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 indoleacetic acid (IAA). However, ethylene synthesis in the diageotropica (dgt) mutant of tomato (Lycopersicon esculentum Mill.) was much less sensitive to IAA than is 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.

The diageotropica (dgt) mutant of tomato is characterized by its diageotropic habit in both shoots and roots, dark green hypostomatic leaves, and lack of lateral roots. These phenotypic characters are pleiotropic effects of a single gene mutation in the parent variety VFN8 (13). Zobel (14) reported that ethylene concentrations as low as 5 nM l⁻¹ would completely normalize the mutant. IAA would also restore the normal morphology, but dgt was 10 times less sensitive to the auxin than was VFN8. Zobel (14) suggested that the aberrant phenotype of dgt might be due to a reduction in auxin-induced ethylene synthesis. When wounded by excision, tissues of both dgt and VFN8 produced a burst of "stress ethylene." These observations suggest that IAA is not involved in the production of wound ethylene.

Waterlogging is also known to increase ethylene synthesis in tomato (2, 6). It has recently been demonstrated that the stimulation of ethylene synthesis in waterlogged plants is due to export of an ethylene precursor, ACC, from anaerobic roots (3). Anaerobic stress accelerates synthesis and accumulation of ACC in the roots, and ACC is transported in the xylem to the shoot where it is rapidly converted to ethylene. It has been proposed that IAA induces the synthesis of the enzyme which converts SAM to ACC in mung bean hypocotyls (11, 12). Little is known, however, about the regulation of stress ethylene synthesis (10).

In this paper, ethylene production rates in response to waterlogging, wounding, ACC, and IAA treatments by dgt and VFN8 tomato plants are compared.

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 greenhouse or for 4 weeks in the field. 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 detached root systems was collected for 3 h under a vacuum (30 mm Hg). ACC was assayed by the method of Lizada and Yang (8). IAA or ACC 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). Petiole ethylene production also fell rapidly and eventually returned to the control value (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...
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>nmol 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
wounding of waterlogging induces (Y. fruit, analogous to an IAAnote, conversion by induced the effect of other varieties with increasing ACC concentration were highly significant for both varieties. Difference in response between varieties is significant at P < 0.005.

Table II. Effects of IAA on Epinasty and C2H4 Production in VFN8 and dgt Tomato Plants

<table>
<thead>
<tr>
<th>Variety</th>
<th>IAA (μM)</th>
<th>Epinasty</th>
<th>C2H4 (nl g⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VFN8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>0.29</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>28***</td>
<td>1.66*</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>28***</td>
<td>4.22***</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>32***</td>
<td>4.22***</td>
</tr>
<tr>
<td>dgt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>-1</td>
<td>0.47</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>1</td>
<td>0.49</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>8</td>
<td>0.56</td>
</tr>
<tr>
<td>100</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.

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 O₂ concentration was above 1%, but ethylene production was only half-maximal at 8–9% O₂ (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 O₂ which are inhibitory to the subsequent conversion to ethylene. Thus, anaerobic stress during waterlogging induces an increase in ACC synthesis in the rusts 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 Cu²⁺ (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 dgt 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 dgt, wounding 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

![Figure 4](image-url)  
**Fig. 4.** Stimulation of ethylene production by ACC supplied through the transpiration stream in VFN8 and dgt. 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. Difference in response between varieties is significant at P < 0.005.

![Figure 5](image-url)  
**Fig. 5.** Time course of ethylene synthesis by petioles excised from shoot cuttings which had been supplied with IAA for 3 h through the transpiration stream. Statistical significance is shown in Table II.

![Figure 6](image-url)  
**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.
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