Note

Possible Role of Phytocassane, Rice Phytoalexin, in Disease Resistance of Rice against the Blast Fungus *Magnaporthe grisea*

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In addition to momilactone, phytocassanes A through E (diterpene phytoalexins) were detected in rice leaves in fields suffering from rice blast. Furthermore, phytocassane accumulation was most abundant at the edges of necrotic lesions, indicating that the phytoalexins prevent subsequent spread of the fungus from the infected site. In pot experiments the pattern of phytocassane accumulation in rice leaves in an incompatible interaction (infection with an avirulent race of *Magnaporthe grisea*) was more rapidly induced than in a compatible interaction (infection with a virulent race of *M. grisea*).

Key words: phytocassane; phytoalexin; rice; disease resistance; *Magnaporthe grisea*

Pathogen-induced defense mechanisms in higher plants may involve *de novo* synthesis of antifungal compounds, known as phytoalexins, which play an important role in the disease resistance of various plant species.1 More than 300 structures of phytoalexins were isolated from approximately 900 plant species,2 including the rice phytoalexins momilactones A and B,3 oryzalexins A through F,4–7 and S,8 phytocassanes A through E,9,10 (diterpenes), and sakuranetine11 (a flavanone). We previously reported that phytocassanes are induced in rice plants or cell-suspension cultures of rice infected with pathogenic fungi or pathogen-derived elicitor and have greater antifungal activity against the rice blast fungus *M. grisea*, than that of other rice phytoalexins.10,12 The objective of this study was to examine the role of phytocassanes in the resistance of rice against *M. grisea*. We found that phytocassanes play an important role in disease responses of rice plants.

Quantitative analysis of phytocassanes and momilactones in rice (*Oryza sativa* L. cv) leaves from a paddy field infested with *M. grisea* was attempted. Leaves of two rice cultivars, Akitakomachi and Domannaka (both cultivars have slightly weak resistance to blast fungus disease in fields), were collected in a paddy field in which the pathogen naturally occurred, in Akita and Yamagata Prefectures, respectively, in July 1995. Each rice leaf was divided into four classes according to the following criteria: healthy leaves, no visible lesion or brown specks; hypersensitive response (HR)-like leaves, visible brown specks; slightly withered leaves, less than ten necrotic lesions; dead leaves. Dead leaves of Akitakomachi were not found in the paddy fields. Twenty grams of samples (about 25 leaves) were cut into pieces and then shaken with 200 ml of ethyl acetate and 200 ml of 0.1N Na2CO3 (pH 10.5) for 18 h. The ethyl acetate fraction was collected and then mixed with 20 ml of 0.02N HCl and centrifuged at 15,000×g for 30 min. To measure the amounts of momilactones and phytocassanes induced, the supernatant was put through high pressure liquid chromatography (HPLC) using a TSKgel ODS-120T column (4.6 mm i.d.×300 mm, Tosoh Corporation, Tokyo, Japan) and eluted with 45% acetonitrile. Phytocassanes were monitored at 280 nm and momilactones were monitored at 215 nm as described before.13 A large amount of phytocassanes and momilactones accumulated in HR-like and slightly withered rice leaves in tested rice leaves (Table 1). Compared with phytocassanes and momilactones, sufficient amount of oryzalexins as other rice phytoalexins, could not be detected by our quantitative analysis. The difference in total amounts of induced phytocassanes and momilactones between HR-like and slightly withered leaves maybe a change due to the size or the number of lesions per leaf. Furthermore, it is plausible that the content of induced phytocassanes and momilactones varied, because of differences of environmental factors such as temperature, hours of sunlight, fertility of soil, and so on. In contrast, both phytocassanes and momilactones...
Table 1. Amounts of Phytocassanes and Momilactones in Rice Plants from the Paddy Field

<table>
<thead>
<tr>
<th>Rice cultivar</th>
<th>Degree of disease severity</th>
<th>Amount of phytoalexin in rice leaves (µg/g F.W.)</th>
<th>Phytocassane</th>
<th>Momilactone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Akitakomachi</td>
<td>HR-like</td>
<td></td>
<td>3.9</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>Slightly withered</td>
<td></td>
<td>27.3</td>
<td>7.2</td>
</tr>
<tr>
<td>Domannaka</td>
<td>Healthy</td>
<td></td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>HR-like</td>
<td></td>
<td>3.9</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td>Slightly withered</td>
<td></td>
<td>18.0</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>Dead</td>
<td></td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values shown are the mean of results from triplicate assays.

Table 2. Amounts of Phytocassanes in Sections of M. grisea-infected Rice Leaves

<table>
<thead>
<tr>
<th>Section</th>
<th>Amount of the induced phytocassane (µg/g F.W.)</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central zone of necrotic lesion</td>
<td>39.4±5.2</td>
<td>16±3.1</td>
<td>5.3±1.0</td>
<td>9.2±1.6</td>
<td>7.1±0.8</td>
<td></td>
</tr>
<tr>
<td>Edge of necrotic lesion</td>
<td>57.3±9.8</td>
<td>19.6±4.1</td>
<td>6.8±1.8</td>
<td>17.6±3.1</td>
<td>8.2±0.9</td>
<td></td>
</tr>
<tr>
<td>Outside of necrotic lesion</td>
<td>2.4±0.3</td>
<td>0.8±0.1</td>
<td>0.3±0.1</td>
<td>1.1±0.2</td>
<td>0.2±0.1</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean of results from triplicate assays±SD.

were hardly detected in healthy or dead leaves. These results may indicate something more than that phytocassanes accumulated in infected rice leaves in a paddy field, but these results also mean that these compounds may play a role in disease resistance in rice in much the same way as momilactones.

We then examined the site of phytocassane induction in M. grisea-infected rice. The sections from typical necrotic lesions and non-lesion tissue of slightly withered leaves of Akitakomachi (refer to Table 1) were divided into three classes: the central zone and edge of the lesion and beyond the lesion, as demonstrated in Fig. 1. The phytocassane content in each specimen (0.2 g) was measured by HPLC analysis as described. As shown in Table 2, the levels of phytocassanes were higher at the edge than in the central zone of necrotic lesions. In contrast, phytocassanes beyond the lesion were present only in small quantities. One interpretation of this result is that they might prevent the spread of subsequent fungal proliferation in the infection site. This phenomenon seems to be correlated with the HR as a plant defense mechanism. HR is characterized by localized cell death of infected cells, accompanied by the formation of a necrotic lesion to enclose the pathogen at the infection site. HR has also been shown to be associated with coordinated induction of salicylic acid (SA) and jasmonic acid (JA), and synthesis of pathogenesis-related (PR) proteins as well as accumulation of phytoalexins.14) Recently, various endogenous molecules, including SA and JA, have been proposed to be involved in the complex network of signaling pathways that lead to disease resistance.15,16) We recently reported that JA induces the accumulation of phytocassanes in cell-suspension cultures of rice,17) but the relationship between defense signal molecules and phytocassanes in HR mechanism remains unclear. Nevertheless, this result suggests that phytocassanes function as an actual defense response in the HR.

Next we analyzed the pattern of phytocassane accumulation in rice leaves between an incompatible and a compatible interaction. Because some defense reactions have been reported to be induced more rapidly after inoculation in an incompatible than in a compatible interaction,18,19) the course of phytocas-
Phytocassane in Disease Resistance of Rice

Fig. 2. The Course of Phytocassane Accumulation in Rice Leaves in Incompatible and Compatible Interactions.

Phytocassane accumulation in rice plants was examined in both types of interaction after inoculation. When the fifth leaf of rice plants (Oryza sativa, L cv. Akitakomachi) cultivated in a phytotron was fully expanded, the leaves were sprayed with a spore suspension of either an avirulent race 031 or a virulent race 007 of M. grisea, inoculated rice leaves at given d were assayed for phytocassane with HPLC. Values shown are the mean of four measurements.

Several aspects of rice disease resistance cannot be explained by phytoalexins alone, but phytoalexins may contribute to disease resistance to varying degrees. Dillons et al. already reported that momilactone, sakuranetin, and oryzalexin as rice phytoalexins are important factors using rice genotypes of different susceptibility to the blast fungus. Here we suggest that phytocassanes are involved in disease resistance of rice plants to the blast fungus M. grisea.

Acknowledgments

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