Induction of *N*-Hydroxycinnamoyltyramine Synthesis and Tyramine *N*-Hydroxycinnamoyltransferase (THT) Activity by Wounding in Maize Leaves

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Both *N*-p-coumaroyl- and *N*-feruloyltiyramine accumulated in response to wounding in leaf segments of maize. The amount of *N*-hydroxycinnamoyltiyramines started to increase 3–6 h after wounding and peaked at 12 h. Thereafter, the amount of *N*-p-coumaroyltiyramine decreased rapidly, while the *N*-feruloyltiyramine content remained at a high level. The accumulation of *N*-hydroxycinnamoyltyramines was accompanied by an increase in the tyramine *N*-hydroxycinnamoyltransferase (THT) activity. This increase was initially detected 3 h after wounding and reached a maximum at 36 h, the level of activity being 40 and 11 times that in the leaves before wounding and in the control leaves, respectively. Partial purification of THT from wounded leaves by (NH₄)₂SO₄ precipitation and subsequent two steps of anion-exchange chromatography resulted in a 12.5-fold increase in specific activity. Kinetic studies with this partially purified enzyme revealed that the best substrates were tyramine and feruloyl-CoA, although tryptamine and sinapoyl-CoA also efficiently served as substrates. The apparent native molecular weight of the enzyme was determined by gel filtration as 40 kDa.

Key words: hydroxycinnamoyltiyramine; tyramine *N*-hydroxycinnamoyltransferase; wound; *Zea mays*

Plants respond to environmental stress through a variety of biochemical reactions. Among these, the activation of secondary metabolism including the phenylpropanoid pathway is a general reaction to stimuli. Hydroxycinnamic acids derived from this pathway usually occur as conjugates with sugars, cell wall carbohydrates, and organic acids. The hydroxycinnamic acid amides with tyramine and octopamine are also common types of conjugates. The formation of the amides serves a defensive purpose in solanaceous plants such as potato, tobacco and tomato. Various forms of stress, including physical injury, infection with pathogens and elicitor-treatments, induce the accumulation of hydroxycinnamic acid amides. It has been suggested that these amide compounds are integrated into the cell wall and eventually form a phenolic barrier against pathogens and herbivores. Tyramine *N*-hydroxycinnamoyltransferase (THT; EC 2.3.1.110), which is responsible for the formation of these amide compounds, has been purified to homogeneity from potato and tobacco, and cDNAs encoding both enzymes have been cloned. In addition to Solanaceae, the involvement of hydroxycinnamic acid amides in stress response has been found in Papaveraceae and Liliaceae, suggesting the generality of this reaction.

Another family of plants in which hydroxycinnamonic acid amides have been suggested to be involved in stress responses is Gramineae. The presence in maize of amides of hydroxycinnamic acid with tyramine and tryptamine has been demonstrated in the grain. However, the behavior of these compounds in stressed plants has not been investigated to date. In this paper, we describe the induction of hydroxycinnamic acid amides and the THT activity in response to wounding in maize leaves.

Materials and Methods

Plant materials. Maize (*Zea mays* L. cv. Snowdent 108, Yukijirushi Shubyo, Sapporo, Japan) seeds were immersed in water at 4°C for 12 h. The seeds were then sown in wet vermiculite, and maintained at 24–26°C for 8 d under continuous fluorescent lighting (60 W m⁻²). The 8-day-old primary leaves were excised with a razor blade. After removing the midrib, the lower epidermis of the leaf segments was peeled off as a wounding treatment. These leaf segments were floated on 10 ml of distilled water in a Petri dish with the peeled surface in contact with the water. To compare the induced compounds and THT

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Abbreviations: HHT, hydroxynitrilacate *N*-hydroxycinnamoyltransferase; THT, tyramine *N*-hydroxycinnamoyltransferase; TrTHT, tryptamine *N*-hydroxycinnamoyltransferase
activity among different hybrid lines, all seeds apart from Snowdent 108 were obtained from Japan Forage Seed Association.

**Chemicals.** N-Hydroxycinnamoyltyramines and related amides were synthesized by a condensation reaction between the respective hydroxycinnamic acid and amine by using dicyclohexylcarbodiimide as described by Villegas and Brodelius. Hydroxycinnamoyl-CoA thioesters were prepared by transesterification of the respective hydroxycinnamoyl N-hydroxysuccinimide ester. The concentration of each obtained CoA thioester was calculated and adjusted on the basis of the known molar extinction coefficient.

**Analyses of N-hydroxycinnamoyltyramines in wounded maize leaves.** Wounded maize leaves (250 mg) frozen in liquid nitrogen were ground to powder and extracted with 3 ml of methanol. The mixture was centrifuged at 12,000 g for 10 min. The resulting precipitate was extracted with 3 ml of methanol and centrifuged again. The combined supernatant was diluted with 1.5 ml of distilled water. After passing through a Sep-pak C18 (Waters) cartridge pre-equilibrated with methanol:water (4:1, v/v), the solution was evaporated to dryness in vacuo. The residue was dissolved in 2 ml of distilled water, and applied to a Sep-pak C18 cartridge equilibrated with water. The cartridge was washed with 3 ml of methanol:water (1:4) and then eluted with 5 ml of methanol:water (1:1). This 20–50% methanol fraction was evaporated to dryness, and the resulting residue was dissolved in 6.2 ml of chloroform: methanol (30:1, v/v). The solution was passed through a Sep-pak Silica (Waters) cartridge pre-equilibrated with chloroform:ethanol (30:1) and then the cartridge was washed with 5 ml of chloroform: methanol (20:1). N-Hydroxycinnamoyltyramines were eluted in the chloroform:ethanol (30:1) and chloroform:ethanol (20:1) fractions. After evaporating the combined fractions, the residue was dissolved in 0.5 ml of methanol. This solution was analyzed by HPLC as described next. When 3.3 nmol of N-feruloyltyramine were added to 0.2 g of maize tissue, and the mixture extracted and analyzed, the recovery was approximately 63%. The reported values for N-hydroxycinnamoyltyramines were adjusted to account for losses during extraction based on this recovery.

The identity of each compound was confirmed by a reverse-phase HPLC analysis [Wakosil II 3C18H8 column, 150 × 4.6 i.d. (Wako, Osaka, Japan); detection at 320 nm], and from UV and ion-spray MS spectra. The retention times of the induced compounds matched those of authentic N-p-coumaroyltyramine and N-feruloyltyramine, respectively, in two different solvent systems: (1) elution for 45 min with a 30% to 70% (v/v) methanol linear gradient in water containing 0.5% TFA at a flow rate of 0.8 ml/min; (2) isocratic elution with 35% (v/v) methanol in water containing 0.5% TFA at a flow rate of 0.8 ml/min. The retention times with system 1 and 2, respectively, of N-p-coumaroyltyramine were 15.2 and 11.8 min, and of N-feruloyltyramine were 17.1 and 13.9 min. In subsequent analyses, solvent system 2 was used. The UV spectra were obtained with a photodiode array detector (Shimadzu SPD-M 10Avp) combined with a Shimadzu 10A HPLC system. LC-MS analyses were carried out with a Perkin-Elmer ScieX API-165 instrument (ion-spray voltage, 5 kV; orifice voltage, 30 V; nebulizer gas, air; curtain gas, nitrogen; scan range, m/z 140–400) combined with the HPLC system. N-p-Coumaroyltyramine. UV λ_max (relative intensity): 310 (98), 292 (100); ion-spray MS m/z (relative intensity): 284 (100, [M+H]^+), 147 (34, [M-C_6H_12O_4]^+). N-Feruloyltyramine. UV λ_max (relative intensity): 318 (100), 293 (87), 284 (8); ion-spray MS m/z (relative intensity): 314 (100, [M+H]^+), 177 (39, [M-C_8H_12O_6]^+).

**Enzyme extraction and assay for THT activity.** Enzyme extraction was carried out at 0–4°C. Frozen maize leaf segments were ground in 4 volumes (v/w) of a 100 mM sodium phosphate buffer (pH 7.5) containing 14.4 mM mercaptoethanol (buffer A). After centrifugation (12,000 g for 10 min at 4°C), the supernatant was used as a crude enzyme solution. The reaction mixture consisted of 10 μl of the crude enzyme, 10 μl of 1 mM feruloyl-CoA, 10 μl of 10 mM tyrosine, and 70 μl of a 100 mM Tris-HCl buffer (pH 8.5). After a 10-min incubation at 30°C, the reaction was stopped by adding 20 μl of acetic acid. The mixture was filled up to 500 μl with methanol, and a 10-μl aliquot was analyzed by HPLC. Protein content was determined according to the method of Bradford.

**Partial purification of THT.** Wounded maize leaves (7.7 g) were frozen in liquid nitrogen and ground to powder with sea sand (40–80 mesh), before being homogenized in 30 ml of buffer A. After centrifugation (12,000 g for 10 min at 4°C), the supernatant was fractionated by (NH_4)_2SO_4 precipitation (30–45% saturation) and dialyzed against a 20 mM Tris-HCl buffer (pH 8.5) containing 14.4 mM mercaptoethanol (buffer B). The enzyme was loaded into a DEAE Sepharose column (Pharmacia) that had been equilibrated with buffer B. The proteins were eluted in a gradient mode (0–40 ml, 0 mM NaCl; 40–360 ml, 0–300 mM NaCl). After diluting the active fractions with buffer B, the enzyme solution was applied to a Mono-Q HR 5/5 column (Pharmacia) that had been equilibrated with buffer B. The bound proteins were eluted with an NaCl linear gradient in buffer B (0–10 ml, 130 mM NaCl; 10–40 ml, 130–400
mm NaCl). The active fractions were combined and concentrated by ultrafiltration (Centriplus-10, Amicon). After adding 1 volume (v/v) of glycerol, the enzyme solution was stored at −30°C prior to its use for characterization.

*Gel filtration.* Part of the active fractions obtained by anion-exchange chromatography on Mono Q was applied to a Superdex 75 HR 10/30 column to estimate the apparent molecular weight of THT. Proteins were eluted with buffer A at a flow rate of 0.5 ml/min. The following proteins were used as molecular weight markers: bovine serum albumin (67 kDa), ovalbumin (43 kDa), myoglobin (17.6 kDa), and ribonuclease A (13.7 kDa).

*HPLC conditions.* To investigate the substrate specificity of THT, various amines and hydroxycinnamoyl-CoA esters were used as substrates. The quantity of amides formed by the enzymatic reaction was determined by HPLC (Wakosil II SC18 HG column, 150 × 4.6 mm i.d.; flow rate of 0.8 ml/min; detection at 320 nm), using synthesized compounds as standards. Two different mobile phases were used: (1) 22% acetonitrile in water containing 0.1% trifluoroacetic acid for analysing N-feruloyltyramine (retention time: 14.3 min), N-feruloyldopamine (8.7 min), N-p-coumaroyltyramine (12.3 min), N-caffeoyltyramine (7.6 min), and N-sinapoyltyramine (13.9 min); (2) 35% acetonitrile in water containing 0.1% trifluoroacetic acid for N-feruloylphenethylamine (10.0 min), N-feruloyltryptamine (10.8 min), and N-cinnamoyletyramine (9.3 min).

**Results**

*Induction of N-hydroxycinnamoyltyramine synthesis in wounded maize leaves*

The lower epidermis of maize leaves was peeled off as a wounding treatment. After fractionating methanol extracts of the leaves with Sep-pak cartridges, both the N-p-coumaroyl- and N-feruloyltyramine contents were measured by HPLC. The identity of compounds corresponding to N-p-coumaroyl and N-feruloyltyramine was confirmed by comparing the retention time for HPLC, and the UV and ion-spray MS data between the induced compounds and authentic amides that had been chemically synthesized. N-p-Coumaroyltyramine was hardly detectable before wounding, whereas a substantial amount of N-feruloyltyramine (2.05 nmol/g fr. wt) was present in the healthy leaves. The level of N-hydroxycinnamoyltyramines started to increase 3–6 h after wounding and reached their highest level after 12 h (Fig. 1). Thereafter, the p-coumaroyltyramine content decreased rapidly, dropping to 20% of the maximum after 24 h. In contrast, the N-feruloyltyramine content remained high until 72 h after wounding, being 70% of the maximum value. In the control leaves, the levels of both compounds gradually increased, and more N-feruloyltyramine had accumulated than N-p-coumaroyltyramine at 72 h. N-Feruloyltryptamine and N-p-coumaroyltryptamine, which have been identified in maize grain with N-hydroxycinnamoyltyramines, were not detected in either the wounded or control leaves.

*Induction of THT activity by wounding*

To optimize the assay method for THT, the dependence on pH of the THT activity was examined by using 100 mM Tris-HCl (pH 7.5–9.5) buffers and GTA buffers (50 mM 3,3-dimethylglutaric acid, 50 mM Tris, and 50 mM 2-amino-2-methyl-1,3-propanediol at pH 5–10). THT activity was detected between pH 5.5 and 10, with maximal activity being observed at pH 8.5 in both buffers.

Figure 2 shows time-course plots of the THT activity in maize leaf segments after wounding. Although
little THT activity was evident in the maize leaves before wounding (6.39 pkat/mg protein), the activity markedly increased after wounding, starting to increase 3 h after wounding and reaching a maximum after 36 h. The maximal activity (270 pkat/mg protein) was 40 and 11 times that in leaves before wounding and in the control leaves, respectively. The wound-induced THT activity remained at a high level throughout the course of the experiment (up to 72 h post-wounding). The marginal but significant increase in THT activity observed in the control leaves was probably due to the damage caused by excision.

**Partial purification and characterization of THT**

Wound-induced THT was partially purified for characterization. The crude extracts prepared from leaves 36 h after wounding were fractionated by salting-out with (NH₄)₂SO₄. Thirty one percent of the total activity was recovered in the fraction precipitated between 30% and 45% of (NH₄)₂SO₄ saturation, while only marginal activity was detected in the other fractions. After dialyzing the 30–45% fraction against a 20 mM Tris-HCl buffer (pH 8.5, with 14.4 mM mercaptoethanol), the enzyme solution was subjected to two steps of anion-exchange chromatography on DEAE Sepharose and Mono Q HR 5/5, resulting in a 12.5-fold purification (Table 1 and Fig. 3). Throughout these chromatographic steps, only one peak of THT activity was apparent. Trpyamine N-hydroxycinnamoyltransferase (TrHT) activity was also monitored in the fractions obtained by anion-exchange chromatography, using feruloyl-CoA as an acyl donor. TrHT activity varied in step with THT activity (Figs. 3a and b).

To estimate the apparent molecular weight, an aliquot of the enzyme solution that had been obtained after the anion-exchange chromatography with the Mono Q column was applied to a gel filtration column. A single peak of THT and TrHT activities was observed, corresponding to a molecular mass of 40 kDa.

Partially purified THT was characterized with regard to its kinetic properties by using amine and CoA thioester substrates. Based on the $K_m/V_{max}$ values, the best substrate was tyramine among the four amines tested (Table 2). Tyramine also served as an acyl acceptor, although it has a unique aromatic ring structure. Among the hydroxycinnamoyl-CoA esters, feruloyl-CoA was converted to its respective amide most effectively, being followed by sinapoyl-CoA (Table 3). Although the $K_m$ value for $p$-coumaroyl-CoA was smaller than that for either feruloyl-CoA or sinapoyl-CoA, the $V_{max}$ value was much smaller. Caffeoyl-CoA also acted as an acyl donor to some extent, and no conversion of cinnamoyl-CoA was detected.

In view of the possibility that the same enzyme

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**Table 1. Partial Purification of THT from Wounded Maize Leaves**

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Protein (mg)</th>
<th>Total activity (pkat)</th>
<th>Specific activity (pkat/mg protein)</th>
<th>Yield (%)</th>
<th>Purification factor (fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude enzyme</td>
<td>61.4</td>
<td>10.8</td>
<td>176</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(NH₄)₂SO₄ precipitation (30–45% saturation)</td>
<td>16.8</td>
<td>3.40</td>
<td>203</td>
<td>31.4</td>
<td>1.15</td>
</tr>
<tr>
<td>DEAE Sepharose</td>
<td>4.23</td>
<td>3.38</td>
<td>796</td>
<td>31.1</td>
<td>4.52</td>
</tr>
<tr>
<td>Mono Q</td>
<td>1.33</td>
<td>2.94</td>
<td>2206</td>
<td>27.1</td>
<td>12.5</td>
</tr>
</tbody>
</table>

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**Table 2. Substrate Specificity of THT for Amines**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>Relative $V_{max}$ (%)</th>
<th>$V_{max}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyramine</td>
<td>130</td>
<td>100</td>
<td>0.76</td>
</tr>
<tr>
<td>Phenethylamine</td>
<td>570</td>
<td>76</td>
<td>0.13</td>
</tr>
<tr>
<td>Dopamine</td>
<td>340</td>
<td>68</td>
<td>0.20</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>59</td>
<td>31</td>
<td>0.53</td>
</tr>
</tbody>
</table>

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**Table 3. Substrate Specificity of THT for Hydroxycinnamoyl-CoA Thioesters**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>Relative $V_{max}$ (%)</th>
<th>$V_{max}/K_m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sinapoyl-CoA</td>
<td>8.3</td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td>Feruloyl-CoA</td>
<td>4.8</td>
<td>69</td>
<td>14</td>
</tr>
<tr>
<td>Caffeoyl-CoA</td>
<td>82</td>
<td>13</td>
<td>0.16</td>
</tr>
<tr>
<td>$p$-Coumaroyl-CoA</td>
<td>3.7</td>
<td>5.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Cinnamoyl-CoA</td>
<td>n.d.</td>
<td>n.d.</td>
<td>—</td>
</tr>
</tbody>
</table>

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n.d., not detectable.
could catalyze the condensation of both tryptamine and tyramine, we examined the effect of each of these amines on the THT and TrHHT activities. THT activity was inhibited by tryptamine at 100 μM and 500 μM concentrations, and a similar effect was observed on TrHHT activity by tyramine (Figs. 4a and b). In both cases, Lineweaver-Burk plots showed increased K_m and unchanged V_max in the presence of the second amine, indicating competition between tryptamine and tyramine in the conjugation reactions.

**Induction of N-hydroxybenzylamine synthesis and THT activity in various hybrid lines of maize**

The induction of N-hydroxybenzylamine synthesis and THT activity was respectively investigated in various hybrid lines 12 h and 36 h after wounding (Table 4). With all the hybrid lines tested, the induction of N-hydroxybenzylamine synthesis and of THT activity was observed, although the extent depended on the line used.

**Discussion**

The present study has demonstrated that N-hydroxybenzylamines accumulated in maize leaves after wounding. This accumulation was accompanied by an upregulation of THT activity, indicating that the accumulation of N-hydroxybenzylamines was a result of enzymatic synthesis from hydroxybenzyl-CoA esters and tyramine in response to wounding. The induction of both N-hydroxybenzylamine accumulation and THT activity was observed in all the hybrid lines of maize tested, suggesting the generality of the reaction to wounding in maize. In respect of the control leaves, the N-hydroxybenzylamine content and THT activity gradually increased, probably due to damage caused by excision of the leaves, because neither an accumulation of N-hydroxybenzylamines nor an enhancement of THT activity was apparent in non-excised maize leaves at this stage of development.

The occurrence of hydroxybenzylamines linked covalently to an amine function is scattered throughout the plant kingdom, including cereals. The series of hydroxybenzylamines conjugated with alkylidene-polyamines appears to be distributed in meristematic tissues, reproductive organs and mature seeds in many plant species. Avenanthramides, substituted N-cinnamoylanthranilates, occur in oat groats and hulls as constitutive components. A hydroxybenzylamine conjugate of agmatine has been isolated from germinated barley as well as the coupled dimers, hordatine A and B. The occurrence of hydroxybenzylamine amides with tyramine and tryptamine has been reported in maize grains. The distribution of hydroxybenzylamine amides with amines suggests a developmental regulation of their synthesis, although their physiological functions are not clear. In addition, the involvement of hydroxybenzylamine amides in stress response has increasingly been found in Gramineae. Avenanthramides have been characterized as oat phytoalexins. The induction of the enzyme which catalyzes the synthesis of avenanthramides from hydroxyanthranilic acids and hydroxybenzyl-CoA congeners has been demonstrated in elicitor-treated oat leaves as well as other biosynthetic enzymes. In barley, treatment with methyl jasmonate and infection with an arbuscular mycorrhizal fungus induced the synthesis of p-coumaroylaminoglutamine. The accumulation of p-coumaroylhydroxyammatin has also been found in epidermis tissue after its infection with the powdery mildew fungus. The accumulation of hydroxybenzylamine

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**Table 4. Induction of N-Hydroxybenzylamine Synthesis and THT Activity by Wounding in Primary Leaves from Various Hybrid Lines Maize**

<table>
<thead>
<tr>
<th>Hybrid line</th>
<th>N-p-Coumaroylamine (nmol/g fr. wt)</th>
<th>N-Feruloylamine (nmol/g fr. wt)</th>
<th>THT activity (pkat/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wounded</td>
<td>Control</td>
<td>Wounded</td>
<td>Control</td>
</tr>
<tr>
<td>Mi29 × Na50</td>
<td>69.5</td>
<td>4.41</td>
<td>238</td>
</tr>
<tr>
<td>Mi19 × H95srm</td>
<td>10.3</td>
<td>3.06</td>
<td>431</td>
</tr>
<tr>
<td>(Te9 × To15) × (W79A × RB262)</td>
<td>98.9</td>
<td>1.86</td>
<td>178</td>
</tr>
<tr>
<td>(914-2 × CM174) × (Ho3 × Ho4)</td>
<td>56.3</td>
<td>3.12</td>
<td>374</td>
</tr>
<tr>
<td>(N19 × To38) × (W41A × W79A)</td>
<td>39.3</td>
<td>1.85</td>
<td>411</td>
</tr>
</tbody>
</table>
acid amides may be a general defence reaction to stress in Gramineae, although the stimulus that triggers the amide production seems to depend on the species.

It has been indicated that \( N \)-hydroxyccinnamoyltryptamines are integrated into the cell wall in potato,\(^7\) tobacco,\(^3\) \textit{Lycopersicon peruvianum}\(^8\) and opium poppy\(^3\) through the activation of peroxidase.\(^3\) These deposited amides are thought to form a phenolic barrier against pathogens and herbivores. It is thus possible that \( N \)-hydroxyccinnamoyltryptamines in maize are analogously metabolized to reinforce the cell wall after wounding. In support of this, the time-course experiments showed that the amounts of \( N \)-hydroxyccinnamoyltryptamines, especially of \( p \)-coumaroyltryptamine, decreased after reaching a maximum, while the enzyme activity remained at a high level throughout the course of the experiment. Furthermore, the increase of \( N \)-feruloyltryptamine in the control leaves can be related to the metabolism of the compounds. The amount of \( N \)-feruloyltryptamine in the control leaves was comparable to that in the wounded leaves at 72 h post-treatment, while the THT activity in the control leaves remained at a lower level than that in the wounded leaves. The activation of amide metabolism may not have occurred in the control leaves. Accordingly, the metabolism of the produced \( N \)-feruloyltryptamine may have been slow in the control leaves that an apparent accumulation occurred even when there was only weak THT activity.

An interesting kinetic property of wound-induced THT in maize is its broader substrate specificity for amines than in other plants. The maize enzyme converted tyramine, phenethylamine and dopamine to their respective amides, while the activity for dopamine was scarcely detectable in THT from barley and wheat.\(^3\) In addition, wound-induced THT in maize accepted tryptamine which has a completely different ring system with comparable efficiency to tyramine. This is in contrast to THT from potato, for which tryptamine is a poor substrate.\(^3\) Unfortunately, kinetic constants for tryptamine of other THTs from barley, wheat and tobacco are not available. The possibility that the activity observed with tryptamine of THT in maize was due to heterogeneity of the enzyme preparation cannot be excluded, because we used a partially purified enzyme in this study. However, it is most likely that the enzyme was responsible for both the TrHT and THT activities, since the two activities changed in parallel during the two steps of anion-exchange chromatography and gel filtration. Moreover, tryptamine competitively inhibited the conjugation of tyramine and hydroxyccinnamoyl-CoA, and vice-versa. Although no accumulation of \( N \)-hydroxyccinnamoyltryptamines was detected in the wounded leaves, these compounds are present in maize reproductive organs, as well as \( N \)-feruloyl- and \( N \)-\textit{p}-coumaroyltryptamine.\(^10\) Thus, tryptamine probably acts as a substrate for THT at specific developmental stages, like tyramine.

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**References**

11) Negrel, J. and Javelle, F., Purification, characterization and partial amino acid sequencing of hydroxyccinnamoyl-CoA:tyramine \( N \)-hydroxyccinnamoyl transferase from tobacco cell-suspension cultures.
Wound-induced N-Hydroxycinnamoyltyramine Synthesis in Maize


