *Magnaporthe grisea* infection in the rice Sekiguchi lesion mutant. J. Phytopathol. 148, 197-203.

Arase, S., Ueno, M., Toko, M., Honda, Y., Itoh, K. and Ozoe, Y. (2001) Light-dependent accumulation of tryptamine in the rice Sekiguchi lesion mutant infected with *Magnaporthe grisea*. J. Phytopathol. 149, 409-413.

Iedome, M., Arase, S., Honda, Y. and Nozu, M. (1995) Light-dependent necrosis formation by *Magnaporthe grisea* toxin(s) in rice cv. Sekiguchi-asahi. J. Phytopathol. 143, 325-328.

Imaoka, A., Ueno, M., Kihara, J. and Arase, S. (2006) Role of chloroplasts in light-dependent Sekiguchi lesion formation. Jpn. J. Phytopathol. 72, 69 (Abstr).

Kinoshita, T. (1995) Report of committee on gene symbolization, nomenclature and linkage groups. Rice Genet. Newsl. 12, 9-153

Kiyosawa, S. (1970) Inheritance of a particular sensitivity of the rice variety Sekiguchi Asahi, to pathogens and chemicals, and linkage relationship with blast resistance genes. Bull. Nat. Inst. Agr. Sci. 21, 61-72.

Marchetti, M. A., Bollich, C. N. and Uecker, F. A. (1983) Spontaneous occurrence of the Sekiguchi lesion in two American rice line: its induction, inheritance, and utilization. Phytopathology. 73, 603-606.

Ueno, M., Shibata, H., Kihara, J., Honda, Y. and Arase, S. (2003) Increased tryptophan decarboxylase and monoamine oxidase activities induce Sekiguchi lesion formation in rice infected with *Magnaporthe grisea*. Plant J. 36, 215-228.

Ueno, M., Kihara, J., Honda, Y. Isota, J. and Arase, S. (2004) DNA fragmentation in Sekiguchi lesion mutants of rice infected with *Magnaporthe grisea*. J. Gen. Plant Pathol. 70, 321-328.