Stress-induced Ethylene Production in the Ethylene-requiring Tomato Mutant Diageotropica

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ABSTRACT
Ethylene synthesis in vegetative tissues is thought to be controlled by indole-3-acetic acid (IAA). However, ethylene synthesis in the diageotropica (dgt) mutant of tomato (Lycopersicon esculentum Mill.) was much less sensitive to IAA than in the normal variety (VFN8). Yet, mechanical wounding stimulated ethylene production by the mutant. The dgt tomato provides an opportunity to study the regulation of stress ethylene independent of IAA effects. Waterlogging (i.e. anaerobic stress) stimulated production of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), in the roots. The ACC was transported to the shoot where it was converted to ethylene. The dgt mutant efficiently utilized ACC for ethylene synthesis under aerobic conditions. The results confirm that the genetic lesion in dgt is located at a step prior to the formation of ACC. Furthermore, induction of ethylene synthesis by anaerobic or mechanical stresses in this mutant is independent of IAA action.

MATERIALS AND METHODS
Seeds of both the dgt and VFN8 isolines of tomato (Lycopersicon esculentum Mill.) were obtained from Dr. C. M. Rick of the University of California at Davis. Plants from the original seeds were grown in a greenhouse and the selfed progeny seeds were used in these experiments. The dgt plants displayed the characteristic phenotypic syndrome described by Zobel (15).

The growth conditions, waterlogging treatments, ethylene measurements, xylem sap collection, and ACC feeding, identification, and assay techniques have been described in detail previously (3). Briefly, plants of both dgt and VFN8 were grown for 5 weeks in a growth chamber for waterlogging studies, or in a greenhouse for ACC or IAA feeding experiments. Ethylene production was estimated by sealing excised petioles in test tubes and periodically sampling the gas phase (5). Data are expressed on a fresh weight basis. Petiole angles were measured with a transparent protractor. Xylem sap from detopped root systems was collected for 3 h under a vacuum (50 mm Hg). ACC was assayed by the method of Lizada and Yang (8). ACC or IAA was fed through the transpiration stream to shoot cuttings.

VFN8 and dgt plants were generally compared in the same factorial experiment, allowing single degree of freedom tests in the analysis of variance. Since ACC levels in xylem sap of control plants were generally below detection, error bars indicating ± 1 SE have been used on ACC curves for flooded plants to give an estimate of experimental error.

RESULTS
The responses of dgt and VFN8 tomato plants to 48-h flooding were qualitatively identical (Table 1). In both varieties, epinasty, ethylene production, and ACC export from the root increased dramatically due to flooding. The appearance of ACC in the xylem sap preceded the increase in ethylene synthesis (Fig. 1). Development of epinasty in flooded plants showed a time course similar to that for ethylene production (data not shown). When dgt plants were flooded for 30 h and then drained, ACC export from the root system fell from 0.75 nmol h⁻¹ to below detection (<0.02 nmol h⁻¹) within 6 h (Fig. 2). Petiolar ethylene production also fell rapidly and eventually returned to the control rate (Fig. 2). The time courses of ACC transport and ethylene production in VFN8 were virtually identical to those of dgt shown in Figures 1 and 2 (3).

Evidence for a quantitative difference in ethylene production between the two isolines is shown in Figure 3. In this case, ethylene production by petioles from control and flooded plants was measured for 3.5 h following excision. Both VFN8 and dgt showed a typical increase in ethylene synthesis due to excision (5, 10). However, the magnitude of the increase is much greater in dgt than in VFN8. Flooding and wounding appear to have additive effects on ethylene production between 0.5 to 1.5 h following excision. Subsequently, the production rates for both flooded and

1 This work was supported in part by National Science Foundation Grant PCM 78-98278.
2 Abbreviations: ACC: 1-aminocyclopropane-1-carboxylic acid; SAM: S-adenosylmethionine.
Table 1. Effects of 48-h Flooding on Epinasty, C2H4 Production, and ACC Flux in VFN8 and dgt Tomato Plants

Epinasty indicates the increase in petiole angle of the third leaf from zero time. Ethylene production refers to the rate by petioles during the first 30 min following excision. ACC refers to the concentration of the compound found in the xylem sap. ACC flux is calculated from the concentration in the sap times the exudation rate. Values are means of four plants per treatment.

<table>
<thead>
<tr>
<th>Variety</th>
<th>Treatment</th>
<th>Epinasty</th>
<th>C2H4</th>
<th>ACC</th>
<th>ACC Flux</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>degrees</td>
<td>nl g⁻¹ h⁻¹</td>
<td>μm</td>
<td>nmmol h⁻¹</td>
<td></td>
</tr>
<tr>
<td>VFN8</td>
<td>Control</td>
<td>6</td>
<td>0.17</td>
<td>&lt; 0.02</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td></td>
<td>Flooded</td>
<td>45***</td>
<td>0.89*</td>
<td>2.3***</td>
<td>1.8***</td>
</tr>
<tr>
<td>dgt</td>
<td>Control</td>
<td>6</td>
<td>0.27</td>
<td>&lt; 0.02</td>
<td>&lt; 0.02</td>
</tr>
<tr>
<td></td>
<td>Flooded</td>
<td>32***</td>
<td>1.19**</td>
<td>3.3***</td>
<td>0.8***</td>
</tr>
</tbody>
</table>

* Difference from control significant at P < 0.05; ** difference from control significant at P < 0.01; *** difference from control significant at P < 0.001.

control petioles fall rapidly. Differences in ethylene production rates between VFN8 and dgt were apparent in shoot cuttings fed ACC through the transpiration stream (Fig. 4). Mutant tissues were more efficient in converting ACC to ethylene, as evidenced by the significantly different slopes in the dose-response curves of the two varieties. Similar results are derived from flooding experiments (e.g. Fig. 1) in which dgt produced as much ethylene as VFN8, although the ACC flux in dgt was smaller than that in VFN8.

When IAA was fed through the transpiration stream, VFN8 cuttings became severely epinastic and ethylene production increased within 3 h (Table II). Measurements were made at a time when epinastic growth was rapidly occurring. Concentrations of IAA up to 10-fold higher had only a slight effect on either parameter in dgt (Table II). In VFN8, petioles fed higher concentrations of IAA (50 or 100 μM) continued to produce ethylene at high rates for several hours following excision (Fig. 5). In contrast, only the highest IAA concentration (100 μM) caused an increase in ethylene production by dgt, which soon returned to the control rate (Fig. 5). ACC, on the other hand, greatly stimulated ethylene production in dgt, but the rates of synthesis declined rapidly following excision of the petioles (Fig. 6). Similar time courses were observed in experiments with VFN8 (3), but the absolute rates were higher in dgt (e.g. Fig. 4). These results emphasize the substrate role of ACC as compared to the presumed enzyme induction caused by IAA (11, 12).

**DISCUSSION**

It is now clear that the primary effect of waterlogging is to create anaerobic conditions in the root zone (2, 6). Since the conversion of ACC to ethylene requires O2 (1), ACC accumulates in anaerobic roots and is transported to the shoot (ref. 3; Figs. 1 and 2). Inasmuch as ethylene production by roots is equal to or only slightly greater than that of shoots (2), merely blocking the normal root ethylene synthesis from ACC could not account for the large increase in shoot ethylene production induced by flooding (Fig. 1 and Table I). Kawase (7) also reported a stimulation of ethylene production by anaerobic stress in sunflower stems. Saltveit and Dilley (9) showed that an anaerobic stress would induce...
Fig. 4. Stimulation of ethylene production by ACC supplied through the transpiration stream in VFN8 and dgf. Following a 6-h uptake period, petioles were excised for measurement of ethylene production rates. Data represent ethylene synthesis rates by petioles during the first 30 min following excision. Differences in uptake of the solutions between varieties were not significant. Linear trends with increasing ACC concentration were highly significant for both varieties. Differences in response between varieties is significant at $P < 0.005$.

Table II. Effects of IAA on Epinasty and $C_2H_4$ Production in VFN8 and dgf Tomato Plants

IAA solutions were supplied through the transpiration stream to shoot cuttings. At the end of 3-h feeding period, epinasty and ethylene production rates were measured. Epinasty indicates the increase in petiole angle from zero time. Ethylene production refers to the rate by petioles during the first 30 min following excision. Values are means of three plants per treatment.

<table>
<thead>
<tr>
<th>Variety</th>
<th>IAA (μM)</th>
<th>Epinasty (degrees)</th>
<th>$C_2H_4$ (nl g$^{-1}$ h$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFN8</td>
<td>0</td>
<td>-1</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>28***</td>
<td>1.66*</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>28***</td>
<td>4.22***</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>32***</td>
<td>4.22***</td>
</tr>
<tr>
<td>dgf</td>
<td>0</td>
<td>-1</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>8</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>6</td>
<td>1.16*</td>
</tr>
</tbody>
</table>

* Difference from control significant at $P < 0.05$; *** difference from control significant at $P < 0.001$.

Fig. 5. Time course of ethylene synthesis by petioles excised from shoot cuttings which had been supplied with ACC for 3 h through the transpiration stream. Statistical significance is shown in Table II.

Fig. 6. Time course of ethylene synthesis by petioles excised from shoot cuttings which had been supplied with ACC for 6 h through the transpiration stream. Linear trend in ethylene production with ACC concentration for 30 min data significant at $P < 0.001$.

A rise in ethylene production similar to that which followed physical wounding of pea epicotyls. Both wounding and anaerobiosis increase ethylene synthesis in excised tomato roots (6). In mung bean hypocotyls, the ethylene-synthesizing system could be induced by IAA if the O$_2$ concentration was above 1%, but ethylene production was only half-maximal at 8–9% O$_2$ (4). Because IAA induces ethylene production by stimulating the synthesis of ACC (12), these data are interpreted to suggest that ACC synthesis can occur at low levels of O$_2$ which are inhibitory to the subsequent conversion to ethylene. Thus, anaerobic stress during waterlogging induces an increase in ACC synthesis in the roots analogous to the effect of other stresses caused by mechanical wounding of citrus peels, by slicing of preclimacteric avocado fruit, and by chemical injury of mung bean hypocotyls with Cu$^{2+}$ (Y. Yu and N. E. Hoffman, unpublished). The additive effect shown in Figure 3 could be due to induction of a wound response in the petioles by excision in addition to the supply of ACC from the anaerobic roots. Although $dgf$ is relatively insensitive to IAA with respect to ethylene production (Fig. 5), the mutant is capable of efficiently converting ACC to ethylene (Fig. 6). In fact, it is more efficient in this respect than is VFN8 (Fig. 4). Thus, IAA must exert its stimulatory effect on ethylene production at a biosynthetic step prior to the conversion of ACC to ethylene. This supports the scheme of Yu et al. (11, 12), who concluded that IAA stimulated ethylene production by inducing the synthesis of the enzyme involved in the conversion of SAM to ACC. The biochemical basis for this varietal difference with respect to IAA effects on ethylene synthesis is unknown.

Although the biosynthetic pathway of stress ethylene is thought to be identical to that of normal ethylene, little is known about the regulation of stress ethylene synthesis (10). Recent results obtained in this laboratory (Y. Yu and N. E. Hoffman, unpublished) indicate that the step in the sequence of ethylene biosynthesis which is stimulated by mechanical wounding and chemical injury is the conversion of SAM to ACC, the same step at which IAA exerts its effect. In $dgf$, woundng or anaerobiosis, but not IAA, fulfills this function. Since active enzyme can be synthesized in response to stress, the lesion may be in a regulatory region of the DNA. Alternatively, a defective IAA receptor site might also
be responsible. While further work is required to distinguish between these alternatives, the VFN8-dgt isolines provide excellent materials for studies of the regulation of IAA- and stress-induced ethylene synthesis.

Note Added in Proof. After submission of this paper, M. B. Jackson (1979. Physiol Plant 46:347–351) also reported increased ethylene production and reversion to upright growth in dgt tomato plants during waterlogging.

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LITERATURE CITED