Induction of Furano-terpene Production and Formation of the Enzyme System from Mevalonate to Isopentenyl Pyrophosphate in Sweet Potato Root Tissue Injured by *Ceratocystis fimbriata* and by Toxic Chemicals

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**ABSTRACT**

When sweet potato (*Ipomoea batatas*) root tissue was infected by *Ceratocystis fimbriata*, activity of the enzyme system from mevalonate to isopentenyl pyrophosphate, especially of pyrophosphate-lyase, was incorporated into the infected region, preceding the furano-terpene production in the infected region. Cutting and incubation of sweet potato slices did not produce furano-terpenes, and only slightly increased the activity of the enzyme system from mevalonate to isopentenyl pyrophosphate.

The enzymic activity in diseased tissue was localized in the soluble fraction, and was higher in the tissue from the surface to a depth of about 5 mm with gradual decrease toward the inner part.

Mercuric chloride (0.1%, w/v) and sodium dodecyl sulfate (1.0%, w/v) were utilized as model inducers of furano-terpenes and pyrophosphate-lyase decarboxylase. The mercuric chloride- or sodium dodecyl sulfate-induced response was inhibited by administration of cycloheximide to the discs together with the inducer immediately after disc preparation. When cycloheximide or blasticidin S was applied together with the inducer, to the discs 9 hours or more after disc preparation, the induction was not inhibited but rather stimulated.

Farnesol pathway in the same way as sterol biosynthesis, and that the terpene biosynthetic system involving the acetate-MVA-farnesol pathway is activated in the tissue in response to fungal infection. However, the triggering mechanism of the enzyme formation has not been elucidated. The induction mechanism of the terpene production may give a clue to elucidating physiological responses of plants to external stimuli.

This paper reports the correlation between the increase in activity of the enzyme system from MVA to IPP, especially MVAPP decarboxylase and terpene production, in sweet potato root tissue infected by *C. fimbriata* or treated with toxic chemicals. Effects of actinomycin D, cycloheximide, and blasticidin S on terpene production and formation of MVAPP decarboxylase are also described.

**MATERIALS AND METHODS**

**MATERIALS**

Sweet potato (*Ipomoea batatas* Lam. cv. Norin 1) roots were harvested at Kariya Farm, Aichi in the autumn and stored at 10 to 14°C until used. [3-14C]lactone and [3-14C]lactone were purchased from the Radiochemical Center, England. Actinomycin D, cycloheximide, and blasticidin S were products of Boehringer Mannheim GmbH, Sigma Chemical Co., and Kakenkagaku Corporation, respectively.

**INDUCTION TREATMENT**

**Method A.** Sweet potato roots were sliced vertically 2 cm thick and divided into two groups. One group was inoculated on cut surfaces with a spore suspension (1-2 × 10^7 spores/ml) of *C. fimbriata* Ell. and Halst. The tissues were incubated at 29°C for 45 hr or 48 hr and are called diseased tissue. The other group of slices was incubated under the same conditions, but without inoculation, and are called cut tissue.

**Method B.** Discs (4 mm thick and 20 mm in diameter) were prepared from parenchymatous tissue of sweet potato roots and inoculated for 0, 9, or 24 hr at 29°C. Deionized H₂O or the solution containing an antibiotic was infiltrated in vacuo (40 mm Hg for 1 min) into the discs. After vacuum infiltration, inducer solution (0.08-1 ml/disc) with or without antibiotic was applied to the discs, which were then incubated at 29°C. In the case of discs which were treated with the solution immediately after disc preparation, a second application of inducer solution was made to the discs after 24 hr, and incubation was continued for another 24 hr.

**Method C.** Discs (4 mm thick and 20 mm in diameter) which

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ABBREVIATIONS: MVA: mevalonate; HMG CoA: 3-hydroxy-3-methylglutaryl CoA; IPP: isopentenyl pyrophosphate; MVAPP: pyrophosphate-lyase; MVAP: pyrophosphate-lyase.

1. Abbreviations: MVA: mevalonate; HMG CoA: 3-hydroxy-3-methylglutaryl CoA; IPP: isopentenyl pyrophosphate; MVAPP: pyrophosphate-lyase; MVAP: pyrophosphate-lyase.
were preincubated without treatment for 20 hr at 29 C were stirred in deionized H₂O or a solution containing an antibiotic at room temperature for 1 hr. After 30 min standing, the discs were treated with inducer solution (0.1 ml inducer solution/disc) with or without antibiotic and incubated for another 24 hr.

The discs treated with inducer solution by method B or C were not contaminated by bacteria (14, 20), and are called treated tissue.

**SAMPLING OF SWEET POTATO ROOT TISSUE**

After a certain incubation period at 29 C, slices (1–1.5 mm thick) treated with method A, B, or C were taken from the noninfected (or noninjured) tissue adjacent to the infected (or inducer-treated injured) necrotic region. As control (cut tissue), slices (1–1.5 mm thick) were taken from the internal tissue, toward the inner part from a region of 0.2 to 0.3 mm thick below the surface.

**ENZYME PREPARATIONS**

Five g of the above mentioned tissue was mixed with 12.5 ml of 50 mm K-phosphate buffer (pH 7.5) containing 40 mm L-cysteine and 1 mm EDTA (20 mm EDTA was used when 0.1% HgCl₂ solution was applied to the discs as inducer) and 1 g of Polyclar AT, and homogenized twice (30 sec each) in a blender (Sakuma Co., Tokyo, Japan) at maximum speed. The homogenate was squeezed through four layers of cotton gauze and centrifuged at 20,000g for 30 min. The supernatant solution was passed through a Sephadex G-25 column (20 × 1.9 cm), pre-equilibrated with 50 mm K-phosphate buffer (pH 7.5) containing 1 mm EDTA. The protein fraction thus obtained (Sephadex G-25 fraction) was brought to 45% ammonium sulfate saturation by the addition of solid ammonium sulfate. The resulting precipitate was collected by centrifugation at 15,000g for 30 min, the pellet was suspended in 5 mm K-phosphate buffer (pH 7.5), and the suspension was passed through a Sephadex G-25 column (0.8 × 6 cm) pre-equilibrated with 5 mm K-phosphate buffer (pH 7.5) to remove ammonium sulfate. The protein fraction thus obtained (ammonium sulfate fraction) was used for enzyme assay.

When Sephadex G-25 fraction was immediately used for enzyme assay, the supernatant solution was passed through a Sephadex G-25 column (1.9 × 20 cm), pre-equilibrated with 20 mm K-phosphate buffer.

**CELLULAR FRACTIONATION**

After 48 hr incubation, diseased tissue (15 g) was mixed with 37.5 ml of 50 mm K-phosphate buffer (pH 7.5) containing 0.5 M sucrose, 1 mm EDTA, 40 mm L-cysteine, and 3 g of Polyclar AT, and homogenized in a blender once (15 sec) at maximum speed, and twice (30 sec each) at low speed. The homogenate was squeezed through four layers of cotton gauze and centrifuged at 300g for 10 min. The supernatant liquid was passed through a Sephadex G-25 column (4.0 × 50 cm) pre-equilibrated with the above medium. The protein fraction thus obtained was centrifuged at 7000g for 20 min to sediment the mitochondrial fraction. The mitochondrial fraction was washed once with 50 mm K-phosphate buffer (pH 7.5) containing 0.5 M sucrose and 1 mm EDTA (washing medium). The supernatant solution was centrifuged at 105,000g for 2 hr to sediment the microsomal fraction. The microsomal fraction was washed once with the washing medium. These particulate fractions were suspended in 6 ml each of 5 mm K-phosphate buffer (pH 7.5) and frozen until used. The supernatant fraction was brought to 45% ammonium sulfate saturation by the addition of solid ammonium sulfate. The resulting precipitate was collected by centrifugation at 15,000g for 30 min. the pellet was suspended in 5 mm K-phosphate buffer (pH 7.5), and ammonium sulfate contained in the suspension was removed through a Sephadex G-25 column pre-equilibrated with 5 mm K-phosphate buffer (pH 7.5). The protein solution was used as the supernatant fraction.

**PREPARATION OF R-5-PYROPHOSPHOMALEVATE**

Nine ml of the ammonium sulfate fraction from a 24-hr diseased tissue (equivalent to 45 g fresh weight of tissue) was incubated at 30 C for 10 min with 9 μmol (7.05 μCi) of r-s-mevalonate-2,4-14C (1.0 ml of 42 μM K-phosphate buffer (pH 7.5) containing 0.45 mmol of KF, 0.225 mmol of monooctaoacetamide, 0.45 mmol of ATP, and 0.225 mmol of MgCl₂. After boiling for 2 min, the denatured protein was removed by centrifugation. The supernatant was chromatographed on six columns (1 × 7 cm) of Dowex 1 (formate form) in a stepwise gradient of 4 n formic acid (6 × 110 ml) and 0.4 M ammonium formate in 4 n formic acid (6 × 70 ml). Fractions containing MVAPP (420 ml) were combined, chromatographed on a Dowex 50W × 8 (H⁺) column to remove NH₄⁺, and then lyophilized. Paper chromatography in a solvent (tert-butanol-formic acid-water, 40:10:15) of the lyophilized sample revealed a single radioactive peak at 0.33 of Rₗ value which accorded with that of MVAPP reported previously (1). Thus, the sample was identified as MVAPP. Since the plant enzymes convert r-MVA to r-MVAPP (7), we believed that the sweet potato enzymes also show the same stereospecificity.

**ASSAY AND ISOLATION OF PHOSPHORYLATED DERIVATIVES OF MEVALONIC ACID**

The incubation mixture for enzymatic synthesis of phosphorylated derivatives from MVA was composed of 0.2 μmol of r-s-MVA-2,4-14C (0.47 μCi), 50 μmol of K-phosphate buffer (pH 7.5), 10 μmol of ATP, 5 μmol of MgCl₂, 10 μmol of KF, 5 μmol of monooctaoacetamide, and the enzyme solution (the ammonium sulfate fraction, equivalent to 1 g fresh weight of tissue) in a total volume of 1.2 ml. The enzymic reaction was carried out at 30 C for 10 min or 20 min, then stopped by heating the reaction tube in boiling water for 2 min. After removal of denatured protein by centrifugation, metabolic products of MVA in the supernatant solution were separated on a Dowex 1 (formate) column (0.7 × 5 cm) by the method of Suzue et al. (22). The following series of media were used for elution: 25 ml of H₂O, 25 ml of 2 n formic acid, 50 ml of 4 n ammonium formate, 50 ml of 0.4 M ammonium formate in 4 n formic acid, and finally, 50 ml of 0.8 M ammonium formate in 4 n formic acid.

**PYROPHOSPHOMALEVATE DECARBOXYLASE ASSAY**

**Method I.** The reaction mixture containing r-MVAPP-2,4-14C (0.062 μCi, 5.03 μCi/μmol), 50 μmol of K-phosphate buffer (pH 7.5), 10 μmol of ATP, 5 μmol of MgCl₂, 10 μmol of KF, 5 μmol of monooctaoacetamide, and the enzyme solution (the ammonium sulfate fraction, equivalent to 0.5 g or 1 g fresh weight of tissue in a total volume of 1.2 ml was incubated at 30 C for 10 min. The reaction was stopped by immersing the reaction tube in boiling water for 2 min. The deproteinated supernatant (0.6 ml) was transferred to a Dowex 1 (formate) column and the MVAPP remaining in the reaction mixture was eluted from a column with 50 ml of 0.4 M ammonium formate in 4 n formic acid, then IPP was eluted with 50 ml of 0.8 M ammonium formate in 4 n formic acid.

**Method II.** The enzyme activity was assayed by measuring the evolution of 14CO₂ from mevalonate-1,14C. Incubation mixture contained r-s-MVA-1,14C (0.25 μCi, 6.10 μCi/μmol), 50 μmol of K-phosphate buffer (pH 7.5). 5 μmol of ATP, 5 μmol of MgCl₂.
5 μmol of KF, and the enzyme solution (the Sephadex G-25 fraction, equivalent to 0.06 to 0.17 g fresh weight of tissue) in a total volume of 1 ml. The incubation vessel was a 15-ml vial equipped with a rubber cap and a removable central microtube containing 0.1 ml of 20% KOH. The reaction was started by the addition of enzyme and performed at 30 C for 5 to 20 min and stopped by the injection of 0.3 ml of 6 N HCl through a rubber cap tightly fitted over the vial opening. The incubation was continued at 30 C for 1 additional hr to ensure complete trapping in the central tube of the liberated 14CO2. The central tube was removed, and KOH solution was transferred to a scintillation vial containing Bray’s solution (2). Enzymic evolution of 14CO2 as a function of time of incubation showed a lag before a linear appearance of label, and was determined in the time interval (5–20 min) showing a linear appearance of label, and MVAPP decarboxylase activity was expressed in terms of the amount (nmol) of 14CO2 evolved/min. We can assay activity of MVAPP decarboxylase by either method I or II. However, method II is more convenient than method I.

QUANTITATIVE ANALYSIS OF TERPENES

The terpenes produced in the tissues were assayed by the method of Oguni and Uritani (15). After incubation for a certain period, infected (or inducer-treated injured) necrotic tissue was homogenized in a glass homogenizer with 20 volumes of chloroform-methanol (1:1, v/v). The homogenate was filtered on a glass filter and the residue washed with another 10 volumes of chloroform-methanol. The filtrates were combined, and concentrated in a vacuum evaporator to dryness on a water bath at 40 C. The residue was dissolved in 2 ml of ethanol. The ethanol solution was filtered through a glass filter to remove the insoluble materials, and then the filtrate was made up to 5 ml. An aliquot of the ethanol solution was made to 2 ml with ethanol, after which 1 ml of 10% p-dimethylaminobenzaldehyde in ethanol and 2 ml of 40% (v/v) aqueous H2SO4 were added successively. The mixture was incubated at 30 C for 15 min and optical density at 527 nm was measured with a spectrophotometer (Shimadzu QV-50).

RESULTS

Formation of Enzyme System from Mevalonate to Isopentenyl Pyrophosphate and Induction of Terpene Production in Sweet Potato Root Tissue in Response to Infection by C. fimbriata. As shown in Figure 1a, the activities for synthesis of total phosphorylated products MVAP + MVAPP + IPP were increased markedly in the 12- to 24- or 48-hr period after inoculation, but the production of terpenes (Fig. 1b) was most active in the 24- to 48-hr period after inoculation. Increase in MVAPP decarboxylase activity using MVAPP-2-14C (method I) was greatest in the 12- to 24-hr period after inoculation (Fig. 2). These results indicated that terpenes were produced following increase in activity of the enzyme system from MVA to IPP in response to infection by C. fimbriata. However, cut tissue did not produce terpenes and slightly increased activity of the enzyme system from MVA to IPP (Fig. 1a).

In order to examine the tissue part where activity of the enzyme system from MVA to IPP is increased, tissue slices (1 mm thick) from five different layers of diseased tissue were homogenized separately. The first layer was about half fungus-invaded. Fungus invasion was not observed in the second to the fifth layers. As shown in Figure 3, activity of the enzyme system from MVA to IPP was activated from the surface to a depth of about 5 mm (from the first to fifth layers where terpenes were not detected with gradual decrease toward inner layer).

Data in Table I show that the enzyme system from MVA to IPP is localized mainly in the soluble fraction in diseased tissue.

Effect of Antibiotics on Induction of Terpene Production. As shown above, sweet potato root tissue produces terpenes in response to infection by C. fimbriata, preceded by the increase in the activity of the enzyme system from MVA to IPP. However, the sweet potato-C. fimbriata system is not suitable for investigation of the mechanism of terpene induction in detail, because RNA and protein synthesis in sweet potato cannot be inhibited selectively without an effect on C. fimbriata. We reported that production of terpenes was induced in sweet potato root tissue by some toxic chemicals such as mercuric chloride (24). Therefore, in this study, mercuric chloride and SDS were used as inducers for terpene production. Terpene induction by mercuric chloride or SDS was inhibited by 30 or 50% of control when inducer was applied together with cycloheximide to freshly prepared discs which had been subjected to vacuum infiltration of cycloheximide (Table II). When actinomycin D was applied in the same conditions (Table II), terpene induction was inhibited, although not much. An increase in activity of MVAPP decarboxy-

Fig. 1. Changes in activity of the enzyme system from MVA to IPP and terpene production after inoculation of C. fimbriata. Induction treatment was done by method A. (a) Changes in formation of phosphorylated products from MVA, MVAP, MVAPP, and IPP, respectively, in the case of diseased tissue. (b) Changes in terpene production. ○ and ◇: terpenes in diseased and cut tissues, respectively.
yrase was observed preceding the maximum terpene production in this system as in fungal-induced tissue. Induced increase in MVAPP decarboxylase activity was inhibited completely by cycloheximide (Table III).

Terpenes accumulated after a 24-hr lag period when inducer was applied to freshly prepared discs (Table III), but accumulated within 24 hr after inducer application when discs were preincubated at 29 C for 24 hr before inducer application (Table IV). Terpene induction in 24-hr incubated discs by mercuric chloride or SDS was not inhibited by cycloheximide or actinomycin D, but was rather stimulated by low concentrations of the inhibitors (Tables IV and V). However, neither cycloheximide nor actinomycin D itself induced terpene production (Table V).

Terpene induction by mercuric chloride was not inhibited by cycloheximide and blasticidin S (1 or 5 µg/ml, a potent inhibitor of protein synthesis in both bacterial and mammalian cells [25]), when either inhibitor and inducer were applied to 9-hr incubated discs. Induced increase in MVAPP decarboxylase activity by mercuric chloride was rather stimulated by cycloheximide, as terpenes were stimulated, when it was applied to the discs together with inducer at the 20-hr incubation stage after disc preparation (Table VI). The level of increased MVAPP decarboxylase activity was roughly proportional to the amounts of terpene produced. In this experiment, a low concentration of terpenes was recorded in discs treated only by distilled H2O or

Table 1. Intracellular Distribution of Enzyme System from MVA to IPP

<table>
<thead>
<tr>
<th>Products</th>
<th>MVAP</th>
<th>MVAPP</th>
<th>IPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial fraction</td>
<td>1.10</td>
<td>0.44</td>
<td>0.14</td>
</tr>
<tr>
<td>Microsomal fraction</td>
<td>1.42</td>
<td>0.60</td>
<td>0.26</td>
</tr>
<tr>
<td>Supernatant fraction</td>
<td>15.75</td>
<td>53.12</td>
<td>7.96</td>
</tr>
</tbody>
</table>

Table II. Effects of Cycloheximide and Actinomycin D on Terpene Production by Mercuric Chloride and Sodium Dodecyl Sulfate

Terpenes were extracted from seven discs 48 hr after inducer application to freshly prepared discs.

<table>
<thead>
<tr>
<th>Inducer</th>
<th>Antibiotics</th>
<th>Amounts of Terpenes</th>
<th>µg ml</th>
<th>mg disc</th>
<th>mg g necrotic tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>HgCl2</td>
<td>Cycloheximide (1)</td>
<td>0.291</td>
<td>1.59</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HgCl2</td>
<td>Cycloheximide (5)</td>
<td>0.215</td>
<td>1.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>Cycloheximide (1)</td>
<td>0.134</td>
<td>0.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>Cycloheximide (5)</td>
<td>0.115</td>
<td>0.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HgCl2</td>
<td>Actinomycin D (50)</td>
<td>0.720</td>
<td>5.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HgCl2</td>
<td>Actinomycin D (100)</td>
<td>0.493</td>
<td>3.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDS</td>
<td>Actinomycin D (50)</td>
<td>0.134</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 When treated with HgCl2 or SDS, the tissue in the depth of about 0.2 to 1.0 mm from the surface showed brown-colored necrosis, and is called necrotic tissue.
2 HgCl2 solution (0.1% [w/v]) and SDS solution (1% [w/v]) were treated by method B.

Table III. Effect of Cycloheximide on Mercuric Chloride-induced Terpene Production and MVAPP Decarboxylase Activity Increase

Experimental conditions are the same as described in Table II. MVAPP decarboxylase was assayed by method II.

<table>
<thead>
<tr>
<th>Incubation Time after Induction Treatment</th>
<th>Antibiotics</th>
<th>Amounts of Terpenes</th>
<th>MVAPP Decarboxylase</th>
</tr>
</thead>
<tbody>
<tr>
<td>hr</td>
<td>µg ml</td>
<td>mg disc</td>
<td>mg g necrotic tissue</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>0.032</td>
<td>0.395</td>
</tr>
<tr>
<td>48</td>
<td>0</td>
<td>0.585</td>
<td>4.08</td>
</tr>
<tr>
<td>48</td>
<td>Cycloheximide (5)</td>
<td>0.162</td>
<td>1.46</td>
</tr>
</tbody>
</table>
Cycloheximide, perhaps because the tissue was subjected to some stress during stirring. The concentrations of cycloheximide used in the present experiments seemed to be enough to inhibit the protein synthesis in sweet potato, because the increase in acid invertase (EC 3.2.1.26), phenylalanine ammonia lyase (EC 4.3.1.5), and peroxidase (EC 1.11.1.7) activities in response to wounding was inhibited completely by cycloheximide (2 μg/ml) (14).

**DISCUSSION**

Terpenes such as ipomeamarone are not detectable in normal sweet potato root tissue, but are induced by the infection of some pathogenic fungi. Furthermore, we found that heavy metal ions, fungus extracts, and amino acids also induced terpene production. Therefore, the mechanism of terpene induction is of interest in terms of biochemical and cytological response of plants to external stimuli including fungal infection and chemical treatments.

Cruickshank and Perrin (4) demonstrated that heavy metal ions, when applied to pea endocarp tissue, induced pisatin. They suggested that these metals might be exerting their effects by inhibiting a sulfhydryl-containing enzyme. Cruickshank et al. (5) later suggested that this action could dissociate special inhibitor peptides which complexed and inactivate enzymes essential for pisatin production. An alternative possibility suggested by Cruickshank’s group was that allosteric mechanisms may be involved in determining activity levels of key enzymes of the phytoalexin pathways. In this case, the inducers would interact with small effector molecules such as those which are known to influence the kinetics of numerous enzymes.

Cruickshank et al. (6) reported that cycloheximide did not inhibit induction of phaseollin or phaseollidin by monillicin A in the endocarp of Phaseolus vulgaris.

Recently, Hadwiger and his associates have proposed (8-10, 21), using DNA-alkylating agents, DNA-intercalating compounds, and base analogues as phytoalexin inducers, that increased phenylalanine ammonia lyase and pisatin or phaseollin production occur in pea or bean tissue as a result of gene activation and that such activation can be induced by an array of compounds having the potential to change the conformation of double-stranded DNA.

In the case of terpene induction in sweet potato, many kinds of known terpene inducers cannot be classified into compounds having the potential to change the conformation of double-stranded DNA, although mercuric chloride is known to bind to DNA (26).

The present experimental results indicated that sweet potato root tissue produced terpenes in response to infection by C. fimbriata. preceded by the increase in activity of the enzyme system from MVA to IPP (Figs. 1 and 2). Furthermore, increase in activity of the enzyme system occurred in the region from the surface to a depth of about 5 mm, where terpenes were not detected, with gradual decrease toward the inner part (Fig. 3). However, cut tissue did not produce terpenes, and slightly increased activity of the enzyme system was observed. The question thus arises, is the biosynthesis of terpenes developed by increasing the catalytic efficiency of enzyme systems such as that from MVA to IPP or by synthesizing enzyme molecules involved in the enzyme systems in the terpene biosynthetic pathway? If induction occurs via gene activation, the transcription of genetic information and the synthesis of enzyme protein should be required. Our present results utilizing the inhibitor of protein synthesis, cycloheximide, indicated that induced terpene production and increase in MVAPP decarboxylase activity were both inhibited when inhibitor and inducer were applied to discs immediately after disc preparation (Table III), but were not inhibited but rather stimulated when inhibitor was applied to the discs together with the inducer at a 20-hr incubation stage after disc preparation (Table VI). These results may suggest that: (a) synthesis of the enzyme protein is required for the induction of terpene production in sweet potato root tissue; (b) synthesis of the precursor protein, not active enzyme, is induced by cutting...
without induction treatment such as fungus infection and application of toxic chemicals; and (c) the additional different cell injury such as fungus invasion and chemical diffusion is required for the modification of the enzyme protein from a latent form to an active form, which participates in terpene biosynthesis. The final conclusion remains to be solved. An approach to solving the mechanism may be to clarify whether the coapplication of some inhibitors such as cycloheximide with terpene inducers as HgCl₂ to precubated discs stimulates the formation of the necrotic tissue, increasing the release of the tissue-originating stimulus to elicit the terpene production.

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LITERATURE CITED