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# Light-dependent tryptamine accumulation in cytosolic fraction of Sekiguchi lesion mutant of rice infected with *Magnaporthe grisea*

Atsuko IMAOKA[a], Makoto UENO[b], Junichi KIHARA[b] and Sakae ARASE[b]

**Abstract** We investigated the subcelluler localization of tryptamine in *sl* mutant of rice infected with *M. grisea*. Under the light condition, tryptamine accumulation was observed in the cytosolic fraction prepared from leaves infected with *M. grisea*, but not in chloroplast fraction. However, no accumulation was observed in the both fractions prepared from *M. grisea*-inoculated leaves kept in the dark. This result suggested that tryptamine was light-dependently biosynthesized in cytosol of *sl* mutant of rice infected by *M. grisea*.

**Keywords**: Light-enhanced resistance, *Magnaporthe grisea*, Sekiguchi lesion, tryptamine

## Introduction

The rice cv. Sekiguchi-asahi was identified as a lesion mimic mutant derived from cv. Asahi (Sekiguchi and Furuta 1965). This propagation type mutant shows a unique response forming orange to orange-brown lesions (Sekiguchi lesion) without pathogen attack. Recently, Arase et al. (2000) demonstrated that rice Sekiguchi lesion (*sl*) mutant, cv. Sekiguchi-asahi was highly resistant to *Magnaporthe grisea* infection under the visible light conditions. 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 inhibited not only spore germination and appressorium formation of *M. grisea* at high concentration (> 600 μg/ml), but also infection hypha formation in onion cells at low concentration (150-300 μg/ml). Tryptamine is well known as an intermediate of tryptamine pathway which is one of indole-3-acetic acid (IAA) biosynthesis pathways. Tryptamine is biosynthesized from tryptophan by tryptophan decarboxylase (TDC) and oxidized by monoamine oxidase (MAO). Ueno et al. (2003) demonstrated that increased TDC and MAO activities induce the Sekiguchi lesion formation in *sl* mutant infected with *M. grisea* under the light condition. Recently, Imaoka et al. (2004) demonstrated that chloroplasts play an important role in the Sekiguchi lesion formation. However, relationship between light dependency of tryptamine biosynthesis and chloroplasts is not yet investigated. In this study, we show tryptamine biosynthesized in cytosolic fraction of *sl* mutant infected with *M. grisea*.

## Materials and Methods

### Plant and pathogen

The rice *sl* mutant (cv. Sekiguchi-asahi) was grown in a greenhouse as described previously by Arase et al. (2001). *M. grisea* (Strain Naga 69-150, race 007) was grown on a rice bran agar medium at 26°C for 14 days. The growth plates were kept at 26°C for about 2 days with near-UV illumination after aerial hyphae on the medium were washed away by distilled water. Thus, synchronously formed spores were used as inocula.

### Inoculation and light irradiation

Detached leaves of rice *sl* mutant at the 4-5 leaf stage were inoculated with a spore suspension ($5\times10^5$ spores $ml^{-1}$) of *M. grisea* (strain Naga 69-150). As a control, distilled water (DW) was sprayed. Inoculated or treated leaves were kept in moist plastic cases under the light from fluorescent lamps (FLR40SW, Mitsubishi Co., Osram, Yokohama, Japan) or in the dark at 26-28°C. After 120 h, these leaves were harvested for chloroplast isolation and tryptamine analysis.

[a] The United Graduate School of Agricultural Science, Shimane University, Matsue 690-8504, Japan

[b] Laboratory of Plant Pathology, Faculty of Life and Environmental Science, Shimane University, Matsue, 690-8504, Japan (Correspondence to S. Arase. E-mail: arase@life.shimane-u.ac.jp)

### Isolation of chloroplasts from rice leaves

DW-treated and *M. grisea*-inoculated leaves were used for chloroplast isolation. Detached leaves were ground with homogenize isolation buffer (0.01M Tris, 0.01M NaCl, 0.4 M Surcrose, pH 7.8). The homogenates were filtered with gauze and then centrifuged at 700 *g* for 2 min at 4°C. The supernatants were centrifuged at 5000 *g* for 15 min at 4°C. The pellets were reextracted with homogenize isolation buffer (0.01M Tris, 0.01M NaCl, 0.4 M Surcrose, pH 7.8) and centrifuged again at 5000 *g* for 15min at 4°C. Pellets and supernatants were used for tryptamine analysis as chloroplatst and cytosolic fractions, respectively.

### Extraction of tryptamine

DW-treated and *M. grisea*-inoculated leaves were extracted with 80 % ethanol, and ethanol was evaporated at 35°C under reduced pressure. The volume of the aqueous solution were adjusted to initial leaves weight for example, when a sample extracted from 1g fresh weight of leaves, the aqueous volume was adjusted to 1 ml. 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. Ten micro liters each extracts and the authentic tryptamine solution were spotted onto silica gel thin layer chromatography (TLC) plates (Silica gel 60, Merck AG, Darmstadt, Germany) and then deveroped using an *n*-BuOH, AcOH, $H_2O$ (4∶1∶1, v/v) solvent system. After development, TLC plates were sprayed with ninhydrin solution and then heated at 100°C.

Using above method, tryptamine was also isolated from chloroplast and cytosolic fractions

## Results and Discussion

When the *sl* mutant was inoculated with *M. grisea*, Sekiguchi lesion formation was induced under light, but not in the dark. In DW-treated leaves, no Sekiguchi lesion formation was observed, regardless of the light conditionss. Tryptamine accumulation was observed in leaves inoculated with *M. grisea* under light, but not in the dark, as demonstrated by TLC analysis. In DW-treated leaves, no accumulation of tryptamine was observed, regardless of the light conditionss. In order to investigate the localization of tryptamine in leaves infected with *M. grisea*, chloroplast and cytosolic fractions were prepared from leaves inoculated with *M. grisea*, and then, tryptamine was extracted from these fractions. As shown in Fig. 1, tryptamine accumulation was observed in cytosolic fraction prepared from leaves with Sekiguchi lesions under light, but not in that from leaves without Sekiguchi lesion in the dark. On the other hand, tryptamine accumulation was not observed in chloroplast fractions prepared from leaves kept under light or in the dark. No accumulation of tryptamine was observed even in cytosolic fractions prepared from DW-treated leaves kept under light.

In *sl* mutant, Sekiguchi lesions were induced in leaves without *M. grisea* inoculation during the cultivation. Tryptamine accumulated in the leaves with naturally-formed Sekiguchi lesions, but not in those without Sekiguchi lesions. Furthermore, tryptamine accumulation was observed in cytosolic fraction prepared from leaves with Sekiguchi lesions, but not in chlo-

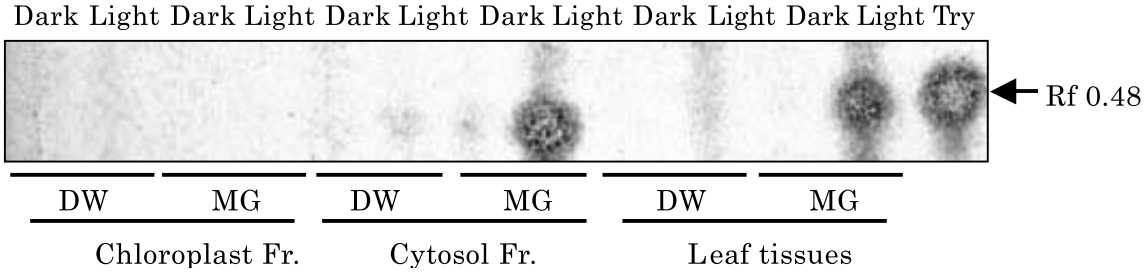


**Fig. 1 Tryptamine accumulation in *sl*-mutant infected with *M. grisea*.**

*M.grisea*-inoculated (MG) and distilled water - treated (DW) leaves were kept under light or in the dark at 26°C. After 120 h, inoculated or treated leaves (Leaf tissues) were extracted with 80 % methanol and then used for tryptamine analysis. On the other hand, chloroplast and cytosolic fractions prepared from inoculated or treated leaves were also used for tryptamine analysis. Tryptamine was detected as ninhydrin positive spot on the TLC plates. As a control, authentic tryptamine (Try) was also used.

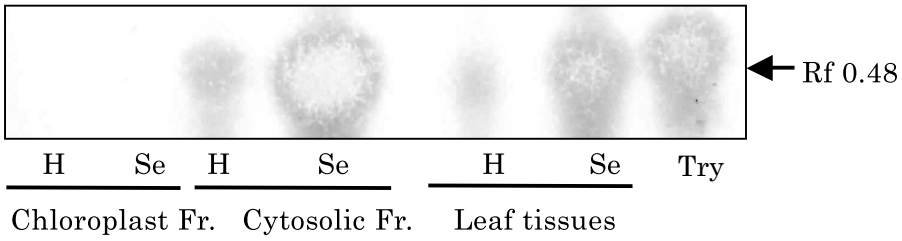


**Fig. 2 Tryptamine accumulation in naturally-induced Sekiguchi lesion of *sl*-mutant.**

Leaf tissues with (Se) or without (H) naturally-induced Sekiguchi lesions were extracted with 80 % ethanol and then extracts were used for tryptamine analysis. Cytosolic and chloroplast fractions prepared from leaf tissues with (Se) or without (H) naturallyinduced Sekiguchi lesions were also used for tryptamine analysis. Each fraction was extracted with ethyl acetate (EtoAc). These extractions were spotted on to the TLC plate and then developed using *n*-BuOH, AcOH, $H_2O$ solvent system. As a control, authentic tryptamine (Try) was also developed. After development, tryptamine was detected as nynhydrin positive spots.

roplast fraction. No tryptamine accumulation was observed in both fractions prepared from leaves without Sekiguchi lesions (Fig. 2).

Arase et al. (1997, 2000, 2001) reported that Sekiguchi lesion formation and tryptamine accumulation in *sl* mutant inoculated with *M. grisea* were enhanced under light, but not in the dark. Moreover, we also demonstrated previously that the tryptamine pathway is playing an important role in light-dependent Sekiguchi lesion formation and tryptamine accumulation (Ueno et al. 2003). In this study, it was demonstrated that tryptamine accumulation was observed in cytosolic fraction of *sl* mutant infected with *M. grisea*. This result suggested that tryptamine biosynthesis site was cytosol, but not chloroplast. On the other hand, Zhao and Last (1996) demonstrated that induction of the tryptophan biosynthetic enzymes is coordinately regulated with the major indolic phytoalexin in Arabidopsis. Recently, we reported that Sekiguchi lesion formation and tryptamine accumulation were significantly suppressed by a tryptophan biosynthesis inhibitor glyhosate even under light, and that suppressive effect by glyhosate pretreatment disappeared by addition of tryptophan (Imaoka et al. 2005). In general, it is well known that tryptophan is biosynthesized in chloroplast (Zhao and Last 1995). These results suggested that role of chloroplasts in light-dependent Sekiguchi lesion formation and tryptamine accumulation was tryptophan supplementation by photosynthesis, but not tryptamine biosynthesis.

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