Abscisic Acid Accumulates at Positive Turgor Potential in Excised Soybean Seedling Growing Zones

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ABSTRACT

Abscisic acid (ABA) accumulated in soybean (Glycine max [L.] Merr. cv Williams) hypocotyl elongating regions when seedlings were transferred to low water potential vermiculite (Ψ = -0.3 megapascals) even though positive turgor is retained in this tissue. Accumulation of ABA in growing zones could occur from de novo biosynthesis within this tissue or transport from adjacent nongrowing zones. Both growing and nongrowing hypocotyl and root tissues accumulated significant levels of ABA when excised and dehydrated to reduce turgor. Surprisingly, excised growing zones (which experienced no water loss) also accumulated ABA when incubated in darkness for 4 hours at 100% relative humidity and 29°C. Induction of ABA accumulation in the excised elongating region of the hypocotyl was not caused by disruption of root pressure or wounding. While excision of hypocotyl elongating regions induced ABA accumulation, no change in either extensin or p33 mRNA levels was observed. Accumulation of extensin or p33 mRNA required more severe wounding. This suggests that ABA is not involved in the response of these genes in wounded tissue and that wound signals are not causing ABA accumulation in excised tissue. Accumulation of ABA in excised elongating regions was correlated with growth inhibition and a decline in turgor to the yield threshold (Ψy = 0.37 megapascals; R Matyssek, S Maruyama, JS Boyer [1988] Plant Physiol 86: 1163–1167). Inhibiting hypocotyl growth by transferring seedlings to lower temperatures or light did not cause ABA accumulation. We conclude that induction of ABA accumulation in growing zones is more sensitive to changes in turgor than the induction which occurs in mature tissues.

Water deficit, sufficient to cause leaves to wilt, generally results in increases on the order of 5- to 50-fold in ABA content within a few hours (23). Based on pressure bomb (18) and penetrating and nonpenetrating osmoticia studies (6), it was concluded that turgor was the critical component of cell water relations modulating ABA levels and reduction of turgor potential to zero was the signal which causes ABA accumulation. While loss of turgor and plasmalemma deformation (1) are correlated with stress-induced ABA accumulation, small changes in cell volume and hence in solute potential could also play a role in induced ABA biosynthesis.

ABA accumulation also occurs under conditions where there is no apparent loss of turgor. For example, flooding (10), fruit removal or stem girdling (11, 20), long photoperiod (22), mineral deprivation (13), and chilling (19) have been reported to induce ABA accumulation. In these examples, wilting was either transient or not observed, suggesting that loss of turgor was transient or did not occur. Less ABA accumulated under these conditions compared with wilted leaves. Some evidence for the induction of ABA biosynthesis by wounding has been obtained from studies on the expression of a maize gene (8) and the proteinase inhibitor II gene in potato and tomato (17). In the latter case, it was demonstrated that mechanical wounding (via a hemostat) caused an increase in ABA levels. It was suggested that ABA may be a mediator of systemic wound responses (17).

We have previously reported on changes in ABA content in the hypocotyl elongating region of soybean seedlings transferred to low water potential vermiculite (Ψ = -0.3 MPa) at 100% RH (2, 5) and the effects of exogenous ABA on growth, polysome status, and gene expression (5). All tissues of soybean seedlings accumulated ABA when seedlings were transferred to -0.3 MPa vermiculite (2, 5). In attempting to determine the origin of ABA in these tissues and the capacity of each tissue to synthesize ABA, we observed that significant ABA accumulation occurs when hypocotyl and root growing zones are excised from seedlings at 100% RH. When these regions are excised, growth ceases and turgor declines to the yield threshold (Ψy = 0.37) (12) instead of zero. Because zero turgor is not reached in these tissues, other signals, such as wounding or growth inhibition, may cause the induction of ABA biosynthesis. In the present study we examined some signals which could cause ABA accumulation under these conditions.

MATERIALS AND METHODS

Plant Material

Soybean (Glycine max [L.] Merr. cv Williams; Illinois Foundation Seed, Champaign, IL) seedlings were grown in the dark at 29°C and 100% RH as previously described (2, 5, 12, 14). The following seedling sections were defined: hook and the zone of elongation the first 5 mm and next 15 mm along the hypocotyl below the cotyledon with the mature region of the hypocotyl the remaining portion of the hypocotyl. The root tip was defined as the terminal 15 mm of the primary root. This section contained a number of structures, including the elongating zone of the root. The remaining portion of the root was defined as the mature root. In most experiments, the elongating region of the soybean hypocotyl (or other sections of 2 d old seedlings) was excised, wrapped in plastic wrap, and stored at 100% RH in darkness at 29°C.

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(2, 5). Under these conditions, no change in fresh weight was detected. In experiments using temperature to decrease growth, seedlings were transferred from 28°C to 20°C. All other conditions were identical. In other experiments, the elongating region of the soybean hypocotyl was wounded by slicing it into 1 to 2 mm sections. In root pressure experiments, seedlings were cut at the root/hypocotyl junction under water. The cut end of the hypocotyl remained in water for 1 h, and then the elongating region of the soybean hypocotyl was excised and incubated as described above.

ABA Determination

ABA was extracted as previously described (2, 5). ABA was quantified using gas chromatography-electron capture detection as previously described except that a DB-1 megabore capillary column (0.53 mm diameter, 1.50 µm phase thickness) was used with the oven temperature programmed from 210 to 230°C at 10°C/min and using He as carrier and Ar/CH₄ (95:5) as makeup gas. Data were acquired and analyzed on a IBM XT Model 286 running Axxi-Chrom model 727 software (Axxiom Chromatography, Inc., Calabasas, CA).

Extensin and p33 Gene Expression

An XbaI restriction fragment from pDC5A1 (carrot extensin genomic clone) (4) containing the coding region for a large portion of the protein was subcloned in pBLUESCRIPT SK (Stratagene). Gel purified insert was used to synthesize random primed radiolabeled probe (300 Ci/mmol [³²P]dCTP, random primers method) using a kit (BRL). For p33 gene expression studies, the insert from EcoRI-cut pDC16 (21) was used in a random primers labeling reaction. RNA blots (10 µg/lane total nucleic acid) were prehybridized and hybridized with 26% formamide, 5 × SSC, 1% SDS, 5 × Denhardt's solution, 100 µg/mL salmon sperm DNA at 42°C (21) with 500,000 cpm/mL probe in the hybridization solution. Blots were washed (twice in 2 × SSC, 0.5% SDS followed by twice in 0.5 × SSC, 0.5% SDS at 42°C), dried, and exposed using Kodak XAR film at −80°C (21).

RESULTS AND DISCUSSION

Excision of Seedling Growing Zones Cause ABA Accumulation

Transfer of soybean seedlings to low water potential vermiculite (Ψ = −0.3 MPa) results in ABA accumulation in root and shoot growing zones (2, 5). ABA which accumulates in these tissues could arise either from de novo biosynthesis or import. Previously, increased accumulation of ABA has been correlated with turgor potential dropping to zero (6, 18). However, this mechanism is unlikely to affect ABA levels in soybean growing zones since turgor does not decrease to zero in these regions when seedlings are grown in low water potential vermiculite (14). To understand the origin of ABA in growing zones, we determined the capacity of growing and nongrowing sections to accumulate ABA (Table I). ABA accumulated in all sections which experienced a 10% loss in fresh weight (Table I), indicating that all sections have the capacity to accumulate ABA to levels observed in seedlings grown in low water potential vermiculite.

Surprisingly, excision of soybean seedling growing regions in the absence of water loss also resulted in ABA accumulation (Table I). The amount of ABA in excised nongrowing mature regions increased two- to threefold while ABA levels in growing zones increased up to eightfold (Table I). ABA accumulation in excised hypocotyl elongating regions was similar to increases observed when tissue was dehydrated such that 10% loss of fresh weight occurred. Excised tissues kept at 100% RH experienced no changes in fresh weight (data not shown) suggesting that no water loss occurred and are consistent with earlier studies (12).

Excision of soybean zones of elongation causes immediate growth inhibition (12). Being deprived of their normal sources of water, cell walls relax (12). In soybeans, this relaxation occurs within 6 min, dropping turgor potential to the yield threshold (0.45 to 0.37 MPa) (12). This new turgor potential remained stable for up to 6 h (12). In contrast, when the mature stem regions (tissue in which no expansive growth occurs) were excised, turgor potential did not change. The large ABA accumulation in excised growing regions could result from decreased growth or reduction of turgor to the yield threshold. Alternatively, loss of root pressure, loss of ABA transport, or a wound signal could trigger the accumulation of ABA upon tissue excision.

Root Pressure, ABA Transport, Growth Inhibition, and ABA Accumulation

It is possible that excision of the elongating region of the soybean hypocotyl from the seedling disrupted root pressure, causing ABA to accumulate. To test this possibility, we excised roots from seedlings under water and incubated the seedling with the cut end in distilled water for 1 to 5 h. Under these conditions, turgor does not decline in the elongating region of the soybean hypocotyl (12). Growth continues at rates similar to those before excision (12), and little ABA accumulates (Table II). This suggests that ABA accumulation induced by excision of growing zones is not due to disruption of root pressure.

Levels of ABA in tissues are controlled by biosynthesis, import, and export. High levels of ABA could accumulate in

| Table I. Effect of Excision on ABA Accumulation in Various Sections of the Soybean Seedling |
|-----------------------------------------------|------------------|------------------|------------------|
| Section                                      | ABA Content       |                  |                  |
|                                              | Con 0 h           | Con + 4 h        | Str + 4 h        |
|                                              | µg/g dry weight   |                  |                  |
| Cotyledon                                    | 0.22 ± 0.03       | 0.43 ± 0.08      | 0.79 ± 0.09      |
| Hook                                         | 1.98 ± 1.23       | 6.05 ± 1.17      | 7.02 ± 1.50      |
| Elongating                                   | 1.27 ± 0.48       | 8.34 ± 1.27      | 10.90 ± 2.01     |
| Mature                                       | 1.52 ± 0.52       | 3.27 ± 1.31      | 7.90 ± 0.45      |
| Mature root                                  | 0.43 ± 0.07       | 1.49 ± 0.30      | 4.80 ± 1.65      |
| Root tip                                     | 1.54 ± 0.39       | 10.83 ± 0.59     | 12.31 ± 0.21     |

Data shown are the mean ± SD for 10 seedlings with two replicates per treatment.
excised elongating regions of the hypocotyl if elongating regions have a high rate of ABA biosynthesis and export was blocked by excision. However, this explanation is unlikely because ABA did not accumulate in excised hypocotyl sections incubated in water (Table II) or in incubation medium containing excised elongating regions (data not shown). When the elongating region of the soybean hypocotyl is excised from rapidly growing seedlings, growth immediately is reduced to 5% or less of the initial rate (3, 12). ABA has been postulated to be a mediator of responses to a wide variety of stresses (23). Consequently, conditions where growth is inhibited might activate signals which cause ABA accumulation to occur. To determine if growth inhibition was causing ABA to accumulate, we shifted 2 d old soybean seedlings from 29 to 20°C. This treatment caused a dramatic decrease in growth (Fig. 1), yet ABA content in the zone of elongation was relatively unaffected. The small increase in ABA levels observed was not unexpected. Transplantation shock might be expected to raise ABA content slightly within 4 h, and ABA levels in well watered seedlings increase approximately fourfold within 36 h (2). Etiolated seedlings transferred to light also exhibited growth inhibition without accumulating ABA in the zone of elongation (data not shown). While the mechanisms of growth inhibition caused by excision, shift in temperature or illumination may differ, these results suggest that growth inhibition, per se, is not sufficient to cause the induction of ABA biosynthesis.

### Wounding and ABA Accumulation

There is some evidence that wounding may produce a signal causing induction of ABA biosynthesis (8, 17). In maize, a gene induced by water stress and ABA is also induced (slightly) by wounding (8). It has also been demonstrated that ABA and wounding can stimulate the expression gene encoding proteinase inhibitor II (17). Exogenous ABA induced proteinase inhibitor II in mutant plants deficient in ABA (17). Wounding caused an increase in ABA (17) and resulted in increased expression of the proteinase inhibitor II gene (17). Based on these results, Peña-Cortés et al. (17) suggested that ABA is a mediator of systemic wound responses.

We tested whether the wound signal which modulates gene expression was also involved in ABA accumulation in excised soybean tissue. Production of wound signals was monitored using two wound inducible genes whose expression in soybeans has been characterized, extensin and the proline-rich protein p33 (21) (similar to SbPRP2 [9] and 1A10-1 [7]). Both extensins, a hydroxyproline rich glycoprotein, and p33 have been reported to accumulate to high levels in wounded tissue (21; Fig. 2), similar to that observed with proteinase inhibitor II (17). In excised elongating regions of the hypocotyl, no change in extensin or p33 mRNA levels was observed in treatments where significant ABA accumulation occurred (Fig. 2). However, if the elongating region of the soybean hypocotyl was extensively wounded by slicing the tissue into 1 to 2 mm sections, ABA accumulation declined slightly and extensin and p33 mRNA levels increased dramatically. If a wound signal is inducing ABA biosynthesis, it is either a signal different from the one which modulates gene expression or excision is sufficient for ABA biosynthesis but not for wound mRNA accumulation. However, since excised sections incubated in water (Table II) do not accumulate ABA, excision is not sufficient to cause ABA biosynthesis. These results suggest that ABA is not directly involved in modulating the response of these genes in wounded tissue.

### Turgor and ABA Accumulation

While the turgor potential in excised elongating regions of the soybean hypocotyl does not decline to zero, it does decline to the yield threshold and growth stops (12). Previously, using mature leaves, it has been shown that the induction of ABA

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**Table II. Accumulation of ABA in Excised Elongating Regions of the Soybean Hypocotyl**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ABA Content in Elongating Region</th>
<th>μg/g dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elongating region excised in air</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, 0 h</td>
<td></td>
<td>0.85 ± 0.07</td>
</tr>
<tr>
<td>Excised, control 4 h</td>
<td></td>
<td>6.05 ± 0.52</td>
</tr>
<tr>
<td>Excised, incubate in water 4 h</td>
<td></td>
<td>1.76 ± 0.21</td>
</tr>
<tr>
<td>Exorse, stress, 4 h</td>
<td></td>
<td>10.41 ± 0.08</td>
</tr>
<tr>
<td>Hypocotyl excised at its base, kept in water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td></td>
<td>0.75 ± 0.05</td>
</tr>
<tr>
<td>5 h</td>
<td></td>
<td>1.26 ± 0.22</td>
</tr>
<tr>
<td>Elongating region excised after hypocotyl</td>
<td></td>
<td></td>
</tr>
<tr>
<td>incubated in water 1 h, incubated air 4 h</td>
<td></td>
<td>5.96 ± 0.33</td>
</tr>
</tbody>
</table>

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**Figure 1.** Elongation growth and ABA content of 2 d old soybean seedlings grown at either 29°C (○) or 20°C (●). Values shown are the mean ± s.d for two sets of 10 seedlings.
biosynthesis occurs at zero turgor (6, 18). Since we have been unable to demonstrate that the induction of ABA biosynthesis in excised elongating regions results from loss of root pressure, growth inhibition, or wounding, our results suggest that the induction of ABA accumulation in growing regions is more sensitive to decreases in turgor potential than in mature leaves. That is, turgor potential in mature leaves must drop to zero to induce ABA biosynthesis, while ABA biosynthesis in growing regions begins at a turgor potential between the yield threshold and full turgor. As the turgor declines to the yield threshold within 6 min (12), it would be difficult to determine the actual potential where ABA biosynthesis is induced. It is possible that a few cells in the elongating region of the soybean hypocotyl could decrease to zero turgor after excision, yet it is unlikely that these cells would provide the amount of ABA which accumulates in excised sections.

Previously, it has been observed that ABA accumulation in the elongating region of the hypocotyl induced by transfer of seedlings to −0.3 MPa vermiculite was biphasic (2). A rapid increase was observed within 2 to 4 h, followed by a slow accumulation reaching a maximum 24 h after transfer to low water potential vermiculite (2). In root tips, a maximum ABA accumulation was reached 4 h posttransfer and then declined to a level approximately 4-fold higher than that found in well-watered seedlings (RJ Bensen, JE Mullet, unpublished results). The rapid increases in ABA observed in growing regions immediately after transfer to low water potential vermiculite could represent ABA made in situ in response to turgor dropping to the yield threshold in inner cortical cells (14, 15). After this time, changes in ABA content probably reflect synthesis of ABA and transport from mature portions of the seedling as their turgor potential decreases to zero (14, 16).

**SUMMARY**

In summary, all tissues of the soybean seedling can synthesize ABA and significant ABA accumulation can occur in excised growing regions without turgor dropping to zero. This induction does not appear to be due to disruption of root pressure, growth inhibition, or wounding. In addition, ABA did not modulate extensin or p33 gene mRNA levels in excised tissues, suggesting that ABA is not directly involved in the modulation of these genes in wounded tissues. Instead, increases in ABA in excised elongating regions are correlated with a decrease in turgor potential to the yield threshold. These results suggest that, compared with mature regions, induction of ABA biosynthesis in growing regions is more sensitive to changes in turgor potential. As ABA can modulate growth (6) and gene expression (5), investigations using excised tissues to determine growth parameters should take into account the potential of these tissues to accumulate ABA.

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**LITERATURE CITED**


