Endogenous IAA Levels in Boron-Deficient and Control Root Tips of Sunflower
Author(s): Ann M. Hirsch, William L. Pengelly, John G. Torrey
Reviewed work(s):
Source: *Botanical Gazette*, Vol. 143, No. 1 (Mar., 1982), pp. 15-19
Published by: The University of Chicago Press
Stable URL: http://www.jstor.org/stable/2474373
Accessed: 03/04/2012 15:50

BOT. GAZ. 143(1):15–19. 1982.

0006-8071/82/4301-0020$02.00

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

ANN M. HIRSCH,[1]* WILLIAM L. PENGELLY,[2]† AND JOHN G. TORREY†

*Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138; and †Maria Moors Cabot Foundation, Harvard Forest, Petersham, Massachusetts 01366

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 (JAWEED and SCOTT 1967; COKE and WHITTINGTON 1968) or questioned (CRISP, COLLIER, and THOMAS 1976; SMIRNOV, KRUPNIKOVA, and SHKOL'NIK 1977) the hyperauxin hypothesis failed to resolve the matter.

We reported (HIRSCH and TORREY 1980) that the addition of 5×10^{-6} M or 5×10^{-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 $\frac{1}{4}$-strength Hoagland's solution (HOAGLAND and ARNON 1950). Three days after germination, the seedlings were transferred to aerated $\frac{1}{4}$-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 +/−B $\frac{1}{4}$-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-

[1] Present address: Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts 02181.

[2] Present address: Department of Chemistry and Biochemical Sciences, Oregon Graduate Center, 19600 Northwest Walker Road, Beaverton, Oregon 97006.

Manuscript received August 1981; revised manuscript received October 1981.

Address for correspondence and reprints: A. M. HIRSCH, Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts 02181.

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.

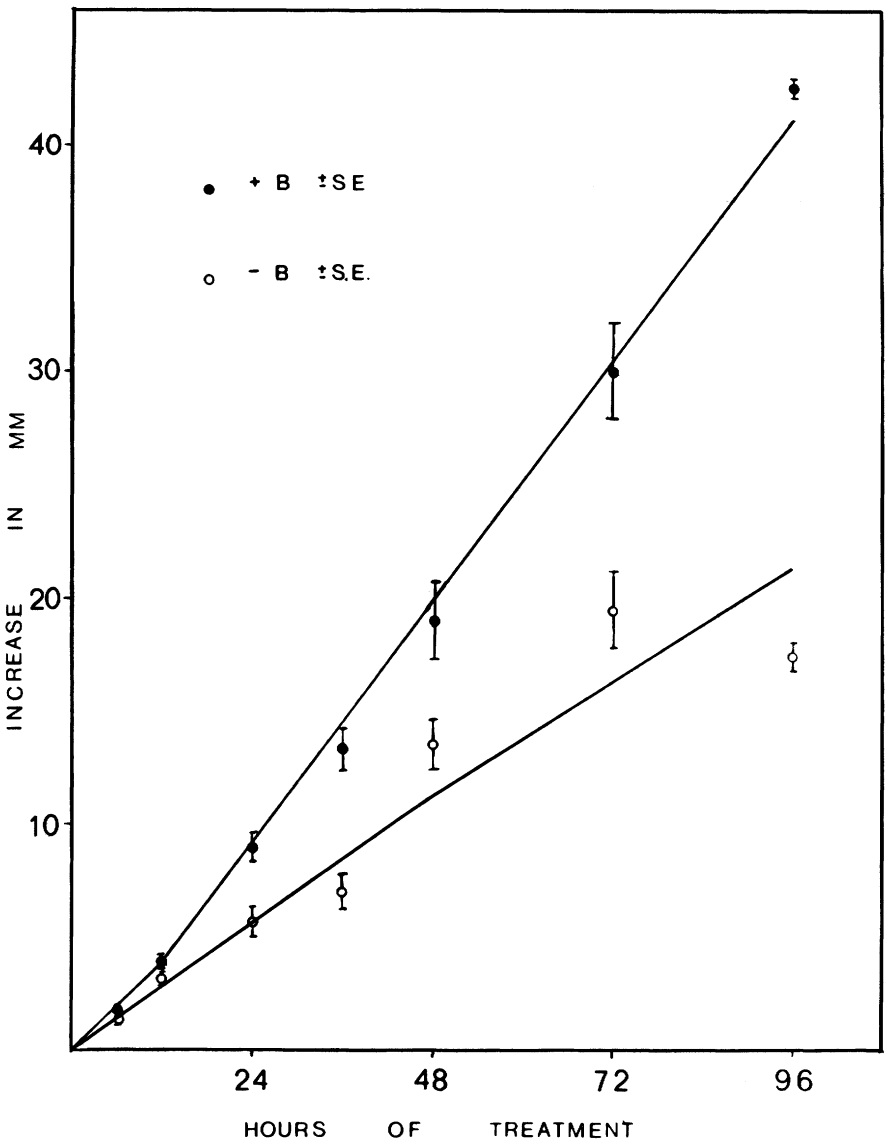


FIG. 1.—Increase in length in millimeters of roots grown in +B and −B nutrient media for 96 h. Boron (B) provided at 0.5 ppm. The lines were "fitted by eye" to be straight lines, but the −B line actually appears to plateau at 72 h.

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.

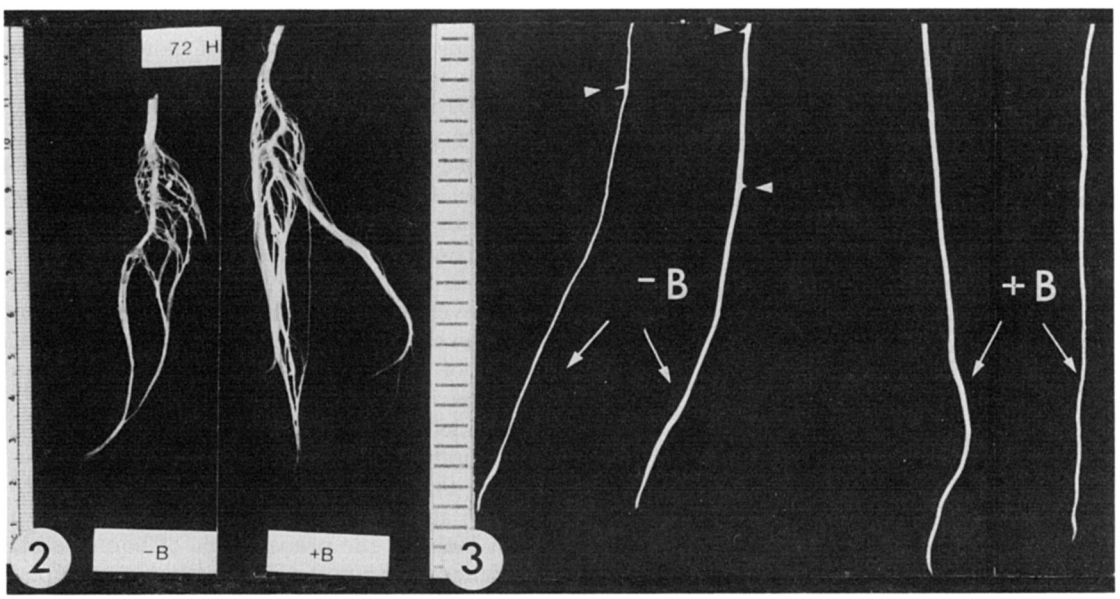
From 6 to 72 h, there was a slight decrease in endogenous auxin in both +B and −B roots. During this period, the growth rate of +B roots increased while in −B roots, the rate decreased (fig. 1). We studied mitotic activity in the two sets of roots, using the method of colchicine block, to determine to 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 the boron-starved cells remained in mitosis after colchicine treatment. Nearly 1.5 × 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

ENDOGENOUS IAA CONCENTRATION (ng/g fresh wt) MEASURED BY RIA IN +B/−B-GROWN ROOTS AT VARYING TIMES

	+B			−B		
TIME PERIOD	Fresh weight (ng)	IAA concentration (ng/g)	Mean IAA concentration (ng/g)	Fresh weight (ng)	IAA concentration (ng/g)	Mean IAA concentration (ng/g)
6 h	321	74	98	309	78	96
		121			114	
24 h	278	91	94	284	119	115
		100			111	
		92			114	
72 h	333	103	86	349	78	78
		74			81	
		82			75	

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 morphological 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 flavenoids (PERKINS and ARONOFF 1956). Exogenously supplied IAA enhances lignification 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 P^{32} 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 similarly 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 concentrations in boron-deficient plants, but their sunflower 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 exhibit 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 concomitantly. 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 experimental conditions. Our sampling periods at 6 and 24 h were chosen to parallel our earlier observations 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 hypothesis (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 expand 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 maintaining membrane integrity (POLLARD, PARR, and

LOUGHMAN 1977; HIRSCH and TORREY 1980; ROTH-BEJERANO and ITAI 1981).

Acknowledgments

This research was supported in part by the Maria Moors Cabot Foundation for Botanical Research of Harvard University, by a Foremost-McKesson (Research Corporation) grant, and by Wellesley College Faculty Awards to A. M. HIRSCH. We acknowledge the expert assistance of HELEN M. MOORE in obtaining the mitotic counts and of KAY LELAND, BARBARA HOWE, and BEVERLY MOQUIN in preparing the photographs and typing the manuscript.

LITERATURE CITED

BOHNSACK, C. W., and L. S. ALBERT. 1977. Early effects of boron deficiency on indoleacetic acid oxidase levels of squash root tips. Plant Physiol. **59**:1047–1050.

BRANDENBURG, E. 1949. Wo stehen wir heute in der Borfrage? Z. Pflanzenkr. Pflanzenschutz. **56**:241–252.

COKE, L., and W. J. WHITTINGTON. 1968. The role of boron in plant growth. IV. Interrelationships between boron and indol-3yl-acetic acid in the metabolism of bean radicles. J. Exp. Bot. **19**:295–308.

CRISP, P., G. F. COLLIER, and T. H. THOMAS. 1976. The effect of boron on tipburn and auxin activity in lettuce. Sci. Hort. **5**:215–226.

HIRSCH, A. M., and J. G. TORREY. 1980. Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. Can. J. Bot. **58**:856–866.

HOAGLAND, D. R., and D. I. ARNON. 1950. The water-culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347.

JAWEED, M. M., and E. G. SCOTT. 1967. Effect of boron on ribonucleic acid and indoleacetic acid metabolism in the apical meristem of sunflower plants. Proc. West Virginia Acad. Sci. **39**:186–193.

JENSEN, W. A. 1955. The histochemical localization of peroxidase in roots and its induction by indoleacetic acid. Plant Physiol. **30**:426–432.

NEALES, T. F. 1960. Some aspects of boron in root growth. Australian J. Biol. Sci. **13**:232–248.

PENGELLY, W. L., and F. MEINS, JR. 1977. A specific radioimmunoassay for nanogram quantities of the auxin, indole-3-acetic acid. Planta **136**:173–180.

PENGELLY, W. L., and J. G. TORREY. 1982. The relationship between growth and indole-3-acetic acid content of roots of *Pisum sativum* L. BOT. GAZ. (in press).

PERKINS, H. J., and S. ARONOFF. 1956. Identification of the blue-fluorescent compounds in boron-deficient plants. Arch. Biochem. Biophys. **64**:506–507.

POLLARD, A. S., A. J. PARR, and B. C. LOUGHMAN. 1977. Boron in relation to membrane function in higher plants. J. Exp. Bot. **28**:831–841.

ROBERTSON, G. A., and B. C. LOUGHMAN. 1974. Response to boron deficiency: a comparison with responses produced by chemical methods of retarding root elongation. New Phytol. **73**:821–832.

ROTH-BEJERANO, N., and C. ITAI. 1981. Effect of boron on stomatal opening in epidermal strips of *Commelina communis*. Physiol. Plantarum **52**:302–304.

SMIRNOV, Y. S., T. A. KRUPNIKOVA, and M. YA. SHKOL'NIK. 1977. Content of IAA in plants with different sensitivity to boron deficits. Fiziol. Rastenii **24**:343–350.

TORREY, J. G. 1953. The effect of certain metabolic inhibitors on vascular tissue differentiation in isolated pea roots. Amer. J. Bot. **40**:525–533.

———. 1965. Physiological bases of organization and development of the root. Handb. Pflanzenphysiol. **15**:1297–1327.