Communication

Effects of Wounding on Cytokinin Activity in Cucumber Cotyledons

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ABSTRACT

Three known physiological responses to exogenous cytokinins were measured in wounded and nonwounded cotyledons from cucumber (Cucumis sativus L. cv Marketer) seedlings grown in darkness. Enhanced cell division, chlorophyll formation, and cotyledon expansion were detected in wounded cotyledons. The data suggest that wounding enhances endogenous cytokinin activity.

Work in this laboratory showed that growth of cotyledons excised from dark-grown cucumber seedlings is promoted by various wounding techniques, including rubbing, abrasion with carborundum, or cutting (11). Similar growth promotion of cotyledons excised from seedlings of cucumber and numerous other dicots is induced by various cytokinins (13), so we suspected that wounding might act by increasing the endogenous level of active cytokinins. In support of this hypothesis, Mitchell and Van Staden (9) found that wounded potato tubers produced increased amounts of cytokinins chromatographically similar or identical to zeatin and zeatin riboside; they concluded that cytokinins were important for enhanced cell division accompanying wound-induced periderm formation in potato tubers. More recently, Giridhar and Thimann (5) found that various wounding techniques delay senescence in excised oat leaves; delayed senescence of excised leaves of various species after addition of cytokinins is a well-known response. Giridhar and Thimann (5) also observed that kinetin was almost ineffective in further delaying senescence of oat leaves that were already wounded, indicating that wounding nearly satisfies the requirement for an exogenous cytokinin. Finally, data of Ross et al. (11) showed that the cytokinin zeatin promotes growth of wounded cotyledons less than of nonwounded cotyledons, again suggesting that wounding satisfies much of the requirement for an exogenous cytokinin. Wound-induced ethylene production and certain other physiological effects of wounding seem unable to account for enhanced cotyledon growth (11).

This report evaluates with three internal bioassays (involving the same species and no tissue extraction) the hypothesis that wounding increases the level of physiologically active cytokinins in wounded cucumber cotyledons. The bioassays studied, all of which seem quite specific to cytokinins, were growth (3, 8, 10), Chl formation (4), and cell division (6, 8). All results are consistent with the hypothesis, but interpretations still suffer from those of most bioassays.

MATERIALS AND METHODS

Plant Material. Cucumber (Cucumis sativus L. cv Marketer) seeds were surface sterilized by quickly rinsing in 1% (w/v) NaOCl, followed by several rinses in distilled H2O. Seeds were germinated and grown 5 d over wet paper towels in darkness at 27°C (11). All studies were done with cotyledons excised from 5-d-old seedlings under a dim green safelight. Ten cotyledons were cultured adaxial side down at 27°C on a layer of Whatman No. 1 filter paper wetted with 20 mM KCl and held in a 9-cm Petri dish (minimum of three Petri dishes per treatment).

Fig. 1. Simultaneous comparisons of the effects of wounding and zeatin on cotyledon expansion and Chl formation in excised cucumber cotyledons grown in the dark. Cotyledons were treated for a 16 h dark, 4 h light period. a, Growth, as measured by increased fresh weight. b, Chl formation. Data shown are means from one experiment, repeated three times with similar results. Vertical bars represent standard deviations.
Table 1. Promotion of Cell Division in Cucumber Cotyledons by Wounding and Zeatin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Cells mean cot⁻¹ × 10⁻⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.97a</td>
</tr>
<tr>
<td>Cut</td>
<td>2.45b</td>
</tr>
<tr>
<td>Abraded</td>
<td>2.57b</td>
</tr>
<tr>
<td>Zeatin (56 μM)</td>
<td>2.73b</td>
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</tbody>
</table>

Method of Wounding. Wounding was performed under a dim green safelight. Usually, excised cotyledons were placed in Petri dishes and cut with a surgical scalp knife into six pieces by making one cut lengthwise and two cuts widthwise. For experiments in which abrasion effects were studied, cotyledons were rubbed with a moist slurry of carborundum (300 mesh, Fisher Scientific) five times between thumb and finger.

Growth Measurements. The average fresh weight of 20 newly excised cotyledons was determined at the beginning of each experiment. Experimental cotyledons were then grown in 2.8 ml of 20 mM KCl solution with or without zeatin for a 16 h dark, 4 h light period, blotted, and reweighed. Fluorescent light was used at an irradiance level of 140 μmol m⁻² s⁻¹ PAR.

Determination of Chl Content. Excised wounded and non-wounded cotyledons were grown as described above. Chl was extracted by placing each group of 10 cotyledons in 5 ml of DMSO at 65°C for 1 h (7). The absorbance of the cooled extract was measured with a Beckman DB-G spectrophotometer at 652 nm. An absorptivity of 36 mg ml⁻¹ cm⁻¹ was used to calculate the sum of Chl a and b.

Estimation of Cell Division. Nonwounded, cut, abraded, and zeatin-treated cotyledons were grown 3 d in 3.2 ml of 20 mM KCl in darkness at 27°C. Each group of 10 cotyledons was blotted and weighed, then shaken in 40 ml of 5% (w/v) Cr₂O₃ at 38°C at 55 oscillations min⁻¹ for 24 h to soften the tissues. Cell separation was accomplished by rapidly stirring the mixture with a magnetic stir bar for 20 to 30 min. Cell counts were performed using a haemocytometer and microscope.

RESULTS AND DISCUSSION

The results of several experiments showed that wounding enhances cotyledon expansion under the conditions tested. In three experiments the average fresh weight increase of control, nonwounded cotyledons was 3.8 ± 0.2 SD mg per cotyledon after 20 h of treatment. Wounded cotyledons had an average fresh weight increase of 8.4 ± 0.2 SD mg per cotyledon during the same period, so wounding increased cotyledon expansion more than 100% above that of nonwounded cotyledons.

Wounding also enhanced Chl formation. Means of three experiments showed nonwounded cotyledons grown for a 16 h dark, 4 h light period contained 1.6 ± 0.4 SD μg of Chl per cotyledon, wounded cotyledons treated for the same period averaged 2.8 ± 0.3 SD μg of Chl. Thus, wounding caused a Chl increase of 75% above that of nonwounded cotyledons. Effects of a series of zeatin concentrations (0.1, 1.0, and 10.0 μM) were studied to determine the concentration that gives effects similar to wounding for enhancement of Chl formation and cotyledon growth. Representative data from one of four experiments are shown in Figure 1. Although it was possible to estimate an equivalent zeatin concentration for each response (Chl formation and growth), the values differed. Wounding resulted in the same amount of Chl formation that occurred in cotyledons treated with approximately 0.1 μM zeatin. Growth of wounded cotyledons was comparable to that of cotyledons treated with approximately 1.0 μM zeatin. The reason for the difference in these results is unclear. Perhaps the experimental conditions were more favorable for growth than Chl formation, or perhaps an additional wound factor suppresses Chl formation (i.e., wounding might produce both a cytokinin and an inhibitor).

We tested the effects of two methods of wounding (cutting and abrasion with carborundum) on cell division and compared them to treatment effects of 56 μM zeatin, a concentration which we had previously determined to induce maximum growth after 3 d in light (CW Ross, unpublished data). The study was set up as a nested design: flasks were nested within treatments, and replicates nested within flasks. Flask within treatment mean square was used as an error term to judge differences between treatments. Table I shows the results of the analysis. The results indicate that wounding caused a significant increase in cell numbers (P = 0.003). No significant differences were detected between the two methods of wounding nor between wounded and zeatin-treated cotyledons.

The three responses caused both by wounding and zeatin suggest that wounded cells either synthesize cytokinins in greater quantities than nonwounded cells or transport them from an inactive to an active location. No other growth regulator is known to cause similar effects in excised (or attached) cotyledons, although certain gibberellins significantly promote growth of excised cotyledons incubated in light (10). If cytokinin synthesis is involved, wounded cotyledons could provide an important system in which to study regulation of cytokinin metabolism. Nevertheless, effects of other wound factors such as trauma (2, 14), protease inhibitor inducing factors (12), and oligosaccharins (1) are worthy of investigation.

LITERATURE CITED

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