Plant Physiol. (1977) 59, 1047-1050

Early Effects of Boron Deficiency on Indoleacetic Acid Oxidase Levels of Squash Root Tips

Received for publication September 16, 1976 and in revised form January 17, 1977

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ABSTRACT

The indoleacetic acid (IAA) oxidase activity of root tips of boron-sufficient, -deficient, recovering, and IAA-treated boron-sufficient squash plants (*Cucurbita pepo* L.) was determined. Apical and subapical root sections displayed an increase in IAA oxidase activity between 6 and 9 hours after boron was withheld, and after 24 hours the activity of the apical sections showed a 20-fold increase over +B controls. Root elongation of −B plants was inhibited before an increase in oxidase activity could be detected. Roots of plants subjected to 12 hours of −B treatment and then transferred to +B treatment for recovery regained normal elongation rates and oxidase activity within 18 to 20 hours. IAA treatment of +B plants increased IAA oxidase activity of apical and subapical root sections and also inhibited root elongation and caused symptoms similar to −B treatments.

These results have demonstrated the earliest enzymic change for intact boron-deficient plants. The results are in agreement with the theory that boron deficiency symptoms may be the result of supraoptimal endogenous levels of IAA. These high levels of IAA may inhibit cell division and lead to an induction of the IAA oxidase enzyme.

One of the more recurrent proposals for a role of boron in higher plants is that it may play a part in IAA metabolism and thereby influence growth (5). Eaton (7) obtained evidence that led him to conclude that boron-deficient plants were also IAA-deficient. Attempts to confirm Eaton's work, however, have been unsuccessful (10, 12). More recently, Neales (14) proposed that instead of boron-deficient plants lacking sufficient IAA, they may actually contain supraoptimal levels which impair normal growth. Shkol'nik *et al.* (19) reported a decrease in the level of free acid auxins and an increase in the bound form in deficient corn and sunflower plants. Other investigators have reported higher levels of IAA in the apical regions of boron-deficient plants and have speculated that the increase in IAA might be due to an inactivation or change in the activity of IAA oxidase (4, 9).

Oxidative enzyme activity appears to be altered by boron deficiency. Higher polyphenol oxidase activity and an increased O_2 consumption by tissue homogenates have been reported for boron-deficient plants (11, 16). Peroxidase activity of tissue homogenates (13) and of root tips (15) has been found to increase, and to the contrary, decrease in stem callus and root cultures (6). IAA oxidase activity has been reported to decrease in plants subjected to several days of boron deficiency (19).

Due to the conflicting nature of these reports and because of its role in IAA metabolism, it was decided to investigate the relationship between boron deficiency and IAA oxidase activity with a system that responds very sensitively to boron deficiency. Work reported in this paper examines early changes in the activity of IAA oxidase during the onset and recovery from boron deficiency in root tips of intact squash plants.

MATERIALS AND METHODS

Plant Culture and Treatment. Squash seeds (*Cucurbita pepo* L. cv. Early Prolific Straightneck), courtesy of Joseph Harris Co., Inc., Rochester, N.Y., were germinated and grown in a complete modified Shive nutrient solution (1), containing 0.1 mg/l boron, for 5 days. Plants of uniform size and appearance were selected for treatment and the roots marked with India ink 1 cm from the tip in order to determine net elongation at the end of treatment. Plants were then transferred to soft glass jars (1.8 l) containing either a complete Shive nutrient solution (0.1 mg/l boron) for +B treatment or with boron omitted for −B treatment. Plants were grown under continuous light (1500 ft-c) at a constant temperature (31 C ±1) and continuous aeration.

Treatment of plants with phenolic compounds, H_2O_2, and IAA was carried out by adding the appropriate amount of chemical (via stock solutions) directly to the nutrient solution.

Determination of IAA Oxidase Activity. The *in vivo* IAA oxidase activity of squash root sections was determined using a modified technique of Galston and Dalbert (8). Fifty each of 5-mm apical and 5-mm subapical root sections were excised and quickly placed in separate incubation flasks containing 10 ml of 2,4-dichlorophenol (10^{-5} M), IAA (40 μg/ml), and citric acid phosphate buffer (pH 5.6) solution. The flasks were placed in a water bath shaker (30 C ± 1 at 130 rpm) for 1 hr. Where results of preliminary experiments indicated considerable enzymic activity, the number of root sections/flask was reduced to 25 and the incubation time to 0.5 hr.

When incubation was complete, the flasks were removed from the water bath and the solution in the flask tested for residual IAA. Aliquots of the solution were placed in test tubes with Salkowski reagent (50 ml 35% perchloric acid-1 ml 0.5 M Fe_2CL_3) and allowed to develop for 1 hr. Absorbance was measured on a Gilford spectrophotometer (540 nm) and residual IAA determined from a standard curve.

Root sections were removed from the flask, dried in an oven (81 C ± 1), and weighed to the nearest 0.1 mg. IAA oxidase activity was expressed as μg IAA destroyed/hr·mg dry wt.

Values for oxidase activity presented represent averages of three to six experiments, except where noted, conducted at different times. Standard errors were calculated for all results and those shown in Table I are typical for all data except for several instances during periods of changing activity when they were up to twice the larger values found in Table I.

RESULTS

Effect of Boron Deficiency on Plant Growth. One of the earliest symptoms of boron deficiency of squash plants is the

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Table I. IAA Oxidase Activity and Elongation of Squash Root Sections from Plants Grown in + B Nutrient Solution and 10^{-6}M IAA for Varying Periods of Time. Roots Were Measured at the Beginning of Auxin Treatment and at the Time of Sampling.

Each value represents an average of three experiments with the S.E. of the Mean. Control values for + B sections: Apical 5.0 mm section = 0.85 ± 0.03 and subapical section = 2.2 ± 0.3 μg IAA destroyed. hr^{-1}. mg dry wt^{-1}.

Hours of Treatment	Apical 5.0 mm Section	Subapical 5.0 mm Section	Net Elongation
	μg IAA destroyed. hr^{-1}. mg dry wt^{-1}		mm
3	1.1 ± 0.1	1.6 ± 0.2	1.0
6	1.7 ± 0.2	2.8 ± 0.2	1.4
9	1.7 ± 0.1	4.2 ± 0.3	1.9
12	2.5 ± 0.1	5.5 ± 0.6	3.7
24	10.9 ± 0.5	11.4 ± 0.3	7.3

inhibition of root growth. Squash plants receiving −B treatment displayed inhibition of root growth which was measurable after 3 hr of treatment. Roots of these plants elongated at a reduced rate until elongation ceased at about 24 hr (Fig. 1). The average 24-hr elongation for −B roots was 8 mm and no plant was used for experimentation or enzyme assay if elongation exceeded 10 mm in 24 hr. Many of the boron-deficient roots displayed a slight deflection or bending near the apex, indicating a loss of the geotropic response. Roots of plants remaining on −B treatment longer than 24 hr developed a brownish color terminally and produced emergent root primordia near the root apex. Roots of +B plants elongated in an almost linear fashion with respect to hr of treatment, with elongation varying between 35 to 50 mm in a 24-hr period. While differences in root elongation between + and −B treatments could be detected very early, inhibition of shoot growth did not become apparent until 48 hr.

Plants subjected to 12 hr −B treatment and then transferred to +B nutrient solutions immediately made a partial recovery in root elongation which lasted for 18 hr after transfer to the +B medium (Fig. 1). Thereafter, the rate of elongation increased to a value approximately equal to that of roots kept continuously in +B solutions. It was noted that recovery of root systems of 12-hr-stressed plants was uniform in terms of elongation, virtually all roots showed complete recovery. Plants stressed for 18 and 24 hr and then transferred did not display this uniformity and total recovery of individual roots was about 85 and 60%, respectively.

IAA Oxidase Activity of + and −B Root Sections. The effect of + and −B treatments on IAA oxidase activity is shown in Figure 2. Root sections from +B plants displayed a fairly uniform level of oxidase activity over a 36-hr period. The 5-mm apical sections had slightly more than one-third the activity of the 5-mm subapical sections with average activity being 0.85 and 2.3 μg IAA destroyed/hr·mg dry wt, respectively.

Apical and subapical sections of −B roots showed little variation in oxidase activity from +B sections during the first 6 hr of treatment. Between 6 and 9 hr, activity began to increase slowly and a dramatic rise in activity was observed after 12 hr on −B treatment. This rise in activity reached a maximum at 18 hr., the apical sections showing a 20-fold increase over the control and the subapical sections a 9-fold increase. The normal relationship between the apical and subapical oxidase activity was reversed in boron deficiency with the apical section possessing more activity than the subapical.

That this destruction of IAA represented enzymic activity and not uptake of IAA by the root sections in the reaction flasks was verified by making crude enzyme extracts of + and −B roots. Activity was found to be 21.7 and 8.4 μg IAA destroyed/hr·ml of crude extract for −B and +B roots, respectively. Boiling the extract completely destroyed the activity. That the enzyme involved was IAA oxidase was further supported by testing the effect of phenolic regulators of IAA oxidase activity in the reaction flask with the root sections. A concentration of 10^{-5} M 2,4-dichlorophenol in the reaction flask enhanced activity of the sections approximately four times, while a concentration of 10^{-5} M caffeic acid inhibited oxidase activity by almost one-half when compared to the activity of root sections run with only citric acid-phosphate buffer.

The effect of +B recovery treatment on the elevated oxidase levels due to −B treatment is shown in Figure 3. During recovery, apical and subapical sections showed a steady decline in oxidase activity with time on +B treatment. After 24 hr, oxidase levels returned to the +B control values. It was observed that plants subjected to 18 hr of −B treatment took substantially longer (48 hr) to return to normal oxidase levels.

Effect of IAA on Root Growth and Oxidase Activity. Root

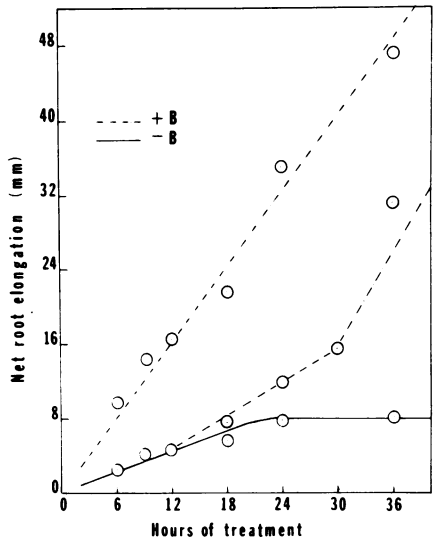

FIG. 1. Effect of boron deficiency and recovery on the elongation of roots of squash plants. For recovery, boron was added after 12 hr on −B treatment.

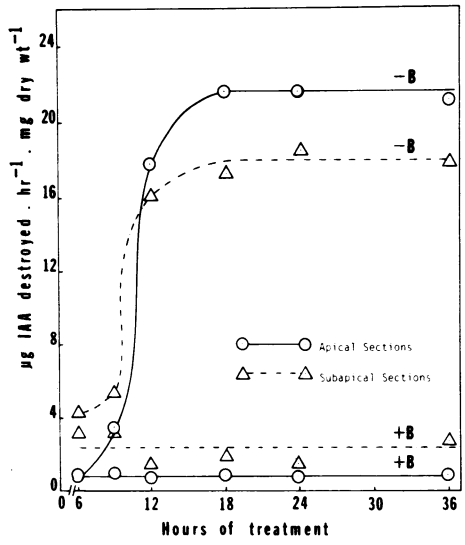

FIG. 2. IAA oxidase activity of apical and subapical 5-mm sections of roots of squash plants grown on + and −B nutrient solutions for varying periods of time.

systems of plants treated with 10^{-6} M IAA showed a severe inhibition of elongation during the first 24 hr (Table I). Roots frequently displayed a loss of geotropic response, initiation of lateral root primordia near the apex, and a swelling of the terminal portion. If IAA was not renewed in the nutrient solution after 24 hr this inhibition of growth was gradually relieved and normal growth resumed. Results also showed that IAA treatment induced IAA oxidase activity in both apical and subapical root sections (Table I). Increases were observable between 3 and 6 hr, and activity continued to rise with time of treatment. Approximately a 10-fold increase was seen after 24 hr, the activity of apical and subapical sections being almost equal at this time.

Effects of Phenolic Compounds and Hydrogen Peroxide on IAA Oxidase Activity and Root Elongation. The inclusion of phenolic regulators of IAA oxidase activity and H_2O_2 in the nutrient solutions was carried out in an attempt to alter oxidase activity and thereby influence boron deficiency symptoms (Table II). Of the inhibitors of oxidase activity (caffeic acid and chlorogenic acid) tested, only 10^{-4} M caffeic acid significantly lowered the *in vivo* activity of this enzyme, with a concomitant inhibition of root elongation. A concentration of 10^{-5} M chlorogenic acid was also inhibitory to elongation but did not inhibit oxidase activity. Dichlorophenol (DCP), an enhancer of oxidase activity, inhibited elongation and oxidase activity at a concentration of 10^{-5} M but inhibited only elongation at 10^{-6} M-H_2O_2 severely inhibited oxidase activity and elongation at 10^{-4} M and to a lesser extent at 10^{-6} M.

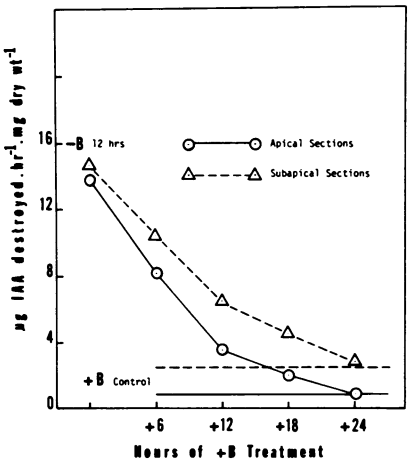

FIG. 3. IAA oxidase activity of apical and subapical 5-mm sections of roots of squash plants during recovery from 12 hr of −B treatment.

DISCUSSION

Results obtained in this study have demonstrated that IAA oxidase activity increases very early during the onset of boron deficiency and that there are similarities between induced IAA toxicity and naturally occurring boron deficiency. It was also observed that the inhibition of root elongation occurs prior to a rise in oxidase activity. A sequence of events leading to this increase may be viewed as follows: IAA concentration in the apical region increases, mitosis stops, elongation ceases, and IAA oxidase is induced. That this sequence is plausible is supported by the observation that under +B and 10^{-6} M IAA treatment, apical sections showed induced oxidase activity after 6 hr of treatment. It was demonstrated previously that this enzyme is inducible in plant tissue in the presence of IAA (8). The slow increase (lag) in oxidase activity observed in −B root sections between 6 and 9 hr is compatible with that of an induced enzyme. Also, the amount of inhibited root elongation due to added IAA was the same after 24 hr as the −B treatment alone. In addition, IAA-treated roots frequently displayed initiation of lateral root primordia near the apex and a loss of geotropism such as observed under −B conditions. Thus, if endogenous levels of IAA in the apical meristem of −B roots increase during the first 6 hr of deficiency, this could lead to an induction of IAA oxidase such as observed in −B roots.

Table II. Effect of Caffeic Acid, Chlorogenic Acid, 2,4-Dichlorophenol and Hydrogen Peroxide included in + and - B Nutrient Solutions on IAA Oxidase Activity and Elongation of Squash Roots. Treatment Time = 24 Hours.

Each value represents an average of two experiments. Control values for oxidase activity of 5.0 mm apical sections: + B = 1.0 and - B = 18.9 µg IAA destroyed. hr^{-1}. mg dry wt^{-1}.

Treatment	Oxidase Activity of 5.0 Apical Section		Elongation	
	+ B	- B	+ B	- B
	µg IAA destroyed. hr^{-1}. mg dry wt^{-1}		% of Control	
10^{-4} M Caffeic Acid	0.8	9.6	-63.2	-52.9
10^{-6} M Caffeic Acid	1.1	19.0	+ 1.6	+15.3
10^{-7} M Caffeic Acid	1.1	20.5	+ 1.6	+ 0.4
10^{-5} M Chlorogenic Acid	1.0	22.3	- 9.6	- 1.6
10^{-6} M Chlorogenic Acid	0.7	21.8	- 4.9	+ 1.6
10^{-5} M DCP	1.0	15.6	-52.0	-37.8
10^{-6} M DCP	1.1	19.7	-18.8	- 4.5
10^{-4} M H_2O_2	0.8	2.8	-33.1	-16.7
10^{-6} M H_2O_2	0.6	16.4	-23.6	-13.6

If the IAA concentration increases in the apical region in boron-deficient roots, this could lead to a change in the normal distribution of the IAA oxidase enzyme as was observed. Under +B conditions, a higher level of oxidase was found in the differentiated subapical sections than in the meristematic apical sections. Galston and Dalbert (8) reported a similar distribution of oxidase activity in pea roots. However, under −B conditions, due to increasing levels of IAA in the apical region, induced oxidase activity rises and finally reaches a higher level in the apical region.

Since higher levels of oxidase activity are associated with differentiating cells, boron-deficient roots may display premature differentiation. Cohen (3) reported that mitosis in −B roots ceased after 6.5 hr with elongation ceasing a short time thereafter. This cessation of mitosis and rise in oxidase activity of −B roots after 6 hr may represent an early stage of differentiation caused by a rising level of IAA. Differentiation and lignification of xylem elements into the apical region is a frequently reported aspect of −B roots (1, 2, 21). Torrey (22) has reported that IAA treatment of cultured pea roots caused lignification into the meristem as well as lateral root initiation. Since IAA oxidase may be part of an oxidizing complex of polyphenol oxidase and peroxidase (17), and further, since peroxidase has been implicated in lignification (20), the observed IAA oxidase increase under −B conditions could lead to an increase in lignification of the apical region.

The results of this work are in general agreement with previous reports of increases in oxidative enzyme activity in −B plants (11, 13, 15, 16). These results are contrary to the observations of Shkol'nik *et al.* (19) who reported a decrease in IAA oxidase activity in boron-deficient sunflower plants which had been subjected to several days of boron deficiency. The length of time to which a plant is subjected to −B stress, however, is crucial to the interpretation given to any subsequent physiological analysis. The more closely one is able to correlate physiological changes with the inhibition of elongation, the more the likelihood of pinpointing the metabolic processes for which boron is essential. Analyses conducted after several days of deficiency may reflect secondary changes far removed from the initial metabolic malfunction.

Attempts to alter the oxidase activity of intact plants and thereby influence deficiency symptoms were generally not successful. Reported inhibitors of oxidase activity, chlorogenic and caffeic acids, increased inhibition of root growth at 10^{-5} M and 10^{-4} M concentrations but only 10^{-4} M caffeic acid had any subsequent inhibition of oxidase activity. Surprisingly, 2,4-dichiorophenol also inhibited elongation and oxidase activity. This is difficult to reconcile because of the well documented evidence that this compound stimulates IAA oxidase activity. It may be that the concentrations used were too high and were toxic to other essential metabolic processes. H_2O_2 has been reported to overcome some aspects of boron deficiency under certain conditions (18). No alleviation of deficiency symptoms by H_2O_2 was noted in squash plants at the concentrations tested, although severe inhibition of elongation and oxidase activity were observed in −B roots at high concentrations.

The increase in IAA oxidase activity of −B root apical sections occurring between 6 and 9 hr after withholding B represents the earliest enzymic change yet to be reported for intact deficient plants. Roots of plants treated with toxic amounts of IAA show similar increases in oxidase activity and are similar in appearance to boron-deficient roots. Several investigators have postulated that boron deficiency symptoms are the result of the accumulation of toxic amounts of IAA, caused perhaps by the inhibition of IAA oxidase (4, 9). The results presented in this paper tend to confirm that high endogenous levels of IAA may exist in boron-deficient plants. These high levels, however, are not the result of an inhibition of IAA oxidase but instead apparently cause the induction of this enzyme. Further investigation is still needed to elucidate the mechanism by which boron controls endogenous levels of IAA.

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