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ENDOGENOUS IAA LEVELS IN BORON-DEFICIENT AND
CONTROL ROOT TIPS OF SUNFLOWER

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Boron deficiency in sunflower resulted in abnormal root morphology. Six hours after transfer to boron-deficient nutrient medium, root elongation was inhibited; numerous ultrastructural symptoms, such as altered cell wall deposition and perturbations in the plasma and mitochondrial membranes, were evident. These early responses to boron deficiency are similar to those caused by exogenously supplied optimal indoleacetic acid (IAA). Using a sensitive radioimmunoassay for IAA, we found no significant difference in free IAA between boron-deficient and control root tips after treatment for 6, 24, and 72 h. The growth rate of boron-starved root tips was less than control root tips, but both showed similar levels of endogenous free IAA. Thus, in sunflower, early boron deficiency symptoms cannot be correlated with elevated levels of endogenous IAA.

Introduction

When grown in the absence of the micronutrient element boron, plant roots may show growth abnormalities as early as 3–6 h after boron is withheld. Symptoms of boron deficiency usually include the inhibition of root elongation, a change in the direction of cell expansion from longitudinal to radial, abnormal cell wall deposition, and finally death of the main root tip. Many of the morphological changes caused by boron deprivation are similar to alterations in growth caused by exogenously applied, supraoptimal concentrations of auxin (TORREY 1965) and have led some researchers (BRANDENBURG 1949; NEALES 1960) to propose that the removal of boron from the culture medium leads to increased endogenous levels of indoleacetic acid (IAA) in the plant. BOHNSACK and ALBERT (1977) described an increase in the levels of auxin oxidase in squash roots as early as 6 h after boron was withheld. They hypothesized that elevation of auxin oxidase levels could be attributed to enzyme induction resulting from an increase in IAA caused by the removal of boron. Data by other workers which might have either supported (JAWED and SCOTT 1967; COKE and WHITTINGTON 1968) or questioned (CRISP, COLLIER, and THOMAS 1976; SMIRNOV, KRUPNIKOVA, and SHKOLNIK 1977) the hyperauxin hypothesis failed to resolve the matter.

We reported (HIRSCH and TORREY 1980) that the addition of $5 \times 10^{-6}$ M or $5 \times 10^{-7}$ M IAA to sunflower root tips, while inhibiting root elongation and causing abnormal root-tip swelling, does not elicit the same symptoms visible at the ultrastructural level as does boron deficiency alone. Cell wall thickening is evident in boron-starved sunflower root tips as early as 6 h, whereas roots grown with exogenously supplied IAA have cell walls similar to the controls. In addition, other ultrastructural modifications apparent in boron-deficient root cells—such as loss of mitochondrial membrane integrity, increase in paramural bodies, accumulation of electron-dense deposits within the vacuoles, and loss of polysomes—are not observed in roots exposed to supraoptimal IAA levels. These observations strongly suggest that removal of boron does not act primarily through elevated IAA levels.

Here we provide further support for this conclusion by actual measurement of endogenous IAA content using a specific radioimmunoassay (RIA) for IAA (PENGELLY and MEINS 1977).

Material and methods

Sunflower seeds (Helianthus annuus L. 'Mammoth,' Lot 15349, W. Atlee Burpee Co.) were germinated in perlite and watered with $\frac{1}{2}$-strength Hoagland's solution (HOAGLAND and ARNON 1950). Three days after germination, the seedlings were transferred to aerated $\frac{1}{2}$-strength Hoagland's solution (0.5 ppm B) in plastic pans to acclimate them to water culture. After 48 h, the seedlings were transferred to fresh $+/-(1/2$-strength Hoagland's solutions. Both primary and lateral roots were marked with India ink at 5 mm behind the root apex at the time of transfer, and net root elongation was determined at selected time intervals by measuring the displacement of the India ink mark. Unless roots were obviously damaged by the marking procedure, all marked roots were measured, includ-
ing those growing in boron-deficient medium which did not respond to the lack of boron as quickly as other roots. Using this procedure, we observed a gradual slowing of growth rate (see fig. 1) for \(-B\) roots rather than the very rapid inhibition of root growth reported for squash (Bohnsack and Albert 1977). We took this approach because large numbers of root tips (600–900) were needed for the RIA. Seedlings were maintained in growth chambers with continuous light (mixed cool-white fluorescent and incandescent lights) at a constant temperature of 25°C.

After 6, 24, and 72 h of treatment, the terminal 5-mm tips of the roots were excised and immediately frozen in liquid nitrogen. Between 600 and 900 tips (0.2–0.35 g) were frozen per treatment. The root tips were stored under liquid nitrogen until the analyses were performed. The tissues were extracted in 80% (vol/vol) methanol, and tissue extracts were purified and analyzed for IAA by RIA as described in Pengelly and Torrey (1982).

Fresh weights of roots from various times and treatments were obtained by collecting five to 10 samples of 50 5-mm root tips on a moist Millipore filter and weighing the roots on an analytical balance. To obtain accurate mitotic counts, roots growing in either +B or -B medium for 6, 24, or 72 h were treated during the final 6 or 8 h with 0.02% colchicine before fixing for cytological analysis. After staining with acetoorcein, squashes were made and photographed. The number of cells in mitosis was scored from the photographs and expressed as a percentage of the total number of cells counted.

![Graph showing the increase in length of roots grown in +B and -B nutrient media for 96 h.](image)
Results

Under the conditions of boron deficiency in our experiments, roots failed to elongate at consistent rates so that the range of variation in length was quite large at any particular time point. For this reason, we chose to include all roots marked in an experiment except those which were clearly damaged by the marking procedure. A minimum of 60 roots in each +/−B sample was included at the different time points.

Sunflower roots grown in Hoagland's solution without boron showed a decline in elongation rate as early as 6 h after boron was withheld (fig. 1). In addition, the expected morphological symptoms, such as swelling and browning of root tips and expansion of lateral roots, are obvious in many roots after 24 h of −B conditions and almost universally present in 72 h boron-deficient roots (figs. 2, 3).

Fresh weight determinations show that boron-deficient roots typically weigh slightly more than boron-sufficient roots. However, little difference was observed between +B and −B roots in levels of endogenous IAA at a specific time point except at 24 h where there is a slight increase in endogenous IAA in −B root tips (table 1). At 72 h, both populations again have similar levels.

From 6 to 72 h, there was a slight decrease in endogenous auxin in both +B and −B roots. During this period, the growth rate of +B roots increased while in −B roots, the rate decreased (fig. 1). We studied mitotic activity in the two sets of roots, using the method of colchicine block, to determine to what extent cell division was influenced by the boron treatments and whether this could be correlated with the observed decrease in endogenous IAA. Six hours after transfer to fresh +/−B culture media, ca. 13% of the −B root tips were blocked in mitosis, whereas 16.5% of the control tips were observed. After 72 h of treatment, ca. 17% of theboron-starved cells remained in mitosis after colchicine treatment. Nearly 1.5 X that number (24.4%) were present in control root tips. The mitotic counts appear to correlate with the increase in growth rate in +B roots and with the inhibition of growth in −B roots (fig. 1). The decrease in endogenous IAA may be correlated with a depletion of nutrients other than boron, but we did not pursue this possibility further.

### Table 1

<table>
<thead>
<tr>
<th>Time Period</th>
<th>+B Fresh Weight (ng)</th>
<th>+B IAA Concentration (ng/g)</th>
<th>Mean IAA Concentration (ng/g)</th>
<th>−B Fresh Weight (ng)</th>
<th>−B IAA Concentration (ng/g)</th>
<th>Mean IAA Concentration (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 h</td>
<td>321</td>
<td>74</td>
<td>98</td>
<td>309</td>
<td>78</td>
<td>96</td>
</tr>
<tr>
<td>24 h</td>
<td>278</td>
<td>91</td>
<td>94</td>
<td>284</td>
<td>119</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>92</td>
<td>111</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>72 h</td>
<td>333</td>
<td>103</td>
<td>86</td>
<td>349</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>82</td>
<td>81</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note.—The average fresh weight per root tip is given for each time and treatment.

Figs. 2, 3.—Fig. 2, Sunflower root systems grown in −B and +B nutrient medium for 72 h. Fig. 3, Individual sunflower roots grown in −B and +B nutrient media for 72 h. Lateral roots (arrows) have begun to emerge on −B-grown roots.
Discussion

Our results indicate that, although control and 
—B-grown roots exhibit very different morpholo-
gical features, the levels of endogenous IAA as
determined by RIA are similar. This conclusion is
in direct contradiction to a proposal that boron-
deficiency symptoms are the result of supraoptimal
auxin concentrations (Brandenburg 1949; Neales
1960). Neales (1960) based his hypothesis on earlier
reports in the literature and on the fact that boron-
deficient roots accumulate caffeic and chlorogenic
acid and other flavonoids (Perkins and Aronoff
1956). Exogenously supplied IAA enhances lignifi-
cation and peroxidase activity in isolated pea root
tips (Torrey 1953) and in bean roots (Jensen
1955). Robertson and Loughman (1974) observed
increased peroxidase activity in —B Vicia faba
roots and also noted that both auxin treatment and
boron deficiency had an effect on P32 uptake.

Several reports exist concerning the amounts of
IAA present in boron-deficient tissues. A substance
extracted from boron-deficient bean roots proved to
be more inhibitory in a bean-root bioassay than
extracts from control roots (Coke and Whittington
1968). This substance was identified tentatively as
IAA on the basis that it chromatographed and
reacted with Ehrlich and Salkowski reagents similar-
ly to IAA. The extracts were obtained from roots
grown for 42 h in a boron-deficient medium. Jaweed
and Scott (1967) also reported increased IAA con-
centration in boron-deficient plants, but their sun-
flower plants had grown for 4 days or longer in —B
medium.

Conflicting reports were presented by Crisp et al.
(1976), who investigated lettuce leaves grown in
boron-deficient medium. No increase in general IAA
levels was seen until 66 days after the start of the
experiment, at which time an increase in activity of
one class of auxin was observed. This was prior to
the onset of visible symptoms of tipburn. These
data led Crisp et al. (1976) to suggest that boron
content may affect the balance of endogenous auxins
and that tipburn is mediated by several factors,
including auxin metabolism. Smirnov et al. (1977)
reported that the roots of—B corn plants and
hypocotyls of sunflower seedlings contained less
IAA than control tissues and that bound IAA in
bean roots increased significantly. In addition, roots
of wheat, a plant which is not very sensitive to
boron deficiency, had increased levels of free and
bound IAA. Because boron-deficient wheat plants
that are able to grow to reproductive maturity ex-
hibit a threefold accumulation of bound IAA with
little deleterious effect and because several plants
show an actual decrease in IAA content, Smirnov
et al. felt that boron deficiency is not equivalent to
IAA toxicity.

Our results show a slight increase of endogenous
IAA in —B roots over the controls at the 24-h
sampling period (115 ng/g vs. 94 ng/g). Bohnsack
and Albert (1977) reported an elevation of auxin
oxidase levels 6–18 h after boron was withheld from
squash roots. This result implied that IAA levels in
—B roots had increased either prior to 6 h or con-
comitantly. The delay exhibited in sunflower roots,
such that a difference in endogenous IAA was not
observed until after 6 h, may in part reflect the more
protracted response to boron stress under our ex-
perimental conditions. Our sampling periods at 6
and 24 h were chosen to parallel our earlier observa-
tions regarding the ultrastructural changes brought
about by boron deficiency (Hirsch and Torrey
1980). After 6 h of boron deprivation, sunflower
roots exhibit ultrastructural changes in cellular
membranes, but at the same sampling time, we did
not observe a difference in endogenous IAA level in
these roots compared to the controls. For this
reason, we conclude that elevated IAA levels are a
consequence, rather than a cause, of boron-deficiency
symptoms.

After 24 h of treatment, we measured a slight
increase of IAA in —B roots, lending support to our
conclusion. Our earlier study on adding exogenous
IAA to +B-grown sunflower root tips, in which we
found little correspondence between this treatment
and boron deficiency, also substantiates our hy-
pothesis (Hirsch and Torrey 1980). Although we
did not measure auxin oxidase, we suspect that levels
of this inducible enzyme would be elevated after
24 h of boron stress. We base this judgment on our
72-h measurements in which both +B and —B-
grown root tips contain similar levels of endogenous
IAA.

Under the experimental conditions used, sunflower
root tips show symptoms of boron deprivation,
namely, inhibition of root elongation and membrane
perturbations, as early as 6 h after treatment has
commenced. As we have reported here and will ex-
pand in a future paper, differences in root elongation
between +/—B can be correlated with an inhibition
of mitotic activity with time in boron-deficient root
cells. However, the very early responses to boron
stress do not correlate with changes in endogenous
IAA level, as evidenced by RIA measurement or
addition of exogenously supplied hormone. Increased
levels of endogenous IAA is thus a secondary
response in sunflower. We therefore conclude that
boron deficiency does not act primarily on auxin
levels influencing root cell elongation. Rather, we
believe that it is to the early events, notably to the
specific alterations of cellular membranes, that we
must look for the primary role of boron in plants.
Evidence is accumulating that boron may play an
important role in membrane transport or in main-
taining membrane integrity (Pollard, Parr, and

Acknowledgments

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LITERATURE CITED


