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Effect of tryptamine on the infection behavior of *Corynespora cassiicola*

Makoto UENO*, Ami HAIDA, Kengo MORIO, Junichi KIHARA, Sakae ARASE

Abstract Antifungal activity of tryptamine was tested using a causal agent, *Corynespora cassiicola,* of Corynespora leaf spot of cucumber. Tryptamine inhibited spore germination of *C. cassiicola* at dosage of over 1250 μg/mL. When cucumber plants (*Cucumis sativus* L. cv. Hokushin) at the 2-leaf stage were inoculated with *C. cassiicola* in the presence of tryptamine (2500 μg/mL), Corynespora leaf spot formation was significantly inhibited. Phytotoxicity was not observed even by tryptamine treatment at 2500 μg/mL. Furthermore, tryptamine inhibited Corynespora leaf spot lesion formation in other cucumber cultivars. These results suggest that use of exogenous or endogenous tryptamine in cucumber contributes to protection of many diseases of cucumber.

Keywords: Tryptamine, Fungicidal activity, *Corynespora cassiicola,* Cucumber

Introduction

The plant pathogenic fungus, *Corynespora cassiicola* (Burk. & Curt.) Wei is well known as a causal agent of serious leaf spot disease on more than 280 plant species in over 70 countries (Silva et al., 1995). Corynespora leaf spot caused by *C. cassiicola* is one of the most destructive fungal diseases affecting greenhouse cucumber production worldwide.

Although chemical fungicides such as benzimidazoles, dicarboximides, *N*-phenylcarbamates, and QoI fungicides play an important role in Corynespora leaf spot control in Japan, their effectiveness decreased by occurrence of chemical-resistant strains (Hasama, 1991; Hasama & Sato, 1996; Takeuchi et al., 2006; Ishii et al., 2007). Therefore, we have to establish new methods for control of Corynespora leaf spot disease novel antifungal compounds.

Auxin, one of plant hormones, plays an important roles during plant growth and development (Erdmann & Schiewer, 1971). In particular, the principal auxin, indole-3-acetic acid (IAA), has numerous effects on plant development. IAA is required for cell elongation and differentiation, and absorption of IAA into the cell membrane affects the permeability of the membrane. IAA is synthesized from tryptophan via 4 pathways: the indole acetaldoxime pathway (Ludwig et al., 1990; Schmidt et al., 1996), the indole acetamide pathway (Kawaguchi et al., 1993), the tryptamine pathway (Songstad et al., 1990; De Luca et al., 1988), and the indole pyruvic acid pathway (Cooney et al., 1991). The production and role of auxin in plant disease have been studied in relation to fungal and bacterial diseases of plants.

Previously, we demonstrated that a rice mutant (cv. Sekiguchi-asahi) exhibited strong resistance to *Magnaporthe grisea* infection under the light inducing the formation of Sekiguchi lesions (Arase et al., 2000). As a key factor in light-enhanced resistance in this mutant, the indole alkaloid compound tryptamine, was isolated from Sekiguchi lesions (Arase et al., 2001). Tryptamine inhibited not only spore germination and appressorium formation of *M. grisea* at high concentrations (>600 μg/mL) but also infectious hypha formation at low concentrations (150-300 μg/mL) (Arase et al., 2001). These results suggest that tryptamine plays an important role in the suppression of plant diseases. However, usefulness of tryptamine for control of Corynespora leaf spot is not yet elucidated. In a present paper, we report that exogenous tryptamine can inhibit the infection behavior of *C. cassiicola* on cucumber.

Laboratory of Plant Pathology, Faculty of Life and Environmental Science, Shimane University, Matsue 690-8504, Japan
*Corresponding author. E-mail address: makoto-u@life.shimane-u.ac.jp

Materials and Methods

Plant and fungus

Cucumber plants (*Cucumis sativus* cv. Hokushin) were used in this study. Cucumber seeds were germinated in tap water at 26-28° C for 1-2 days and then sowed in plastic pots (diameter, 7.5 cm) containing commercial garden soil (N : P : K=0.4 : 1.9 : 0.6 g/kg; Kureha Chemicals, Tokyo, Japan). In this study, cucumber plants were grown in a glasshouse and used at the 2-leaf stage.

C. cassiicola was grown on rice bran agar medium at 26℃ for 7 days and then at 26℃ for about 2 days under near-ultraviolet radiation provided by fluorescent lamps (FL20s B-B; Panasonic, Osaka, Japan) to induce abundant sporulation.

Antifungal activity of tryptamine on spore germination of *C. cassiicola*

A spore suspension (5×10^4spores/mL) of *C. cassiicola* with tryptamine at different concentrations of 0, 18.75, 37.5, 78, 156, 312, 625, 1250, or 2500 μg/mL was placed onto glass slides and incubated in a moist chamber at 26℃. After 24 h, the percentage of spore germination was determined using a light microscopy.

Effect of tryptamine on lesion formation by *C. cassiicola*

A spore suspension (5×10^4spores/mL) of *C. cassiicola* was inoculated to cucumber seedlings at the 2-leaf stage in the presence or absence of tryptamine of 2.5 mg/m. Inoculated plants were incubated in a moist chamber at 26-28℃ for 24h and then kept under natural light. Corynespora leaf spot development was determined 4 days after inoculation.

Statistical analysis

Data are indicated as mean ± SD. The results were compared statistically using the Tukey test ($P<0.05$).

Results and Discussion

To check the effect of tryptamine on spore germination of *C. cassiicola,* a spore suspension (5×10^4 spores/mL) was placed onto glass slides in the presence of tryptamine at different concentrations and kept in a moist chamber at 26℃. After 24 h, the spore germination was determined by a light microscopy.

The percentages of spore germination in tryptamine solution at different concentrations of 0, 18.75, 37.5, 78, 156, 312, 625, 1250, and 2500 μg/mL were 99.2%, 97.6%±3.0%, 97.4%±2.0%, 97.3%±3.0%, 93.9%±11.2%, 97.1%±3.0%, 96.9%±1.8%, 32.2%±8.4%, and 0%, respectively (Fig. 1). Spore germination was significantly inhibited at dosages of over 1250 μg/mL.

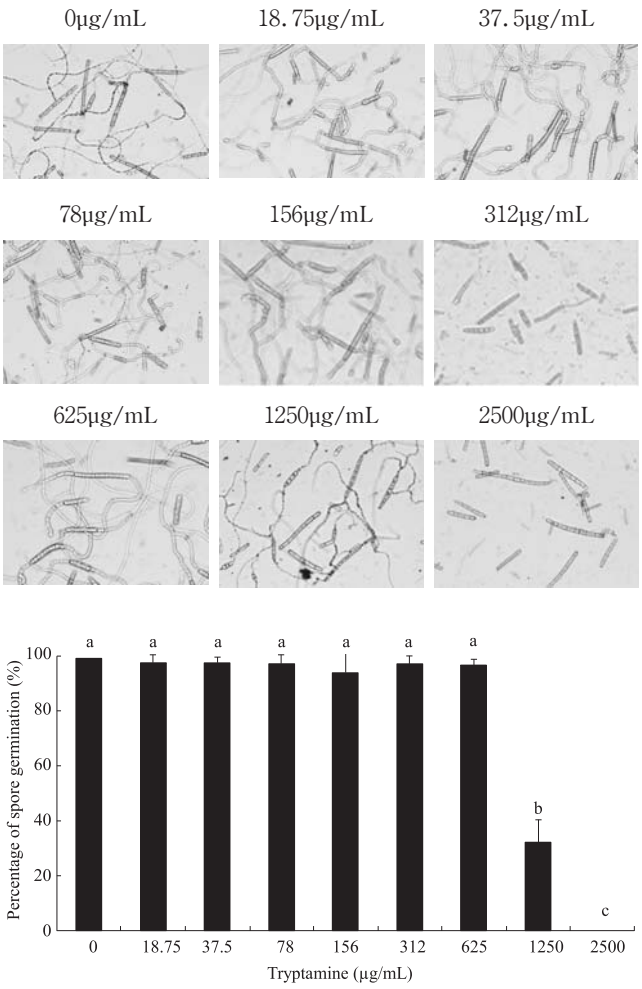

Fig.1 Effect of tryptamine on spore germination of *Corynespora cassiicola.* Spores of *C. cassiicola* were suspended in tryptamine solutions at different concentrations of 18.8, 37.5, 78.0, 156.0, 312, 625, 1250 and 2500 μg/mL and placed onto glass slides. After incubation for 24 h at 26℃ in a moist chamber, spore germination was examined under a light microscope. Experiments were repeated 3 times. Bars represent mean ± SD. Mean values followed by the same letters are not significantly different (Tukey test, $P<0.05$).

To determine the fungicidal activity of tryptamine, cucumber leaves were inoculated with *C. cassiicola* spores in the presence of tryptamine (2500μg/mL). Corynespora leaf spot formation by *C. cassiicola* was significantly inhib-

ited in the presence of tryptamine 4 days after inoculation, as compared with lesion formation in the absence of tryptamine (Fig. 2A). The number of lesions in cucumber inoculated with *C. cassiicola* in the presence and absence of tryptamine was 2.0±3.2 and 105.7±31.7, respectively (Fig. 2B). Phytotoxicity was not observed in cucumber leaves even by tryptamine treatment at 2500 μg/mL (data not shown). Furthermore, inhibition of Corynespora leaf spot formation by tryptamine was observed in other cucumber cultivars (data not shown).

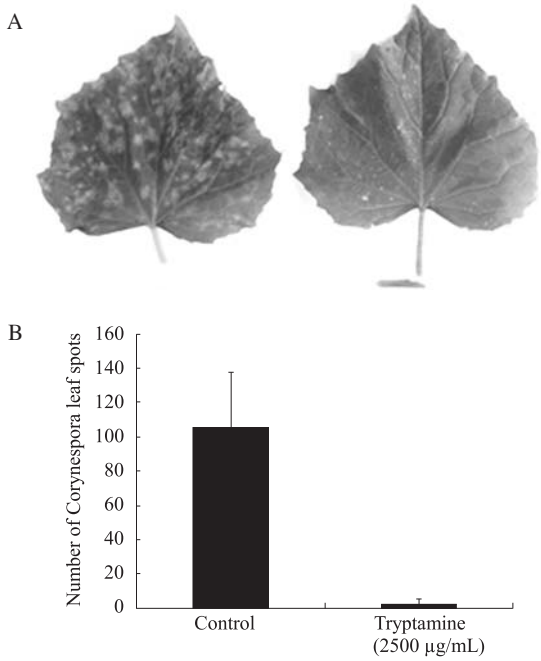

Fig.2 Effect of tryptamine on Corynespora leaf spot formation on cucumber leaves by *Corynespora cassiicola.* Cucumber leaves were inoculated with *C. cassiicola* spores in the presence of tryptamine (2500 μg/mL) and kept in a moist chamber for 24 h at 26-28℃. Development of Corynespora leaf spot (A) and the number of Corynespora leaf spots (B) were investigated 4 days after inoculation. Experiments were repeated 3 times. Bars represent mean ± SD.

Previously, we showed that tryptamine contributes to blast control in rice and barley (Ueno et al., 2004, Ueno et al., 2011). In this study, we found that tryptamine exhibited antifungal activity against *C. cassiicola.* Tryptamine is biosynthesized from tryptophan by tryptophan decarboxylase (TDC). Furthermore, TDC has been isolated from cucumber (Sherwin, 1970). We suggest that transgenic cucumbers expressing tryptophan decarboxylase might also show enhanced resistance. In addition, these result suggested that use of exogenous or endogenous tryptamine in cucumber leaves contributes to protection of many diseases of cucumber.

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