Ribonucleic Acid Content, Boron Deficiency Symptoms, and Elongation of Tomato Root Tips

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Introduction

Boron is recognized as an essential micronutrient for higher plants, but the precise role of this element in plant growth and development is not clear. In Gaugh and Dugger's (3) comprehensive review they presented and discussed a wide spectrum of proposed roles and effects of boron on higher plants. From their own work and their interpretative review of the literature they concluded that “boron may be essential for sugar transport in higher green plants,” and “boron deficiency symptoms are an expression of sugar deficiency in the cambia, stem tips, root tips, and flowers and fruits.” From reports that have appeared since their review was published in late 1954, it appears that the role of boron in sugar translocation per se must be indirect (5, 8, 9, 13, 17), that sucrose does not readily alleviate the symptoms of boron deficiency (1, 6, 7, 15), and that as much or more carbohydrate was found in boron-deficient root tips as in boron sufficient tips (5, 16).

Although the exact function of boron in plant metabolism is unknown, a common feature on which many investigators agree is that boron is essential for the normal growth and functioning of apical meristems (1, 7). Whittington (14) reported that a deficiency of boron caused a cessation of cell division and later suggested (15) that in the absence of boron, cell division ceases because abnormalities in the formation of the cell wall prevent the cell from becoming organized for mitosis. However, Skok (13) concluded from radiosensitivity studies with sunflower plants that boron is required “for some process or processes concerned with cellular maturation or differentiation rather than with cell division.”

Albert and Wilson (1) found that external symptoms of boron deficiency of roots of intact tomato plants were detected within 24 hours after boron was withheld from the nutrient solution. These symptoms consisted of the cessation of root elongation as early as 6 hours after boron was withheld and the subsequent development of a brown color and loss of fluorescence in the terminal portion (2 mm) of the root tips. Brown root tips did not resume elongation when again supplied with an adequate amount of boron. However, roots that ceased elongation in minus boron solutions will resume elongation if boron is supplied before the tips turn brown. As the brown color develops, the intensity of the characteristic white fluorescence from this same area of the root tip decreases and finally disappears. Internal symptoms were observed to occur in the post-meristematic region of the tip and were apparent as a darker staining of the cytoplasm followed by its disintegration, resulting in empty, apparently dead cells. Cell walls of some, but not all, of the dead cells were thicker than cell walls of cells retaining their cytoplasm. These observations emphasize that boron is essential for cellular activity and that the absence of boron from the nutrient medium results in early symptoms of boron deficiency in root meristems. They also indicate that boron is necessary for cellular processes which are essential to the generation or differentiation of new cells.

While this investigation was in progress several reports appeared relating to boron and RNA in plants. Matter and Turian (4) observed that high concentrations of boric acid had antimitotic activity at the apex of the radicle of Lactuca sativa. At high and moderate concentrations of boric acid the nucleolus was enlarged, and boric acid was detected in the nucleolus by cytochemical means. Sherstnev and Kurilenok (12) found that adenine-8-C14 supplied in the nutrient solution to sunflower plants was incorporated into RNA of the upper-most leaves and roots of plants supplied with boron to a greater extent than in boron-deficient plants. These results supported previous reports from their laboratory that a lowering of nucleic acid content occurs when plants are grown in nutrient media deficient in boron. Also, Shkolnik and Kositsyn (11) followed the incorporation of P32 into nucleic acids in the presence and absence of boron and concluded that boron deficiency strongly inhibits the synthesis of nucleic acid and supports their hypothesis that boron plays an important role in the synthesis of nucleic acids.

The present investigation developed from an attempt to isolate and identify the fluorescent material in healthy tomato root tips which disappeared and was replaced by a brown color as boron deficiency symptoms progressed. It was found that the RNA content of the root tips of tomato plants decreases and boron deficiency symptoms of roots (loss of fluorescence and browning) occur concurrently when boron is withheld from the nutrient solution. However, root elongation ceases before the decrease in RNA content occurs.

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Materials and Methods

Plant Culture. Tomato plants (*Lycopersicum esculentum* Mill., var. Rutgers) used in these experiments were grown from seed obtained from the W. Atlee Burpee Company. The seeds were germinated in Perlite, a heat expanded volcanic ore product, contained in plastic flats and the seedlings received nutrient solution until they were transplanted. When the seedlings had 2 to 3 small leaves, and most often before the stem length was measurable, they were transplanted to 1 gallon glass jars, 1 plant per jar, containing a complete nutrient solution; aeration of the solutions was started immediately (1). Soft glass containers were used for preparing the solutions and for growing the plants. Nutrient solutions were changed every 5 to 7 days and lateral buds were removed so that all top growth occurred from the apical meristem. Plants ranged from 5 to 20 cm in height (cotyledons to apical meristem) at the time experiments were started. All plants were grown with an adequate supply of boron (0.1 mg/liter) until experiments were initiated, at which time the concentration of boron in the treatment solutions was adjusted to the desired level. Fresh nutrient solutions were always used at the beginning of an experimental period.

Root Elongation Measurements. Root elongation measurements were obtained by marking root tips with India ink marks placed 10.0 mm from the tip and measuring the subsequent net increase in distance from the tip to the ink mark. Measurements of individual roots were made to the nearest 0.5 mm and from 5 to 10 root tips were marked per plant. Additional details of root marking were previously reported (1). At the selected time interval of treatment the roots were measured and the tips harvested for chemical analysis or returned to the solution for later measurement and subsequent analysis.

Observation of Boron Deficiency Symptoms. The degree of boron deficiency was followed by observing the root tips of intact plants at the time of harvest. Observations of fluorescence were made with a Hanovia mercury vapor lamp (Type 30620) fitted with a 250 to 370 nm filter. Fluorescence was recorded as: normal (+ +), weak (+) or absent (0). Associated with the weak and absent ratings of fluorescence is the appearance of the brown color in the normally fluorescent area of the tips.

Nucleic Acid Analysis. A modified Schmidt-Thanhauser method was used for analysis of RNA and DNA (2, 10). The terminal 3 mm of 12 to 20 tips were excised, gently blotted, quickly weighed, and placed in 80% ethanol. Tips were either analyzed immediately or held in 80% ethanol until the completion of an experiment, at which time all tips from all harvests were analyzed at the same time. It was found that tips could be stored in 80% ethanol for at least 8 days without a loss of nucleic acid content. Results from the first experiments showed that using whole tips gave more consistent results (lower standard deviation) than the usual procedure of homogenization and filtration. Whole tips were thereafter routinely used in all analyses. Perchloric acid was used throughout the procedure. Absorbance values were determined with a Beckman DU spectrophotometer and nucleic acid contents were calculated according to the procedure of Bonner and Zeevaart (2).

Results and Discussion

Fluorescent substances present in the terminal 3 mm of healthy tomato root tips were found to be readily soluble in 0.1 N NaOH. Absorption spectra of alkaline extracts gave absorption curves typical for nucleic acid, having a single absorption maximum in the ultra-violet at 260 nm and no absorption in the visible portion of the spectrum.

Based on these observations an experiment was performed to follow the nucleic acid content, root elongation, and boron deficiency symptoms of root tips of intact tomato plants over a 24-hour period. In this experiment 4 plants were used, 2 for the plus boron treatment and 2 from which boron was withheld for the 24-hour experimental period. Root elongation measurements were averaged from each treatment pair and a total of 20 tips were collected from each treatment pair for nucleic acid analysis. These results (table I, exp 1) show nearly a 50% decrease in the RNA content of root tips from plants from which boron was withheld (O B) as compared to the tips receiving an adequate supply of boron (O B). In contrast, the DNA content of tips from both treatments is nearly the same. Associated with the RNA decrease of the O B root tips is a large decrease in the elongation of these tips as compared to the O B tips. The O B tips exhibited typical symptoms of boron deficiency, that is, the tips did not fluoresce and a brown color developed in the normally fluorescent area.

Another experiment was designed to determine how soon the RNA content of the tips decreases after boron is withheld from the nutrient medium, and the relationship of the decrease in RNA to be observed decrease in root tip elongation. It was observed (exp 2) that by 12 hours root elongation in the O B solution had stopped, but the decrease in the RNA content of tips on this treatment was not apparent until the 24-hour harvest. Thus, root elongation in the O B treatment ceased before the RNA content of the tips decreased. Associated with the decrease in fluorescence is a decreased RNA content of the O B root tips. No significance is attached to the variations observed in the DNA content of the tips in this experiment.

To explore these observations more fully harvests were made in the next experiment every 6 hours for 24 hours and 1 harvest was made after 48 hours. It was again observed (exp 3) that root elongation
of the O B treatment stopped before the RNA content decreased. However, the RNA decrease was not apparent until the 48-hour harvest. This experiment was conducted in late summer when the days were long, but the first day of the treatment period turned cloudy and was a very dark, dull day. Under these conditions the development of boron deficiency symptoms are delayed (1) and although root elongation in the O B treatment ceased within 12 hours the decrease in RNA content was not observed until the 48-hour harvest was made. It was again observed that as the tips lose their fluorescence the RNA content of the tips decreases. There were no significant changes in the DNA content of the tips in this experiment.

These experiments demonstrate that root elongation in minus boron nutrient solutions stops before the RNA content of the tips decreases. If RNA analyses and elongation measurements are not made early enough in the treatment period it appears that the RNA content decreases and elongation ceases at the same time (expt 1). However, time-course measurements (expts 2, 3) clearly demonstrate that root elongation ceases before the RNA content of the root tips decreases and before the loss of fluorescence and appearance of browning occurs in the tips.

Although root elongation ceases before the RNA content of the tips decreases when boron is withheld from the nutrient solution, the roots will resume elongation if boron is supplied before the tips turn brown (1). Therefore the cessation of elongation per se does not cause irreversible damage to the tips since boron can restore growth. However, the addition of boron to the solution cannot restore growth once the brown color develops and the characteristic fluorescence disappears from the tip. It is precisely during this time that the RNA content of the tips decreases.

These data and observations indicate that root tips of tomato plants lose their response to boron (failure to elongate in the presence of boron) and concurrently develop symptoms of boron deficiency (browning of tips and loss of fluorescence) as the RNA content of the tips decreases. This pattern of deficiency symptoms coupled with a decrease in RNA content has been repeatedly observed in additional experiments with this system. Thus, it appears that boron has an early effect on RNA metabolism. These data do not indicate whether the effect is direct or indirect. The data demonstrates that when working with tissues which are sensitive to boron, time-course measurements are important to segregate sequential effects following the withdrawal of boron.
Summary

Tomato plants (Lycopersicon esculentum Mill., var. Rutgers) were grown in nutrient solution cultures with adequate boron (0.1 mg/liter) until treatments were started. Treatments consisted of placing plants in fresh nutrient solutions containing adequate boron or in solutions from which boron was withheld. At selected time intervals after initiating treatments root elongation measurements were obtained and tips (3 mm) were analyzed for nucleic acid (RNA and DNA).

The main results and conclusions are: 1) Elongation of root tips ceases before a decrease in RNA content occurs. 2) The RNA content of root tips decreases soon after (24 to 48 hr) boron is withheld from the nutrient solution. 3) Failure of root tips to elongate in the presence of boron and the appearance of typical boron deficiency symptoms occur concurrently as the RNA content of the tips decreases. 4) The DNA content of the tips does not change significantly during the experimental period.

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Literature Cited