Estimation of Osmotic Parameters Accompanying Zeatin-Induced Growth of Detached Cucumber Cotyledons

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ABSTRACT

Water potential (Ψ), the osmotic potential (Ψ₀), and the pressure potential (Ψₛ) of detached cotyledons isolated from Cucumis sativus L. cv Marketer seedlings after 0, 1.5, and 3 days growth with and without zeatin were determined. From zero time to 3 days, cotyledons incubated without exogenous zeatin exhibited a slight decrease in Ψ (from -0.4 to -1.0 bars), while those grown with zeatin developed even more negative values (about -4 bars). Both groups showed rising Ψ₀ values (decreases in solutes per unit volume), but this rise was more dramatic in those treated with zeatin. These data indicate that the capacity of zeatin-treated cotyledons to take up water more rapidly than controls and thus expand faster must be due to wall loosening, as reflected in Ψ, values which declined during 3 days from about +11 bars to about +1.4 bars.

It was also found that freshly detached cotyledons or those grown without exogenous zeatin exhibited osmoregulation in polyethylene glycol (PEG) solutions. That is, while cotyledons initially lost H₂O into certain PEG solutions, their Ψ values decreased over time and they began absorbing water after 1 to 4 hours. After 3 days growth, zeatin-treated cotyledons had lost most of this capacity of osmoregulate. It seems likely that osmoregulation in cotyledons not treated with zeatin is due to wall loosening rather than changes in Ψ₀. Zeatin-treated cotyledons with already loosened walls may not have this option to deal with water stress and thus simply come to equilibrium with external PEG solutions.

Cytokinins promote growth of detached cotyledons of numerous species in light or darkness. The most intensive investigations of this phenomenon have been performed with radish (1, 3, 6, 13, 18) and cucurbits (4, 8, 18). In these species, dry weight changes with or without cytokinin are negligible during growth periods of 3 d and, at least in radish (1, 3), cytokinesis is increased by cytokinins much less than is H₂O uptake. In each species, cytokinins cause wall loosening that apparently contributes substantially to water uptake and thus growth promotion (16, 18). Nevertheless, cytokinin-enhanced production (6, 8) and absorption (4, 13) of osmotically active solutes have also been implicated in growth promotion.

Our objectives were to determine the water potential (Ψ), osmotic potential (Ψ₀), and pressure potential (Ψₛ) values of detached cucumber cotyledons growing with and without zeatin. Such results were expected to help evaluate the relative importance of wall loosening and solute accumulation to growth promotion by zeatin. Other results suggest that wall loosening represents the major and primary cause of H₂O uptake leading to growth promotion of detached cotyledons (16, 18), but that cytokinin-induced absorption of monovalent salts (4, 13) and production of sugars from macromolecules (6, 8) helps minimize loss of turgor accompanying growth.

MATERIALS AND METHODS

Preparation of Cotyledons. Cucumis sativus L. cv Marketer seeds were germinated and grown 5 d in darkness at 27°C, as described previously (16, 18). Cotyledons were then detached under fluorescent laboratory light and placed on wet paper towels. Fresh weights, after blotting off surface H₂O, averaged 18.8 mg, and dry weights (70°C) averaged 7.2 mg.

Growth Methods. After detachment and measurement of fresh weights, some cotyledons (hereafter called zero-time cotyledons) were immediately utilized for measurements of Ψ and Ψ₀ (see below). Others were transferred to 9-cm Petri dishes and allowed to grow, adaxial surface down, under fluorescent laboratory light for 1.5 or 3 d at 25 to 30°C before Ψ and Ψ₀ measurements. Each Petri dish contained one disc of Whatman No. 1 filter paper and 3.5 ml of 20 mM KC₁₅ dishes for zeatin-treated cotyledons also contained 56 µM zeatin.

Measurements of Ψ and Ψ₀. All Ψ values were determined cryoscopically using an Osmette (Precision Systems, Framingham, MA). Techniques were as described previously (18), and results were corrected to 27°C by use of Van't Hoff's equation.

All Ψ values were estimated by the gravimetric method of Ursprung (see Ref. 9). For zero-time measurements, two or three groups of 20 cotyledons were blotted, weighed, and floated adaxial surface down on PEG 4,000 solutions contained in Petri dishes (9 cm diameter). After incubation for 1 to 5 h at 26 to 30°C under 20 µE m⁻² s⁻¹ of laboratory light, they were removed from covered Petri dishes, blotted carefully, and weighed again. For cotyledons previously grown 1.5 or 3 d with or without zeatin, techniques were identical except that two or three groups of only 10 cotyledons were used to determine average weight changes in PEG solutions. In all cases, Ψ values were estimated by determining the Ψ of PEG solutions in which cotyledons neither gained nor lost weight. The Ψ values of PEG solutions were, in turn, estimated from the closely agreeing standard curves of Nabors (12) and Michel and Kaufman (11), both determined by vapor pressure equilibration methods near 25°C.

RESULTS

Estimates of Ψ. We were initially unsure how long cotyledons should be incubated in PEG before measuring weight changes leading to reliable estimates of Ψ, so we first varied incubation times from 1 to 9 h. Time periods of 1 to 2 h provided data that...
we considered most reliable, so $\Psi$ estimates only from such periods are presented.

Figure 1 illustrates fresh weight changes of freshly detached (zero time) cotyledons incubated in various PEG solutions for 1, 2, or 3 h. Such data provide estimates of $\Psi$ values before growth with or without zeatin and indicate that weight changes of such cotyledons depend both upon PEG concentration and incubation time. Cotyledons incubated in relatively concentrated PEG solutions at first lost more $H_2O$ than in more dilute solutions, yet all cotyledons then absorbed $H_2O$ and gained weight. Henceforth, we refer to such loss followed by reabsorption of $H_2O$ as osmoregulation. Recognizing the importance of osmoregulation, we used the 1-h time point from Figure 1 and estimated the initial $\Psi$ of freshly detached cotyledons to have been near $-0.4$ bar. It should be recognized that this value represents a minimum estimate and that the true value might have been even closer to zero.

Cotyledons grown without zeatin for 3 d also showed osmoregulation in PEG solutions. For these, we estimated $\Psi$ values of about $-1$ bar (Fig. 2A). Cotyledons grown similarly with zeatin consistently showed less ability to osmoregulate, and their apparent $\Psi$ values were more negative than for those grown without zeatin (Fig. 2B). Cotyledons grown with zeatin for 3 d had $\Psi$ values near $-4$ bars.

Estimates of $\Psi$, $\Psi_m$, and $\Psi_e$. Table I summarizes our estimates of these three osmotic properties before and after growth with zeatin, ignoring effects of matric potentials. We conclude that cotyledons grown without zeatin did so at $\Psi$ values that became only slightly more negative with time. As one might expect, the faster growing zeatin-treated cotyledons exhibited more negative $\Psi$ values (near $-4$ bars) than did cotyledons grown without zeatin (near $-1$ bar). In general, $\Psi_e$ values increased toward zero during growth, especially with zeatin. Calculated $\Psi_m$ values thus indicated that zeatin-treated cotyledons grew at substantially less positive $\Psi_e$ values than did cotyledons without zeatin, presumably because of zeatin-induced cell wall loosening (18).

Osmoregulation in Cotyledons not Exposed to Zeatin. We were curious about the apparent ability of zero-time cotyledons and cotyledons grown 3 d without zeatin to osmoregulate in PEG solutions. The ability of such cotyledons to absorb $H_2O$ after initially losing it in PEG solutions must result from more negative $\Psi$ values caused either by more negative $\Psi_e$ values or less positive $\Psi_m$ values, or both. Figure 3 shows that $\Psi_e$ values did not change detectably in zero-time cotyledons incubated 1 to 5 h in two PEG solutions differing by approximately 3 bars, yet after 4 h, cotyledons absorbed $H_2O$ from each solution.

DISCUSSION

First, we address potential errors in our estimated $\Psi$ and $\Psi_e$ values, then we evaluate what our data indicate about cytokinin-induced growth of cotyledons.

The gravimetric method of Ursprung is an apparently theoretically sound technique to measure $\Psi$ values (9), although inflitra-
tion of cell walls of some tissues by relatively small solutes (e.g. sucrose and presumably also mannitol) can make estimates too negative (14). Our first problem with this method was error in measuring small weight changes. Our second problem was osmotic adjustment in PEG solutions. The first problem was partially overcome by replication of treatments within an experiment and by repeating experiments. The second was partially overcome by minimizing the time of incubation in PEG, again with repetition of experiments. Overall, we believe that the gravimetric method suited our needs better than psychrometric techniques. The Wes-cor psychrometer limited sample sizes and required equilibration times so long that osmoregulation would have been difficult to evaluate. Our cryoscopic Ψw measurements are probably also somewhat in error because homogenization of cells in H2O leads to changes in solute activity coefficients (well-known dilution and precipitation problems) and because homogenization allows relatively solute-free H2O of cell walls to dilute cell sap (7). Such problems are also common to psychrometric techniques that presumably always involve disruption of all cell membranes (2).

Realizing that small errors exist in measurements of both Ψ and Ψw, we assume that they were similar for tissues treated with or without zeatin. If so, we can estimate the effect of zeatin on each osmotic property and relate that effect to mechanisms by which cytokinins initiate cotyledon expansion. First, we show that zeatin-treated cotyledons attained Ψ values about 3 bars more negative than did controls. The lower Ψ of zeatin-treated cotyledons, then, is the ultimate cause of more rapid water uptake and hence accelerated growth. Next, we show that during cotyledon expansion the Ψw of both zeatin-treated cotyledons and controls rises toward zero, although that rise is greater in cells exposed to zeatin. Apparently, water uptake exceeds generation of internal solutes or absorption of KCl with or without zeatin. However, because the Ψw of zeatin-treated cotyledons becomes less negative during growth than that of controls, this parameter cannot cause the lower Ψ values of zeatin-treated cotyledons. Instead, cell wall loosening leading to lower values of Ψw must be the ultimate cause of a lower Ψ and thus enhanced water uptake in zeatin-treated tissues. A similar conclusion regarding cytokinins and wall loosening of cotyledons was reached by Thomas et al. (18), but without measurements of Ψw values.

The osmotic adjustment exhibited by non-zeatin-treated cotyledons in PEG solutions is probably important in understanding growth mechanisms in general. Interestingly, Meyer and Wallace (9) found almost no such adjustment differences between potato tuber cylinders incubated 5 or 24 h in sucrose solutions, although considerable adjustment might have occurred before 5 h. Alternatively, such nongrowing cells might not be capable of extensive osmoregulation. Kuzmanoff and Evans (7) found substantial osmoregulation by lentil roots within 20 min in mannitol solutions that also contained nutrient salts. Their data suggest that adjustment can occur both by increases in solutes (absorbed or produced from stored foods) and by wall loosening. Other growing tissues also osmoregulate (5, 10, 15, 17). We found (Fig. 3) that tissue osmotic potentials did not change detectably in PEG, even though the tissue water potential decreased from its original value near −0.4 bar to a value as negative as −3.6 bars. We therefore presume that increased wall loosening, not a decrease in osmotic potential, was largely responsible for the decrease in water potential.

The limited osmoregulatory capacity of cotyledons grown 3 d with zeatin may also relate to wall loosening. Thus, walls of zeatin-treated cotyledons might have already undergone so much loosening during growth that they were incapable of further loosening under osmotic stress and simply approached equilibrium with the surrounding PEG solutions.

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LITERATURE CITED

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Table 1. Estimated Values of Ψ, Ψw, and Ψ0

<table>
<thead>
<tr>
<th>Day</th>
<th>Fresh Wt</th>
<th>Ψ</th>
<th>Ψw</th>
<th>Ψ0</th>
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<tr>
<td>0</td>
<td>18</td>
<td>−0.4</td>
<td>−11.1 (0.33)</td>
<td>+10.7</td>
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<tr>
<td>1.5</td>
<td>28</td>
<td>−0.5</td>
<td>−11.5 (1.1)</td>
<td>+11.0</td>
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<tr>
<td>1.5</td>
<td>48</td>
<td>−4</td>
<td>−7.4 (0.38)</td>
<td>+3.4</td>
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<tr>
<td>3</td>
<td>42</td>
<td>−1</td>
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</tr>
<tr>
<td>3</td>
<td>93</td>
<td>−4</td>
<td>−5.4 (0.1)</td>
<td>+1.4</td>
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