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Tryptamine accumulation in Sekiguchi lesion mutant of rice infected with *Bipolaris oryzae*

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Abstract The effects of indole-related compounds on infection behaviors of *Bipolaris oryzae* in onion epidermal cells were investigated. Tryptamine inhibited dose-dependently all stages of the infection behavior such as spore germination and infection-hypha formation. Furthermore, antifungal activity of some indole-related compounds to *B. oryzae* was investigated on the TLC plate. As the result, in the indolyl-3-butryric acid and indole-3-acetnitrile, the larger size growth inhibition zone of *B. oryzae* was observed in comparison to the tryptamine, oxindole, gramine, indole, indole-3-acetic acid and indole-3-acetamide. However, in the tryptophan, growth inhibition zone of *B. oryzae* was not observed on the TLC plate. These results suggested that accumulation of high levels of tryptamine or some indole-related compounds might be utilizable to protect with fungal disease by *B. oryzae*.

Key words: Sekiguchi lesion, Light-enhanced resistance, *Tryptamine*, *Bipolaris oryzae*

Introduction

Recently, we demonstrated that rice Sekiguchi lesion mutant, cv. Sekiguchi-asahi, which produces a lesion mimic symptom, was highly resistant to *Magnaporthe grisea* infection under the visible light conditions (Arase et al. 2000a). 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). Furthermore, we reported that some of indole-related compounds were classified into three groups, based on their effect on the infection behavior of *M. grisea* (Ueno et al. 2005).

On the other hand, Arase et al. (2000b) reported that Sekiguchi lesion was also light dependently induced by inoculation with *Bipolaris oryzae*. However, accumulation of tryptamine in Sekiguchi lesion induced by *B. oryzae* infection and antifungal activities of indole-related compounds have not yet been elucidated.

In the present paper, we show that light-dependent accumulation of tryptamine is observed in rice Sekiguchi lesion mutant inoculated with *B. oryzae* that induced Sekiguchi lesion formation, and tryptamine or some indole-related compounds are able to inhibit infection behavior of *B. oryzae.*

Materials and Methods

Plant and fungus

Rice Sekiguchi lesion mutant, cv. Sekiguchi-asahi, was grown in a green house as described previously (Arase et al. 2001). *Bipolarise oryzae* (Strain D6) was used as the pathogen and assay fungus. *B. oryzae* spores were formed by potato dextrose agar (PDA) medium for 10 days at 26℃.

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Inoculation and light irradiation

Detached leaves of rice Sekiguchi lesion mutant at the 6-7 leaf stage were inoculated with *B. oryzae* spores (10^5 spores /ml). The spore-inoculated leaf blades were kept in moist chamber covered with either plastic film for light treatments or aluminum foil for the dark, and irradiated by 40 W daylight fluorescent lamps (FLR40SW, Mitsubishi Co.) suspended above filters. Under the filters, the light intensities on the leaf blades were adjusted to 405 μW/cm^2 by changing the distance between the lamps and the leaves. The light intensity was measured using a thermopile with a quartz window (MIR-100q, Mitsubishi Yuka Co. Ltd., Tokyo, Japan). After 72 h, the spore-inoculated leaves were harvested for tryptamine analysis.

Analysis of tryptamine

Detached leaves were inoculated with *B. oryzae* spores. At 72 h after inoculation, *B. oryzae*-inoculated leaves kept in light or dark conditions were soaked in heated 80% ethanol, and ethanol was then evaporated at 35°C under reduced pressure. 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 1600, Wako Wakosil-Ⅱ 5C18 AR, column size: 4.6×250 mm) and eluted with methanol : ammonium acetate (8:2 v/v), monitoring at 281 nm. The retention time of authentic tryptamine (Aldrich, Milwaukee, WI, USA) was 2.94 min.

Infection behavior-inhibiting activity of tryptamine

In order to investigate the effects of indole-related compounds on the infection behaviors of *B. oryzae*, onion epidermal strip method was used (Homma et al. 1983). As we can observe the all stages of infection behaviors from spore germination to infection-hypha formation, this method is useful for determining the mode of action of unknown compounds such as new pesticides or host components to fungal pathogens (Yamamoto et al. 2000). A spore suspension (10^5 spores/ml) of *B. oryzae* was dropped onto washed onion epidermal cells in the presence of indole-related compounds at 0, 0.02, 0.04, 0.08, 0.15, 0.3, 0.6, 1.2, 2.5 and 5.0 mg/ml, and kept in a moist chamber at 26°C. After 24 h, the percentages of spore germination and infection-hypha formation were determined.

Detection of antifungal activity to *B. oryzae* by indole-related compounds

Tryptamine, tryptophan (Wako Pure Chemical Industries, Osaka, Japan), oxindol (Wako), gramine (Wako), indole-3-pyruvic acid (Wako), indole (Wako), indole-3-butyric acid (Wako), indole-3-acetic acid (Wako), indole-3-acetnitrile (Wako) and indole-3-acetamide (Wako) were used as indole-related compound. Aqueous solution of the indole-related compounds was made at concentration of 5 mg/ml. Two μl of each aqueous solution were spotted onto the silica gel thin layer chromatography (TLC) plates (Silica gel 60, Merck AG, Darmstadt, Germany). After spotted, the TLC plate was sprayed with a concentrated spore suspension of *B. oryzae* in the presence of 4% PDA powder. Inoculated plates were kept in a moist chamber at 26°C for 2 days in the dark. The antifungal activity of compounds was detected on the TLC plates by growth inhibition assay of *B. oryzae* spores.

Result

Light induces Sekiguchi lesion formation and tryptamine accumulation in Sekiguchi lesion mutant infected with *B. oryzae*

To demonstrate tryptamine accumulation in Sekiguchi lesion, the rice Sekiguchi lesion mutant was kept under light or in the dark for 72 h after inoculation with *B. oryzae*. Under light, Sekiguchi lesion was significantly induced 72 h after inoculation (Fig. 1A), and tryptamine amount in leaves with Sekiguchi lesions also increased significantly (72±10.8 μg/g fresh weight) (Fig. 1B). In the dark, however, many typical brown leaf spots were induced (Fig. 1A), and tryptamine did not accumulate (5.4±2.7 μg/g fresh weight) (Fig. 1B).

Effect of tryptamine on infection behaviors of *B. oryzae*

The effects of tryptamine on infection behaviors of *B. oryzae* were investigated using onion epidermis. Spore germination and infection-hypha formation of *B. oryzae* were not inhibited at the concentration of 0.02-0.08 mg/ml. However, at a high concentration (>0.15 mg/ml), tryptamine inhibited spore germination and infection-hypha formation dose-dependently (Fig. 2).

Effect of indole-related compounds on growth of *B. oryzae* on the TLC plate

Antifungal activity of indole-related compounds (tryp-

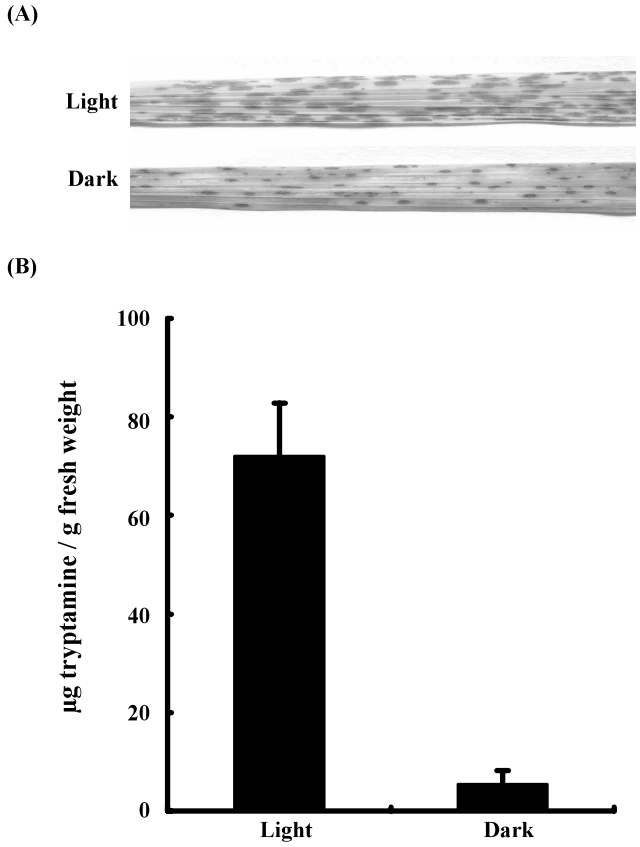


Fig. 1 Light-dependent Sekiguchi lesion formation and accumulation of tryptamine in Sekiguchi lesion mutant infected with *B. oryzae*. Detached rice leaves were inoculated with *B. oryzae*, and kept under light or in the dark condition at 26°C. After 72 h, lesion formation was observed (A). Tryptamine level was determined by HPLC analysis 72 h after inoculation (B).

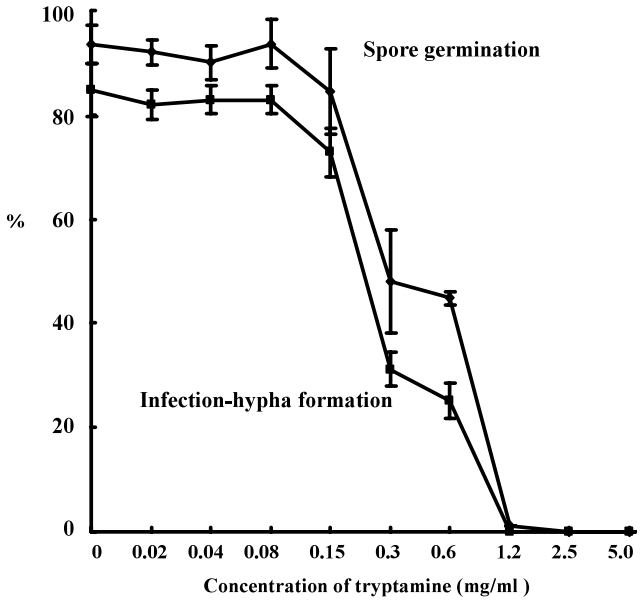
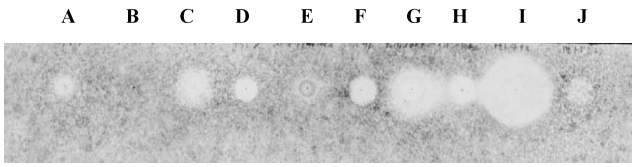


Fig. 2 Effect of tryptamine on infection behaviors of *B. oryzae* spores on the onion epidermis. Spores of *B. oryzae* were suspended in tryptamine solutions at different concentrations and dropped on washed onion epidermal cells. After incubation for 24 h at 26°C in a moist chamber, the infection behavior of the spores was observed under a light microscope. The percentages of spore germination (◆) and infection-hypha formation (■) were determined.



A：Tryptamine　E：Indole-3-pyruvic acid　I：Indol-3-acetnitrile
B：Tryptophan　F：Indole　J：Indole-3-acetamide
C：Oxindol　G：Indolyl-3-butyric acid
D：Gramine　H：Indole-3-acetic acid

Fig. 3 Inhibitory effect of indole-related compounds on growth of *B. oryzae* on the TLC plate. All compounds were dissolved in methanol (5 mg/ml), and then 2 μl of each solution were spotted onto the TLC plate. The TLC plate was sprayed with a concentrated spore suspension of *B. oryzae* in the presence of 4% PDA powder. Inoculated plates were kept in a moist chamber at 26°C for 3 days.

tamine, tryptophan, oxindol, gramine, indole-3-pyruvic acid, indole, indole-3-butyric acid, indole-3-acetic acid, indole-3-acetnitrile and indole-3-acetamide) to *B. oryzae* was estimated by degree of mycelial growth inhibition on the TLC plate. Among them, indole-3-butyric acid and indole-3-acetnitrile showed most strong activity, as demonstrated by the formation of the growth inhibition zone (ca. 2.3 and 3.3 cm in diameter) of *B. oryzae*. Although tryptamine, oxindole, gramine, indole, indole-3-acetic acid and indole-3-acetamide also showed antifungal activity, size of inhibition zone by them was smaller than above two chemicals. In tryptophan, however, growth inhibition zone of *B. oryzae* was not observed on the TLC plate (Fig. 3).

Discussion

In our previous reports (Arase et al. 2000a, b), it was reported that the Sekiguchi lesion formation by *B. oryzae* and

M. grisea was light-dependent in the rice Sekiguchi lesion mutant. We also reported that tryptamine accumulated in a Sekiguchi lesion mutant inoculated with *M. grisea* that induced Sekiguchi lesion formation under light, but not in the dark (Arase et al. 2001). Furthermore, we reported previously that the tryptamine pathway is playing an important role in the light-dependent formation of Sekiguchi lesions induced in Sekiguchi lesion mutant after *M. grisea* infection (Ueno et al. 2003). In this study, it was demonstrated that tryptamine was also light-dependently induced in rice Sekiguchi lesion mutant by infection with *B. oryzae*. This result suggested that tryptamine pathway might play an important role in Sekiguchi lesion formation induced by *B. oryzae* infection.

On the other hand, Arase et al. (2001) reported that tryptamine inhibited not only spore germination and appressorium formation at high concentrations (>0.6 mg/ml), but also the infection-hypha formation of *M. grisea* on the onion epidermis at low concentrations of 0.15-0.3 mg/ml. In this study, we showed that infection behavior of *B. oryzae* such as spore germination and infection-hypha formation was dose-dependently inhibited by tryptamine on the onion epidermal cells. Furthermore, mycelial growth of *B. oryzae* was inhibited in the presence of the indole-related compounds, as demonstrated by the formation of growth inhibition zone on the TLC plates. In a previous paper (Ueno et al. *in press*), based on their effect on the infection behavior of *M. grisea*, some indole-related compounds were classified into three groups. Also, when barley leaves pretreated with IAA, tryptamine and tryptophan, were inoculated with *M. grisea* spores 24 h after chemical pretreatments, both blast lesion and infection-hypha formations were significantly inhibited (Ueno et al. 2004). These results suggested that some indole-related compounds can protect the rice plants from *B. oryzae*.

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