Autoradiographic Examination of Meristems of Intact Boron-deficient Squash Roots Treated with Tritiated Thymidine

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ABSTRACT

Intact roots of boron-sufficient squash (Cucurbita pepo L.) plants, plants entering boron deficiency, and plants recovering from boron deficiency were exposed to tritiated thymidine at the end of the treatment period to label the replicating DNA of root tip cells. Using histological sections, autoradiographs of intact root meristems were prepared. The labeling pattern in +B root tips revealed the presence of a well defined quiescent center. The ability of root tip cells to incorporate label is correlated with the total root elongation during the -B treatment period. A greater amount of total root elongation during boron deficiency and recovery reflects the fact that root tip cells have retained their ability to synthesize DNA and enter mitosis for a longer time. In roots recovering from boron deficiency, cells of the quiescent center were seen to play no part in the recovery process in roots treated for as long as 20 hours in a -B nutrient solution. They were inactive before, during, and after the -B treatment. Cessation of mitosis occurs as early as 6.5 hours after boron is withheld from the nutrient solution while DNA synthesis can occur for as long as 20 hours after withholding boron. It was concluded that boron is essential for continued DNA synthesis and mitotic activity.

The absence of boron results in the cessation of mitosis and DNA synthesis within 20 hours from the time boron is withheld.

Several workers have reported the effects of a lack of boron on root growth. Neales (6, 7) reported that root elongation continued for periods of 48 to 80 hr when corn, bean, and pea were grown in the absence of boron while elongation of flax roots was sustained for only 48 hrs in a boron-deficient medium. Albert and Wilson (1) reported that, in intact tomato plants, root elongation may stop as early as 6 hr after boron is withheld from the nutrient solution. However, observation of the cessation of root elongation is not equivalent to elucidation of biochemical changes which occur in the meristem. In this paper, we report observations of early changes in DNA synthesis and mitotic activity which result in the observed cessation of root elongation.

The earliest symptom of boron deficiency that can be observed in intact squash plants treated in boron-deficient solution culture is the cessation of root elongation (4). It was observed that elongation was virtually terminated during the first 6.5 hr after withholding boron from the growth medium, although a small amount of elongation may occur up to 24 hr. Histological examination of these roots showed that the cessation of root elongation did not result from a cessation of cellular elongation, as this process was observed to continue for at least 98 hrs after boron was withheld from the culture solution. As the period during which the plants were treated in a boron-deficient growth medium increased, cellular elongation and differentiation continued distally in the stele until finally the differentiation extended into the region normally occupied by the apical meristem and the cells of the meristem became differentiated (4). This result strongly suggested that the cessation of root elongation was caused by a cessation of mitosis and cell division.

Johnson (5) reported the results of autoradiographic examination of the mitotic cell cycle of boron-deficient squash root meristems exposed to tritiated thymidine. Due to the disruption of the root tips caused by the squash technique used to study the cells, the results gave no information about labeling patterns in the intact root or which region of the meristem was affected first during the entry into and recovery from boron deficiency.

In the experiments reported herein, tritiated thymidine was introduced at the end of the boron treatment period to label the replicating DNA of squash root tip cells of boron sufficient plants, plants entering boron deficiency, and plants recovering from boron deficiency. Using histological sections, autoradiographs of intact squash root meristems were prepared. This provided more detailed information about early effects of boron deficiency than did the squash technique used to investigate only the mitotic cell cycle.

MATERIALS AND METHODS

Squash (Cucurbita pepo L., Cv. Early Prolific Straightneck) seedlings were grown from seed supplied by Joseph Harris Company, Inc. All seedlings were 5 days old when treated and were grown in +B nutrient solution until treated. The culture solution was a modified Shive’s nutrient solution (1), and all plants were grown under conditions of continuous light (1500 ft-c), constant temperature (30 ± 1 C), and continuous aeration. Net root elongation was determined by marking two roots per plant with a small India ink mark placed 1.0 cm from the root tip. Elongation could then be measured at selected time intervals (1). Upon completion of the treatment period, the terminal 1 cm of each root tip was harvested by excision and immediately placed into a modified Carnoy’s fixative (absolute ethanol-glacial acetic acid, 3:1 v/v). After fixation, the roots

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were dehydrated using an ethyl alcohol series and subsequently embedded in Tissuemat (m.p. 61°C).

To examine uptake of label by boron-sufficient (+B) plants, the roots were immersed into a soft-glass finger bowl containing 100 ml of modified Shive's nutrient solution and 1 mCi/ml tritiated thymidine (New England Nuclear, specific radio-activity 6.7 Ci/mM) for 60 min. The roots were then rinsed to remove any unincorporated label. After rinsing, the terminal 1 cm of each root was excised, immediately fixed, and subsequently embedded.

To examine the entry of plants into boron deficiency, plants were treated in a boron-deficient (−B) culture solution for periods of 3, 6, 12, 15, 18, and 24 hr. The plants were labeled with tritiated thymidine during the last hour of the −B treatment period. The roots were then rinsed, excised, fixed, and embedded as described above.

Recovery from boron deficiency was examined by treating plants in the −B culture solution for selected time periods. At the end of the −B treatment period, the plants were returned to a +B culture solution. Recovery was allowed for selected time periods. The plants were labeled with tritiated thymidine during the last hour of the recovery period. The roots were then rinsed, excised, fixed, and embedded as described above. Elongation measurements were recorded for all treatments.

For each boron-sufficient, boron-deficient, and recovery treatment, a minimum of 40 roots were treated. Each of these was separately embedded, completely sectioned, and the median sections were used for analysis. Suitable median sections were obtained from at least 30 roots from each treatment. The root tips were analyzed as to elongation during the treatment period, whether or not tritiated thymidine was incorporated, and the appearance of the labeling pattern on the autoradiographs.

Microtomed sections (6 to 7 μm) were mounted on slides, and the paraffin was removed with xylene rinses followed by rinses in absolute ethanol, and finally in water. The slides were coated with Kodak NTB2 liquid emulsion and stored in light-proof slide boxes containing a dessicant for periods of 8 to 14 days at 4 C. The emulsion-coated slides were developed using standard Kodak procedures (Kodak Pamphlet No. P-64), and the resulting autoradiographs were air dried.

**RESULTS**

Autoradiographs of the root tips of +B squash plants clearly revealed the presence of a quiescent center as described by Clowes (3) (Fig. 1). The presence of nuclei in which tritiated thymidine was incorporated into newly replicated DNA during the 60-min period of labeling is revealed by the appearance of exposed groups of black silver grains. DNA was synthesized in the central cylinder, cortex, and, to a lesser extent, in the root cap. The region immediately distal to the central cylinder (classically considered to be the meristematic region) showed no incorporation of label into nuclei.

To examine the uptake of label by squash plants entering boron deficiency, autoradiographs of the roots of plants treated in a −B nutrient solution for periods of 3, 6, 12, 15, and 18 hr were prepared. The pattern and amount of label present in these autoradiographs was similar to that seen in +B root tips showing that the plants had not yet entered boron deficiency. Roots excised from plants which had been treated in a −B nutrient solution for a period of 20 hr showed no evidence of incorporation of the label. Elongation measurements showed that the total elongation of these intact roots averaged 4.4 mm during the 20 hr −B treatment period. The results of other experiments showed that a very small amount of label can be incorporated as late as 24 hr after initiation of the −B treatment period if the total root elongation is as high as 5.5 mm during the 24-hr treatment period. Total root elongation was virtually completed during the first 6.5 hr of the −B treatment period. After this time elongation proceeded at a negligible rate, if at all.

**Figs. 1–3.** Autoradiographs of squash root tips. 1: Boron-sufficient root showing quiescent center (Q). × 100. 2: Twenty hr of boron deficiency followed by a 9-hr recovery period. Label is visible only in the periphery of the root cap. × 100. 3: Twenty hr of boron deficiency followed by a 12-hr recovery period. The quiescent center is again visible. × 100.
Table I. Root Elongation and Incorporation of $^3$H-Thymidine in
Boron-sufficient, Boron-deficient, and Recovering Root Tips

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean No. Nuclei Labeled$^1$</th>
<th>Mean Root Elongation$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>+B Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>NR$^2$</td>
<td>2.5</td>
</tr>
<tr>
<td>6 hr</td>
<td>NR</td>
<td>4.8</td>
</tr>
<tr>
<td>9 hr</td>
<td>NR</td>
<td>9.0</td>
</tr>
<tr>
<td>12 hr</td>
<td>NR</td>
<td>13.2</td>
</tr>
<tr>
<td>20 hr</td>
<td>52</td>
<td>29.5</td>
</tr>
<tr>
<td>-B Medium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 hr</td>
<td>58</td>
<td>4.3</td>
</tr>
<tr>
<td>20 hr</td>
<td>0</td>
<td>4.4</td>
</tr>
<tr>
<td>-B Medium 20 hr +B recovery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6 hr</td>
<td>0</td>
<td>0.2</td>
</tr>
<tr>
<td>9 hr</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>12 hr</td>
<td>48</td>
<td>0.7</td>
</tr>
</tbody>
</table>

$^1$ All figures are means of 30 roots.
$^2$ Means of not less than 40 roots.
$^3$ NR: not recorded.

Recovery from a period of 20 hr of boron deficiency was followed for periods of 3, 6, 9, and 12 hr. No incorporation of label was observed during the first 6 hr of recovery. Total elongation of the intact roots during the 3 and 6 hr recovery periods averaged 0 and 0.21 mm, respectively. After a recovery period of 9 hr (Fig. 2), some of the root tips showed a small amount of incorporation of label into cells located in the periphery of the root cap while others showed no incorporation of label. Total elongation of the intact roots during the 9-hr recovery period averaged 0.45 mm. Figure 3 shows a root tip treated for 20 hr in a -B nutrient solution followed by treatment in a +B nutrient solution for 12 hr. A larger amount of label than incorporated previously during the recovery periods was incorporated into the DNA of cells of this root tip. The appearance of the labeling pattern is similar to the pattern of tritiated thymidine incorporation seen in the +B root tip. Label was incorporated into the central cylinder, cortex, and to a lesser extent the root cap. A well defined quiescent center was again visible in the region considered to be occupied by the meristem in classical theories of root tip organization. Total elongation of the intact roots during the 12-hr recovery period averaged 0.72 mm. The measurements cited above are summarized in Table I.

**DISCUSSION**

 Autoradiographic examination of the roots of boron-sufficient squash plants revealed the presence of a well defined quiescent center as seen in Figure 1. Evidence indicates that cells of the quiescent center are inactive only because of their relative position with respect to the active cells and that if the latter are cut away or otherwise nullified, the cells of the quiescent center are fully able to become actively meristematic. They begin to synthesize DNA, divide, and eventually regenerate a new root tip complete with a new quiescent center (2). The withholding of boron from the culture solution of squash plants for a period of 20 hr temporarily suspends the ability of the cells to synthesize DNA. This is evidenced by the lack of incorporation of tritiated thymidine into nuclei after 20 hr of treatment in a -B nutrient solution and during the first 6 hr of recovery in a +B nutrient solution. The evidence from Clowes (2) suggests that this condition would produce the activation of the previously inactive cells of the quiescent center and that these cells would then produce a new root tip complete with a quiescent center. This did not occur up to a period of 20 hr of -B treatment. As the recovery progressed, the labeling pattern that appeared was similar to the pattern seen in +B root tips. The cells of the quiescent center were inactive before, during, and after boron deficiency. Recovery occurred in surrounding cells which are responsible for meristematic activity.

In intact boron-deficient root tips, tritiated thymidine continued to be incorporated into DNA for as long as 20 hr after the start of the -B treatment period. No labeled division figures were seen either during the first 6.5 hr of the treatment period or after this time. Johnson (5) reported that, after 6.5 hr of -B treatment, mitotic figures were observed in 36.5% of squash root tip cells while after 6.5 hr or more of -B treatment, no mitotic figures were observed. The results of the treatments in which boron was withheld for periods of 3 to 18 hr showed that DNA synthesis continued while none to few cells were seen to enter mitosis. Both Johnson's and our results, considered together, show that the cessation of mitosis in boron-deficient roots occurred as early as 6.5 hr from the time that boron was withheld from the nutrient solution while synthesis of DNA continued in some cells for as long as 20 hr after boron was withheld.

Several experiments demonstrated that the ability of the cells of the root tip to incorporate label is correlated with the total root elongation during the -B treatment period. A greater amount of total root elongation during boron deficiency and recovery reflects the fact that the root tip cells have retained their ability to synthesize DNA and enter mitosis for a longer time.

The observations reported above are among the earliest biochemical changes of boron deficiency yet reported. It is now clear that cessation of root elongation observed in boron-deficient plants is a result of biochemical changes which have occurred in the root meristem which prevent the synthesis of DNA and subsequent mitosis. Root growth is attributable to the production of new cells (cell division) and the subsequent elongation of those cells. Under conditions of boron deficiency, cell division ceased; this was followed by the inability of the cells to incorporate tritiated thymidine and cessation of root growth. When boron was resupplied, the cells regained the ability to incorporate tritiated thymidine, cell division ensued, and root growth resumed. It was concluded that boron is essential for continued DNA synthesis and mitotic activity. The absence of boron ultimately results in the cessation of mitosis followed by the loss of ability to synthesize DNA within 20 hr from the time boron is withheld.

**LITERATURE CITED**