Ethylene-Induced Polyamine Accumulation in Rice (Oryza sativa L.) Coleoptiles

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ABSTRACT

Effects of ethylene on free polyamine biosynthesis in rice (Oryza sativa L. cv Taichung Native) coleoptiles were investigated in sealed and aerobic conditions. In sealed conditions, putrescine increased significantly and coincided with ethylene accumulation. Application of ethylene in sealed containers promoted putrescine accumulation over that in sealed controls. This ethylene-enhanced putrescine accumulation was inhibited by the ethylene action inhibitor 2,5-norbornadiene at 4000 μL/L. In aerobic conditions, ethylene and 1-aminoacyclopropane-1-carboxylic acid also induced putrescine accumulation. Activity of arginine decarboxylase (EC 4.1.1.19) and S-adenosylmethionine decarboxylase (EC 4.1.1.50) increased on exposure to ethylene in aerobic conditions. Ornithine decarboxylase (EC 4.1.1.17) activity, however, remained unchanged. The ethylene-induced putrescine accumulation was inhibited by $5 \times 10^{-4}$ M α-difluoromethylarginine, but not by $5 \times 10^{-4}$ M α-difluoromethylornithine. Apparently, arginine decarboxylase, not ornithine decarboxylase, mediates the ethylene-induced putrescine accumulation. The increased S-adenosylmethionine decarboxylase activity, however, did not result in a significant spermidine/spermine accumulation. In ethylene-treated coleoptiles, the accumulation of putrescine paralleled the increase of coleoptile length in both sealed and aerobic conditions. α-difluoromethylarginine inhibited ethylene-induced putrescine accumulation and coleoptile elongation. It seems that putrescine biosynthesis might be involved in the ethylene-induced elongation of rice coleoptiles.

Polymamines, present ubiquitously in a wide range of higher plants (8), are proposed to play important roles in the regulation of plant growth and development, including cell division, morphogenesis, flower initiation, pollen tube growth and senescence (5, 8, 9, 15). Stresses such as oxygen deficiency (19–21), chilling (11), high saline, low pH, and K+ and Mg2+ deficiency (5, 8, 9) increase polyamine contents and their biosynthetic enzyme activities (5, 8, 9). Ethylene was also found to influence polyamine biosynthesis (1, 3, 6, 12, 13, 22, 24); however, whether the effect was inhibition or stimulation depended on the plant system (5). In apical tissues of etiolated pea seedlings, ethylene inhibited the activities of ADC3 (1), SAMDC (13), and lysine decarboxylase (EC 4).

The inhibition of the enzyme activity by ethylene was shown to be correlated with the ethylene-induced reduction of apical tissue activity. In contrast, ADC and SAMDC activities, and also polyamine contents, in deepwater rice stems increased in response to ethylene (3). The increased polyamines were suggested to be involved in the promotion of both cell division and elongation of rice stems (3). Thus, ethylene-mediated alterations in polyamine biosynthesis are parallel to the ethylene-regulated physiological responses (8, 9).

Rice (Oryza sativa L.) plants survive under oxygen-restricted environments like hypoxia (27) and submergence (18, 28); these environments promote coleoptile elongation (14, 18, 27). It has been established that ethylene is an important factor in this stimulation of rice coleoptile elongation (17, 18). Polyamines have been suggested to be involved in the control of cell elongation. Putrescine content and a high ratio of putrescine to spermidine were directly correlated to the elongation rate in pea roots (25) and corn coleoptiles (4). The excessive accumulation of putrescine in coleoptiles of anaerobic-grown rice seedlings was also proposed to be required for coleoptile elongation in anaerobic conditions (20). It raises the possibility that polyamine is involved in the regulation of ethylene-enhanced elongation of rice coleoptiles. The present study therefore examines the effects of ethylene on polyamine biosynthesis, as well as the possible role of polyamine in the processes of ethylene-enhanced elongation in rice coleoptiles.

MATERIALS AND METHODS

Plant Materials and Treatments

Rice (Oryza sativa L. cv Taichung Native) seeds were sterilized with 5% sodium hypochloride for 10 min and then rinsed with distilled water 10 times. The seeds were then germinated in Petri dishes (9 cm in diameter) containing 10 mL of distilled water under darkness at 30°C. After a 3-d incubation, seedlings with 2-mm shoots were used. In the experiment under sealed conditions, 10 seedlings were transferred into a 30-mL flask with 2 mL of $10^{-3}$ M Mes buffer solution (pH 5.5), and the flasks were sealed with silicone rubber caps. Ethylene treatment was administered by injecting 3 mL of 10 μL/L ethylene into the sealed containers to make a concentration of 1 μL/L in each flask. In the experiment under aerobic conditions, 10 seedlings were also sealed in a 30-mL flask as above and fresh air was passed continuously. For ethylene treatment under aerobic conditions, 10 seedlings were sealed in a 30-mL flask, and sterilized air...
containing 1 μL/L ethylene was passed in and out with a flow rate of 25 mL/min.

Putrescine and its biosynthetic inhibitors, DFMA and DFMO, were prepared in 2 mL 10^{-3} M Mes buffer (pH was adjusted to 5.5 with KOH) and were added to the flasks. All experiments were carried out under darkness at 30°C for various time intervals.

**Determination of Ethylene Content**

To determine ethylene content, 1 mL of gas was withdrawn from the sealed flask through the cap and analyzed by a Carle gas chromatograph (model AGC-211, Hach Company, Loveland, CO) with a flame ionization detector, column temperature at 80°C, using a Porapak Q5 aluminum column (1.83 m, 1/bin, 80/100 mesh, Carle Inc., CA). To analyze the ethylene production by ACC-treated seedlings, 20 seedlings were closed in a 10-mL test tube for 30 min after various periods of ACC treatment, and 1-mL gas samples were analyzed. The preliminary results showed that the closure of ACC-treated seedlings in test tubes for 30 min had no effects on coleoptile elongation and polyamine accumulation.

**Extraction and Determination of Free Polyamine**

Tissues (about 0.5 g fresh weight) were frozen in liquid nitrogen and homogenized immediately with 5 mL of freshly prepared 5.25% (v/v) PCA and extracted at 4°C for 24 h. After centrifugation at 15,188g for 20 min at 2°C, the supernatant containing PCA-soluble free polyamines was subjected to benzoylation. The benzoylation method was according to Flores and Galston (7) with some modifications. A 0.5-mL aliquot of extract was mixed with 1 mL of 2 N NaOH and 20 μL of benzoyl chloride in a 10-mL plastic tube. The mixture was vortexed for 20 s and then incubated at 30°C in the dark for 40 min. The benzoylation reaction was stopped by the addition of 2.5 mL of saturated NaCl solution. The benzoylated polyamines were extracted with 3 mL of chilled diethyl ether (LC grade, BDH, Poole, UK), centrifuged at 5000g for 10 min, and 1 mL of the ether fraction was collected and evaporated to dryness with a Savant Speed Vac concentrator (model SVC-100H-115, Savant Inc., Hicksville, NY). The benzoylated polyamines were dissolved in 200 μL of chilled 51% methanol and kept at -20°C until assay.

Polyamines were analyzed by HPLC. A 20-μL sample was injected into a Waters HPLC system (Waters Associates, Inc.) with a 5-μm C_{18} reverse phase column (250 × 40 mm, end-capped; E. Merck, Darmstadt, FRG) and a UV detector at 254 nm. MeOH/1% acetic acid was used as the mobile phase in a gradient program as follows: flow rate, 1.0 mL/min; running gradient, 51 to 64% MeOH in 7 min, 64 to 70% MeOH in 5.5 min, 70 to 87% MeOH in 4.5 min, 87 to 100% MeOH in 3 min, 100% MeOH in 0.5 min, and regeneration to the initial state (51% MeOH) in 10 min. In polyamine analysis, samples were replicated at least four times, and results were expressed in nanomoles per gram fresh weight.

**Extraction and Assay of Polyamine Biosynthetic Enzyme Activity**

The preparation and assay of polyamine biosynthetic enzymes were modified from the method of Cohen and Kende (3). Coleoptiles (0.5 g fresh weight) were ground in a chilled mortar and pestle with 1.5 mL of grinding buffer containing 25 mM potassium-phosphate, 50 μM EDTA, 0.1 mM PMSF, and 25 mM ascorbic acid, and its pH was adjusted to 8.0 with KOH. The homogenate was centrifuged at 15,188g for 20 min at 2°C. The supernatant was collected and dialyzed at 4°C against 1 L of grinding buffer for 24 h in darkness.

Enzyme activity was analyzed by CO₂ evolution from decarboxylation reaction. The reaction buffers for ADC, ODC, and SAMDC assay were 100 μL of 200 mM Tris (pH 8.5), 200 mM Tris (pH 8.0), and potassium-phosphate (pH 7.5) buffers, respectively. After preincubation of 50 μL of dialyzed enzyme extract and reaction buffer at 0°C for 5 min, 10 μL of the respective substrate solution, 3.66 mM arginine (containing 5 μCi/mL L-[1-^{14}C]arginine), 21.55 mM ornithine (containing 5 μCi/mL L-[1-^{14}C]ornithine), and 0.57 mM SAM (containing 1.25 μCi/mL S-adenosyl-L-[carboxyl-^{14}C]methionine) was added. The 10-mL reaction tubes were then sealed with silicone rubber caps and incubated at 40°C for 120 min with shaking. The released ^{14}CO₂ was trapped by two 2 n KOH-impregnated filter paper discs. The reaction was stopped by injecting 200 μL of 10% (w/v) TCA with a syringe. After trapping for another 60 min, the paper discs were allowed to dry and counted (5-mL solution of 0.35% [w/v] 2,5-diphenyloxazole:2-methoxyethanol = 4:1) with a Beckman LS-1801 liquid scintillation counter (Beckman Instruments, Inc.). The enzyme activity was expressed in nanomoles of ^{14}CO₂ released per milligram protein per hour. Protein content was determined according to Bradford (2) using BSA as a standard.

**Chemicals**

Putrescine, spermidine, spermine, L-arginine, L-ornithine, SAM, pyridoxal phosphate, DTT, EDTA, and ascorbic acid were purchased from Sigma. The radioactive compounds, L-[1-^{14}C]arginine monochloride (1.85 GBq/mmol), L-[1-^{14}C]ornithine monochloride (1.85 GBq/mmol), and S-adenosyl-[carboxyl-^{14}C]methionine (1.85 GBq/mmol), were purchased from Amersham (Buckinghamshire, England, UK). Polyamine biosynthetic inhibitors, DFMA and DFMO, were kindly provided from Dr. P.P. McCann (Merrill-Dow Research Center).

**RESULTS**

**Changes of Coleoptile Length, Ethylene Content, and Free Polyamine Concentration in Sealed Conditions**

Changes of coleoptile length and ethylene concentration in response to sealed conditions were presented in Figure 1. Coleoptiles elongated rapidly in sealed conditions and reached 33.50 mm after 6 d, which was 2.7-fold higher than the controls. The maximal elongation rate of coleoptiles in sealed-grown seedlings occurred between d 1 and d 3 (Fig. 1A). The ethylene content in sealed flasks also showed a marked increase (Fig. 1B), reaching the maximum amount of 0.68 nmol/flask after 6 d.

Temporal changes of free polyamine concentrations are shown in Figure 2. In aerobic controls, putrescine concentra-
Effects of Exogenously Applied Ethylene on Free Polyamine Concentrations of Rice Coleoptiles in Sealed Conditions

After 6 d in sealed containers containing 1 μL/L ethylene, the coleoptile length was 1.6-fold longer than that of the controls (Table I).

Exogenously applied ethylene was found to enhance the accumulation of putrescine and spermidine above sealed controls without added ethylene (Table I). After 6 d, putres-

Figure 1. Changes of coleoptile length (A) and ethylene content (B) in sealed flasks in response to sealed conditions. △, Sealed conditions; ▲, aerobic conditions.

Figure 2. Levels of putrescine (PUT), spermidine (SPD), and spermine (SPM) in etiolated rice coleoptiles in sealed conditions. A, PUT; B, SPD; C, SPM; △, sealed conditions; ▲, aerobic conditions.
putrescine accumulation inhibited ACC Applied retardation comparison. Stimulation of Accumulation 15- and cine and spermidine in ethylene-treated coleoptiles reached levels 15- and 2-fold higher, respectively, than those in aerobic controls. Transfer of sealed-grown rice seedlings to aerobic conditions after 3 d in sealed conditions resulted in a retardation of coleoptile elongation and putrescine accumulation compared to sealed seedlings. Ethylene clearly elicits the accumulation of free polyamines, especially putrescine, in coleoptiles of etiolated rice seedlings.

Effects of Norbornadiene on the Ethylene-Enhanced Coleoptile Elongation and Free Putrescine Accumulation in Sealed Conditions

The ethylene action inhibitor 2,5-norbornadiene at 4000 μL/L inhibited or blocked the enhancement of coleoptile elongation by ethylene and also inhibited ethylene-induced putrescine accumulation (Table II).

Stimulation of Coleoptile Elongation and Free Putrescine Accumulation in Aerobic Conditions by Exogenously Applied ACC and Ethylene

Because the accumulated CO₂ and the declined O₂ in sealed containers have also been identified to enhance the rice coleoptile elongation (14, 18), the sealed system may overestimate the effect of ethylene on coleoptile elongation and accumulated polyamine contents. Therefore, effects of ethylene and its biosynthetic precursor, ACC, were treated in aerobic conditions. ACC stimulated not only ethylene production, but also coleoptile elongation (Fig. 3) and putrescine content (Fig. 4). The maximum stimulation of ethylene production and coleoptile elongation occurred at 10⁻³ M ACC; 10⁻⁴ M had a small promotive effect and 10⁻⁵ M was inactive (Fig. 3). ACC at 10⁻² and 10⁻⁴ M caused a significant accumulation of putrescine, which reached a maximum at 4 d after treatment. ACC at 10⁻⁵ M did not stimulate putrescine accumulation. The spermidine and spermine concentrations of coleoptiles declined in all treatments; however, 10⁻³ M ACC retarded their decline.

To verify the results with ACC, 1 μL/L ethylene was applied by passing the ethylene-air mixture continuously over seedlings in darkness. The application of ethylene in air induced a rapid elongation of rice coleoptiles and stimulated putrescine accumulation up to 6.5-fold higher than the controls at 4 d after treatment (Table III). In both ACC and ethylene treatments, rice coleoptiles showed a significant amount of putrescine accumulation as compared to spermidine and spermine.

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Table I. Effects of Exogenously Applied Ethylene on Coleoptile Elongation and Polyamine Contents of Etiolated Rice (O. sativa L. cv Taichung Native 1) Seedlings in Sealed Conditions

Ethylene was injected into the sealed flask at the beginning of treatment and the ethylene concentration in a flask was 1 μL/L. The coleoptile length and polyamine content were determined 6 d after treatment. The treatment of "sealed → aerobic" was administered by transferring the sealed-grown seedlings to aerobic conditions after 3 d in sealed conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Coleoptile Length (mm)</th>
<th>Polyamine Content (nmol/g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.08 ± 1.12</td>
<td>Putrescine 186.67 ± 11.18 Spermidine 34.84 ± 0.76 Spermine 8.17 ± 0.45</td>
</tr>
<tr>
<td>Sealed, 3 d</td>
<td>19.48 ± 0.95</td>
<td>1493.11 ± 57.06</td>
</tr>
<tr>
<td>Sealed, 6 d</td>
<td>30.93 ± 0.57</td>
<td>1700.83 ± 92.41</td>
</tr>
<tr>
<td>Sealed, 3 d → aerobic</td>
<td>17.60 ± 1.06</td>
<td>246.16 ± 7.95</td>
</tr>
<tr>
<td>Sealed + C₅H₄</td>
<td>47.79 ± 1.08</td>
<td>2774.56 ± 31.05</td>
</tr>
</tbody>
</table>

Means and SE.

Table II. Effects of 2,5-Norbornadiene (NBD) on Polyamine Biosynthesis and Coleoptile Elongation of Etiolated Rice (O. sativa L. cv Taichung Native 1) Seedlings in Sealed Conditions

NBD was injected into sealed flasks at the beginning of sealed-condition treatment and sampled after 60 h. Ethylene was also injected into the sealed flask, and the ethylene concentration in a flask was 1 μL/L at the beginning.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Coleoptile Length (mm)</th>
<th>Polyamine Content (nmol/g fresh weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>18.21 ± 1.03</td>
<td>Putrescine 1100.93 ± 17.69 Spermidine 139.62 ± 10.70 Spermine 13.65 ± 1.09</td>
</tr>
<tr>
<td>NBD</td>
<td>24.49 ± 0.72</td>
<td>1865.90 ± 23.40</td>
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<tr>
<td>NBD, 2000 μL/L</td>
<td>16.17 ± 0.39</td>
<td>242.74 ± 3.65</td>
</tr>
<tr>
<td>NBD, 4000 μL/L</td>
<td>15.71 ± 0.59</td>
<td>143.50 ± 7.81</td>
</tr>
<tr>
<td>C₅H₄ + NBD, 2000 μL/L</td>
<td>23.05 ± 0.94</td>
<td>26.70 ± 0.37</td>
</tr>
<tr>
<td>C₅H₄ + NBD, 4000 μL/L</td>
<td>17.29 ± 1.02</td>
<td>10.33 ± 2.75</td>
</tr>
</tbody>
</table>

Means and SE.
Figure 3. Changes of ethylene production rate (A) and coleoptile length (B) of etiolated rice seedlings in response to exogenous ACC in aerobic conditions. ▲, 10^{-3} M ACC; ■, 10^{-4} M ACC; ●, 10^{-5} M ACC; □, control.

Figure 4. Effects of exogenously applied ACC on polyamine contents of etiolated rice coleoptiles in aerobic conditions. PUT, putrescine; SPD, spermidine; SPM, spermine. ▲, 10^{-3} M ACC; ■, 10^{-4} M ACC; ●, 10^{-5} M ACC; □, -ACC.

Activities of Polyamine Biosynthetic Enzymes in Ethylene-Treated Coleoptiles under Aerobic Conditions

The involvement of polyamine biosynthetic enzymes, ADC, ODC, and SAMDC, in ethylene-stimulated polyamine accumulation was assayed in ethylene-treated coleoptiles under aerobic conditions. In response to ethylene, a rapid in-
crease of ADC activity above the aerobic-grown controls was observed in the initial 12 h and reached a maximum after 48 h that was 3.5-fold above the controls (Fig. 5). In contrast, ODC activity in ethylene-treated coleoptiles was slightly inhibited during the early periods (Fig. 5). SAMDC activity in the controls showed a slight decline with the increase of growth time (Fig. 5). SAMDC activity was enhanced by exposure to ethylene and reached a maximal level 3-fold higher than the controls after 24 h. SAMDC activity decreased to the control levels after 48 h (Fig. 5).

**Possible Mediation of Putrescine in the Ethylene-Enhanced Elongation of Rice Coleoptiles**

Because the above results implied that the enhancement of putrescine biosynthesis may be involved in ethylene-enhanced coleoptile elongation of rice seedlings, putrescine was applied in aerobic conditions to etiolated rice seedlings. However, putrescine at concentrations from 10^{-3} to 10^{-6} M had little effect on coleoptile elongation.

Another way to question whether or not putrescine acts as a mediator of ethylene-enhanced rice coleoptile elongation is to use inhibitors of ADC and ODC, DFMA and DFMO, respectively. After 60 h, DFMA at 5 \times 10^{-4} M reduced or prevented the ethylene-induced increase of putrescine and actually reduced it about 10-fold compared to ethylene treatment alone (Table IV). Similarly, DFMA also inhibited the ethylene-enhanced elongation of coleoptiles. There was no effect of 5 \times 10^{-4} M DFMO on inhibition of ethylene-enhanced coleoptile elongation and putrescine accumulation (Table IV).

**DISCUSSION**

The enhancement of free putrescine biosynthesis by ethylene in rice coleoptiles is supported by the following evidence: (a) the parallelism between the increased tissue putrescine concentrations and the accumulation of ethylene in sealed containers; (b) the induction of a large increase in putrescine concentration by ethylene applied in sealed containers; (c) the inhibition of ethylene-enhanced putrescine accumulation by 2,5-norbornadiene, a specific inhibitor of ethylene action (23); (d) a similar putrescine accumulation pattern in response to exogenously applied ethylene and ACC.
in aerobic conditions; and (e) a reduction of ethylene-enhanced putrescine accumulation by DFMA, a specific inhibitor of ADC. On exposure to ethylene, the metabolism of putrescine and spermidine/spermine was greatly different. First, putrescine showed a greater accumulation than spermidine/spermine in ethylene-treated coleoptiles (Fig. 4; Table III). Second, the putrescine showed a continuous increase after ethylene treatment, whereas the spermidine/spermine concentrations, although higher than the controls, decreased as controls did. These findings contrast with the results from the deepwater rice system (3), where significant accumulation of spermidine was observed in response to ethylene, but putrescine showed only a relatively small increase. The observation that in etiolated pea seedlings polyamine biosynthesis is inhibited by ethylene shows even more of a contrast (1). Moreover, ethylene inhibits growth in pea, whereas it promotes growth in both types of rice. Apparently, the effect of ethylene on polyamine metabolism depends on the plant system.

In plants, putrescine can be synthesized by either the ADC- or ODC-mediated pathway (5). In general, ADC links to stress environments, whereas ODC is likely related to cell division (5, 8, 9). In this study, ADC activity, but not ODC activity, was observed to increase in ethylene-treated rice coleoptiles. The earlier increase in ADC activity rather than putrescine accumulation supports the suggestion that ethylene-induced putrescine accumulation in rice seedlings results from synthesis through the ADC pathway. The reduction of ethylene-induced putrescine accumulation by DFMA application, but not by DFMO, provides further evidence to support the view. Similarly, ADC, but not ODC, mediates putrescine accumulation in deepwater rice stems in response to ethylene (3). Because ADC activity in ethylene-treated coleoptiles decreased gradually after 48 h, factors other than ADC are apparently involved in the maintenance of higher putrescine concentrations during later periods of ethylene treatment used here. Possibly, the significant decrease of SAMDC activity after 24 h reduces the conversion of putrescine to spermidine/spermine, resulting in putrescine accumulation.

The immediate increase of SAMDC activity following ethylene treatment (Fig. 5) could increase spermidine/spermine concentration; however, the relative small increases in free spermidine/spermine concentrations observed (Table III) are not consistent with the large-scale increase in SAMDC activity. Therefore, the free spermidine/spermine resulting from the increased SAMDC activity may be converted to a conjugated form or be oxidized. More experiments are needed to clarify the role of SAMDC activity in spermidine/spermine metabolism in ethylene-treated coleoptiles.

It has been established that ethylene can accelerate coleoptile elongation of etiolated rice seedlings (16, 18, 23). The present results from both sealed and aerobic conditions also support this finding. In sealed containers, the accumulation of ethylene paralled the increase in the elongation rate of coleoptiles. As in another report (23), the inhibition of the ethylene-enhanced coleoptile elongation by the specific inhibitor of ethylene action, 2,5-norbornadiene, indicates that ethylene plays a role in the stimulation of coleoptile elongation of etiolated rice seedlings. This conclusion is further supported by the stimulation of coleoptile elongation by ethylene and ACC in aerobic conditions. Moreover, coleoptile elongation rate was parallel with the ACC-induced ethylene evolution.

We propose that the significant accumulation of free putrescine may mediate ethylene-induced coleoptile elongation. The parallelism between the elongation rate (Fig. 1A) and the relative accumulated putrescine level (Fig. 2A) in ethylene-treated coleoptiles supports this proposal. The inhibition of both the ethylene-induced putrescine increase and coleoptile elongation by DFMA provides further evidence. It has been suggested that putrescine is closely correlated with cell elongation, whereas the spermidine and spermine are associated with cell division (5, 8, 9). The high putrescine concentration or the high putrescine/spermidine ratio is required for cell elongation (25). Other results also indicate that putrescine plays an important role in coleoptile elongation in anaerobic-grown rice seedlings (19–21). The ethylene-induced accumulation of polyamines in deepwater rice stems is suggested to be involved in the enhancement of cell elongation and

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Coleoptile Length</th>
<th>Polyamine Content</th>
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<tbody>
<tr>
<td></td>
<td>mm</td>
<td>Putrescine</td>
</tr>
<tr>
<td>Control</td>
<td>12.67 ± 0.39a</td>
<td>246.53 ± 10.78</td>
</tr>
<tr>
<td>C3H8</td>
<td>18.95 ± 1.19</td>
<td>1102.39 ± 20.73</td>
</tr>
<tr>
<td>DFMA</td>
<td>9.42 ± 0.50</td>
<td>80.92 ± 5.36</td>
</tr>
<tr>
<td>DFMO</td>
<td>13.59 ± 0.81</td>
<td>284.21 ± 2.98</td>
</tr>
<tr>
<td>DFMA + C3H8</td>
<td>10.08 ± 0.21</td>
<td>109.58 ± 8.67</td>
</tr>
<tr>
<td>DFMO + C3H8</td>
<td>19.01 ± 0.52</td>
<td>1312.88 ± 38.77</td>
</tr>
</tbody>
</table>

Table IV. Effects of the Specific Inhibitors DFMA and DFMO on the Ethylene-Induced Coleoptile Elongation and Polyamine Accumulation of Rice (O. sativa L. cv Taichung Native 1) in Aerobic Conditions

The etiolated rice seedlings with 5-mm length shoots were used. All the treatments were conducted in 10−4 M Mes buffer. After 60 h of treatment, coleoptile length and polyamine contents were determined. The concentration of DFMA and DFMO was 5 × 10−4 M. The ethylene concentration was 1 μL/L. The samples in this work were replicated 10 times.
enhance division (3). However, in the present study, the failure to enhance coleoptile elongation with applied putrescine in aerobic conditions demonstrates that exogenous putrescine in aerobic conditions cannot replace ethylene. One possibility is that limited uptake of putrescine into coleoptiles or its compartmentation into nontarget sites limits its availability to active sites. Galston and Kaur-Sawhney (10) mentioned that putrescine has no effect on aerobic elongation of rice coleoptiles; however, Reggiani et al. (19) reported that putrescine does stimulate elongation in anaerobic conditions. A reason for this distinctly different effect of putrescine on rice coleoptile elongation between aerobic and anaerobic conditions is not apparent. It is possible that putrescine applied in aerobic conditions may be oxidized to ineffective metabolites or interact with other unknown factors.

Because free polyamines are the form suggested to be active in the regulation of plant physiological processes (26), only free polyamines were analyzed in this study. There is, however, growing evidence that conjugated polyamines may influence physiological processes (5). Accordingly, changes of conjugated polyamine content in ethylene-treated coleoptiles are now under investigation.

ACKNOWLEDGMENTS

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LITERATURE CITED