Screening of Chemical Compounds for Selectively Inhibiting Haustorial Formation of Powdery Mildew Pathogen in Barley Coleoptile Epidermis

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In our attempts to establish an efficient system for fungal transformation, we have elaborated a microinjection technique for specifically introducing the genes into targeted infection structures^{4,6,9,11)} and demonstrated that the reporter gene (β -glucuronidase gene) flanked to the cauliflower mosaic virus 35S promoter was functionally transcribed in fungal cells^{5,8)}. This microinjection technique was also applicable to the transformation of obligate fungal pathogens such as powdery mildew fungi^{5,6)}. The reporter gene was microinjected most effectively into appressoria of the powdery mildew pathogen inoculated into barley coleoptile epidermis and the expression of the gene was detected in primary haustoria produced by the gene-injected appressoria⁵⁾. These results suggest that the successfully transformed pathogens could be rapidly and effectively detected by checking the survival of the haustoria if some selective markers are integrated in our transformation system. From this point of view, we have focused on the screening of chemical compounds capable of selectively inhibiting the growth of powdery mildew fungi without causing detrimental effect on barley plants. In a series of works for screening chemical agents for soil-borne pathogens of tomato, we found that some indole derivatives (indole-3-propionic acid and indole-3-acrylic acid) were capable of inhibiting the growth of *Pseudomonas solanacearum*^{3,10)}, and these compounds were also inhibitory to some fungal pathogens including *Erysiphe cichoracearum*, the causal pathogen of powdery mildew of tomato. Recently, Miyagawa *et al.*⁷⁾ reported that tryptamine was produced in UV-injured or powdery-mildewed barley leaves, and that the compound inhibited the conidial germination of *E. graminis* f. sp. *hordei*. These results suggest a possible application of some indole derivatives to selective chemical agents for the powdery mildew pathogen. In the present study, therefore, we examined some related compounds of indole-3-

propionic acid and tryptamine for their antifungal activities and selected the compounds which specifically inhibit the haustorial formation of *E. graminis* f. sp. *hordei*.

Coleoptile epidermis was excised from 10-day-old seedlings of barley (*Hordeum vulgare*, L., cv. Kobin-katagi, susceptible to race I) and inner epidermis was used in the following experiment. First, the toxicity of test compounds to host cells was examined by floating the epidermis onto a test solution (0.5% methanol solution containing 10, 50, and 100 μ g/ml of test compounds) at 20°C for 48 hr according to the method of Koga *et al.*²⁾ The survival of coleoptile epidermal cells was determined by microscopically observing their cytoplasmic streaming and the cells maintaining cytoplasmic streaming were regarded as survived cells. The result is given in Table 1. Indole-3-acetamide, tryptamine, tryptophol, and tryptophan did not exhibit significant effect on cell activity (more than 90% in the survival rate) even when the epidermis was treated at the highest concentration (100 μ g/ml). Other six compounds (indole, indole-3-carboxylic acid, indole-3-glyoxylic acid, indole-3-lactic acid, indole-3-propionic acid, and indole-3-butyric acid) also gave high survival rates of host epidermal cells when these compounds were added to the test solution at lower concentrations (10 or 50 μ g/ml). In the present study, therefore, we selected these non-toxic or low-toxic compounds for examining their specific antifungal activity at the concentrations which did not cause the cell death of host coleoptile tissues. Namely, excised inner epidermis of coleoptile was inoculated with fresh conidiospores of *E. graminis* f. sp. *hordei* (race I) by the method described previously⁹⁾ and floated onto the test solution mentioned above. After 48 hr of incubation, the epidermis was vitally observed under an Olympus differential phase contrast microscope to determine the fungal development or host cell survival. Figure 1 shows

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Table 1. Phytotoxic effect of indole and indole derivatives on coleoptile epidermal cells of barley

Test compounds ^{b)}	Survival rates (%) ^{a)}		
	10	50	100 ^{c)}
indole	96.3 (2.5)	95.8 (0.6)	58.0 (6.6) ^{d)}
indole-2-carboxylic acid	5.3 (3.3)	0	0
indole-2-carboxylic acid ethyl ester	74.0 (8.2)	35.0 (1.4)	30.0 (0.3)
indole-3-carboxylic acid	93.1 (0.9)	41.5 (0.2)	0
indole-3-carbinol	0	0	0
indole-3-acetic acid	67.7 (1.2)	13.8 (3.3)	0
indole-3-acetic acid ethyl ester	40.8 (3.6)	25.0 (1.3)	25.0 (6.9)
indole-3-glyoxylic acid	89.4 (3.5)	8.0 (1.1)	0
indole-3-acetamide	100.0 (0.1)	95.8 (0.6)	92.3 (0.8)
DL-indole-3-lactic acid	82.7 (9.1)	12.3 (2.9)	0
3- β -indoleacrylic acid	17.5 (0.3)	0	0
indole-3-propionic acid	89.0 (2.8)	9.0 (4.6)	0
indole-3-butyric acid	98.7 (1.55)	2.0 (1.9)	0
tryptamine	98.7 (0.7)	96.7 (0.1)	95.0 (0.1)
tryptophol	98.3 (0.2)	97.2 (0.9)	97.3 (0.8)
tryptophan	98.3 (1.1)	95.0 (3.2)	95.0 (2.1)

a) Inner epidermis of coleoptile was floated onto a 0.5% methanol solution containing test compounds for 48 hr and cytoplasmic streaming of epidermal cells was microscopically checked. The rates were expressed as percentage relative to the values of coleoptile tissues floated onto a methanol solution (control). The survival rates of the epidermis floated onto water and a methanol solution were 96.9 ± 0.4 and 92.3 ± 0.8 , respectively. The data were given as average of four separate replications.

b) Test compounds were purchased from Sigma, St. Louis, MO., USA.

c) Concentrations ($\mu\text{g/ml}$).

d) Standard error.

Fig. 1. Inhibition of appressorial (A) and haustorial formation (B) of *E. graminis* f. sp. *hordei*, race I, by the treatment of indole derivatives. Inner epidermis of barley coleoptile was inoculated with race I and floated onto a solution containing 10 (□), 50 (▨), and 100 $\mu\text{g/ml}$ (■) of test compounds for 48 hr, and the fungal development was microscopically observed after checking cytoplasmic streaming in epidermal cells infected by the pathogen (infection sites). Three epidermal tissues were used in each experiment and approximately 100 infection sites per tissue were selected at random. The inhibition rates were calculated from the difference between the rates of appressorial or haustorial formation of the pathogen inoculated onto coleoptile epidermis floated onto a methanol (control) and test solution. The data were given as average of four separate experiments. Abbreviation: IA, indole-3-acetamide; TA, tryptamine; TT, tryptophol; TP, tryptophan; IN, indole; IC, indole-3-carboxylic acid; IG, indole-3-glyoxylic acid; IL, indole-3-lactic acid; IP, indole-3-propionic acid; IB, indole-3-butyric acid.

the inhibitory effect of these compounds against the development of the powdery mildew fungi inoculated onto coleoptile epidermis. The formation of appressoria was inhibited by all of the compounds treated, but their inhibition rates were not so conspicuous (less than 50%). However, the haustorial formation was completely inhibited by the treatments with indole-3-acetamide,

tryptamine, tryptophol, indole, and indole-3-propionic acid. Especially, in the coleoptile epidermis treated with tryptamine or indole-3-propionic acid, the haustorial formation was effectively inhibited even when tested at the lowest concentration (10 $\mu\text{g/ml}$). Although the mechanisms of inhibition by these compounds have not been elucidated in the present study, some workers have

reported the production of fluorescent antifungal compounds in barley leaves inoculated with *E. graminis* f. sp. *hordei*¹⁾ and Miyagawa *et al.*⁷⁾ successfully identified one of these compounds as tryptamine. These results strongly suggest that the powdery mildew fungi transformed with a certain gene for detoxifying tryptamine or indole-3-propionic acid could be propagated on barley coleoptile epidermis floated onto the selective solution. Our preliminary investigation indicated that *Pseudomonas solanacearum* was resistant to tryptamine because it was capable of metabolizing the compound and that this resistance could be destroyed by the insertion of a transposon to chromosome of this bacterium (unpublished data). The cloning of the tryptamine detoxifying gene from *P. solanacearum* chromosomal DNA is underway.

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和 文 摘 要

道後充恵・豊田秀吉・松田克礼・松田一彦・国領ゆきえ・大内成志：オオムギ子葉鞘接種うどんこ病菌の吸器形成を特異的に抑制する化学物質の検索

宿主のオオムギ細胞を死滅させず、うどんこ病菌の吸器形成のみを特異的に抑制する物質の検索を試みた。すなわち、切離したオオムギ子葉鞘内表皮にうどんこ病菌を接種し、種々の濃度のインドール化合物の水溶液に浮遊させ、うどんこ病菌の吸器形成に及ぼす影響を調べたところ、トリプタミンが顕著な選択的効果を示した。すなわち、100 µg/ml のトリプタミンを処理した場合でも95%以上の宿主細胞が生存したのに対し、うどんこ病菌の吸器形成は10 µg/ml の処理で完全に阻害された。以上の結果から、トリプタミン無毒化遺伝子のクローニングとこの選抜化合物の併用によって、オオムギうどんこ病菌の形質転換体選抜系が確立されるものと考えた。

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