Note

Accumulation of Tryptamine in Barley Leaves Irradiated with UV Light

Hisashi MIYAGAWA, Hiroshi TODA, Tetsu TSURUSHIMA,* Tamio UENO,** and Jiko SHISHIYAMA***

Pesticide Research Institute, Faculty of Agriculture, Kyoto University, Kyoto 606-01, Japan
*Hannan University, Amami-higashi, Matsubara, Osaka 580, Japan
**Department of Agricultural Chemistry, Kyoto University, Kyoto 606-01, Japan
***Faculty of Agriculture, Kinki University, Nara 631, Japan

Received March 14, 1994

Tryptamine was identified as a stress compound in UV-irradiated barley leaves. Its induction was also shown to occur upon the inoculation of plant-pathogenic fungi. In the interaction between barley and powdery mildew fungus, the induction was related to the degree of resistance and spore germination was inhibited by tryptamine in vivo, suggesting that it acts as a defense substance.

Plants have various kinds of defense reactions against attacks by pathogens. In barley (Hordeum vulgare L.), genetically-determined resistance has been observed against powdery mildew fungus, Erysiphe graminis f. sp. hordei1–3) but its chemical or biochemical basis is still not well understood. Pathological defense responses in plants are often reproduced by abiotic stresses, such as UV irradiation and mechanical injuries. Therefore the examination of these stress responses is expected to give some clue for elucidating the metabolic changes upon the infection by pathogens. Here we report the identification of a stress substance in barley leaves produced after UV irradiation, together with the quantitative analyses of its production in terms of the biological interaction between barley leaves and pathogenic fungi.

Two-week-old barley seedlings (cv. Minorimugi) were UV irradiated for 10 min using a Toshiba germicidal tube GL-15 at an approximate distance of 20 cm. Irradiated seedlings were grown for 24 h at 25°C and the leaves were detached to be extracted with MeOH. Changes in the constituent profile were surveyed by gradient HPLC (column, Cosmosil 5C18-AR, 4.5 × 150 mm; solvent, starting with 20% MeOH in water containing 0.1% H3PO4 for 5 min, then linearly increasing the concentration of MeOH to 80% in 25 min; flow rate, 0.8 ml/min; detection, fluorescence emission at 410 nm with the excitation at 290 nm) and a marked increase of a fluorescent substance (tR 5.7 min) was observed in the extract from the UV-irradiated leaves compared to that from the control.

To characterize this fluorescent substance, irradiated leaves (420 g) were extracted with 80% MeOH and the extract was concentrated in vacuo to be partitioned between EtOAc and water. The aqueous phase was then purified by two steps of preparative HPLC (column, Cosmosil 5C18-AR, 20 × 250 mm; solvents, 20% MeOH in water containing 0.1% TFA (1st) and 15% CH3CN in water containing 0.1% TFA (2nd)) to afford the target compound (10 mg). By comparing its diagnostic spectral data with those of the authentic standard, the compound was identified as tryptamine (1).

Table shows the content of tryptamine in UV-irradiated barley leaves measured by HPLC (column, Wakosil II 5C18 HG, 4.6 × 150 mm; solvent, 20% MeOH in water containing 0.1% H3PO4, 0.8 ml/min; detection, fluorescence emission at 355 nm with the excitation at 290 nm) following MeOH extraction. The amount of tryptamine had increased by about 70 times 24 h after the irradiation. The accumulation also occurred in response to biotic stress 3 days after the inoculation of leaf-blight fungus, Bipolaris sorokiniana. The content in the inoculated leaves was about 10 times higher than that in the control leaves (Table).

Then we examined whether tryptamine production is also related to the disease resistance of barley against powdery mildew fungus. Leaves from the cultivars Goseshikoku (susceptible) and Turkey 290 (resistant) were inoculated with Erysiphe graminis f. sp. hordei race I and analyzed after 8 days. Figure shows the concentration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration after treatment (days)</th>
<th>Content (μg/g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>UV Irradiation</td>
<td></td>
<td>140.0</td>
</tr>
<tr>
<td>Pathogen Inoculation (Bipolaris sorokiniana)</td>
<td>3</td>
<td>19.7</td>
</tr>
</tbody>
</table>

Abbreviations: TFA, trifluoroacetic acid; BV, blue-violet light.
of tryptamine measured by HPLC under the conditions described above. In the leaves of both cultivars, the tryptamine content was increased by the inoculation. Moreover, the inoculated leaves of the resistant cultivar, which had many small necrotic spots caused by hypersensitive cell-death to reject the pathogen, contained about 4 times more tryptamine than those of the susceptible cultivar, which had vigorous sporulation. Although this difference may appear rather small, the actual tryptamine production in the resistant leaves at the infection site can be much higher than that expressed as the concentration in the whole leaf. Because the area of the leaf part interacting with the pathogen is more confined and smaller in the resistant cultivar than in the susceptible one. These findings suggest that tryptamine production is associated with the resistance of barley against powdery mildew. In this context, tryptamine was found to inhibit spore germination of E. graminis f. sp. hordei with an ED50 of about 25 ppm, indicating that it can act as a defense compound, i.e., phytoalexin in the host-parasite interaction. Further work is now in progress to discover the role and significance of tryptamine in the disease resistance of barley.

![Image](image-url)

In barley, the indole compound, gramine (2), is a well-known constituent of young shoots. It has biological activities such as phytotoxicity and antifeedant, suggesting its effectiveness for the rejection of pathogens. Indeed, a large amount of gramine was detected by HPLC in the leaves used in this study as well, but its content was not significantly changed by any of the stresses tested here. In addition, both susceptible and resistant cultivars to powdery mildew contained practically the same level of gramine in their healthy leaves, or the level was even somewhat higher in the susceptible cultivar (data not shown), which may imply that gramine plays no significant role, at least in the resistance to powdery mildew.

In barley leaves infected with powdery mildew, resistant cellular responses have been significantly related to the deposition of autofluorescent material in the cells around the infection site. The nature of this fluorescent material is unknown, except that it is distinct from lignin in the tissue on the basis of histochemical analysis. Although the fluorescent nature of tryptamine suggests its involvement in such a cellular response, its emission spectrum (max at 360 and 690 nm under 290 nm excitation) was different from that deposited in the barley leaves (max at 540 nm under BV excitation) so such observations are not directly attributable to tryptamine formation. However, it is interesting to note that one of the tryptamine derivatives, 5-hydroxytryptamine, has a similar emission spectrum with the maximum of 540 nm. Thus some tryptamine derivatives may be incorporated into the incompatibly reacting cells of barley, little being extracted.

In conclusion, this study demonstrated that tryptamine is a stress substance in barley seedlings, though its general role in disease resistance still remains to be examined. Tryptamine is considered to be produced from anthranilate via tryptophan in plants and some anthranilate-derived substances are also known to be associated with the defense to disease and pests in Gramineae; e.g., averalumins in oats and 4-hydroxy-1,4-benzoxazin-3-ones in a wide range of cereal plants. The regulation of anthranilate metabolism may be important in the stress responses in gramineous plants.

References and Notes

4. Physicochemical data for tryptamine. UV max (MeOH) nm: 278, 218; EIMS (70eV) m/z: 160 (M+), 131, 130, 103, 69, 45. 1H-NMR (90 MHz, MeOH-d6): δ: 7.52–7.68 (m, 1H), 7.35–7.48 (m, 1H), 7.03–7.24 (m, 3H), 3.10–3.30 (m, 4H); 13C-NMR (22.5 MHz, MeOH-d6): δ: 138.4 (s), 128.1 (s), 124.2 (d), 122.8 (d), 120.1 (d), 118.8 (d), 112.5 (d), 110.2 (d), 41.2 (t), 24.5 (t). Signal multiplicities were measured by INEPT experiments.