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Synthesis, Salvage, and Catabolism of Uridine Nucleotides in Boron-Deficient Squash Roots[1]

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ABSTRACT

Previous work has provided evidence that plants may require boron to maintain adequate levels of pyrimidine nucleotides, suggesting that the state of boron deficiency may actually be one of pyrimidine starvation. Since the availability of pyrimidine nucleotides is influenced by their rates of synthesis, salvage, and catabolism, we compared these activities in the terminal 3 centimeters of roots excised from boron-deficient and -sufficient squash plants (*Cucurbita pepo* L.). Transferring 5-day-old squash plants to a boron-deficient nutrient solution resulted in cessation of root elongation within 18 hours. However, withholding boron for up to 30 hours did not result in either impaired *de novo* pyrimidine biosynthesis or a change in the sensitivity of the *de novo* pathway to regulation by end product inhibition. Boron deprivation had no significant effect on pyrimidine salvage or catabolism. These results provide evidence that boron-deficient plants are not starved for uridine nucleotides collectively. Whether a particular pyrimidine nucleotide or derivative is limiting during boron deprivation remains to be examined.

More than 50 years have passed since Sommer and Lipman (32) presented conclusive evidence that boron is an essential micronutrient for a large number of plant species. Nevertheless, a definitive role for boron in plant metabolism still has not been determined. In recent years, it has become apparent that boron's primary role may be that of a necessary cofactor in certain enzymic reactions, one or more of which may influence nucleic acid biosynthesis. The incorporation of [3H]thymidine into the acid-insoluble fraction of root tips of intact 5-day-old squash plants was decreased significantly after only 6 h boron deprivation and was reduced by 66% when boron was withheld for an additional 6 h (19). Similar experiments using autoradiography revealed that the incorporation of [3H]thymidine into root apical meristems of 5-day-old squash plants ceased after 20 h boron deprivation (8). When these plants were returned to a boron-sufficient medium for 12 h, autoradiographs showed that their incorporation was indistinguishable from that of boron-sufficient root tips (8). These observations suggest that DNA synthesis is impaired under conditions of boron deficiency. Several workers (1, 7, 14, 16) have reported decreased RNA content in boron-deficient roots; in addition, measurements of the incorporation of radiolabeled precursors into RNA provide evidence that RNA synthesis is also impaired during boron deficiency. For example, Sherstnev and Razumova (29) reported decreased incorporation of [14C]adenine into RNA of boron-deficient sunflower leaves and roots; while other workers have demonstrated increased incorporation of [14C]uridine (7) and [14C]orotic acid (36) into RNA in boron-deficient mung bean root apices and cotton ovules, respectively. These reports suggest that the utilization or the level of available purine or pyrimidine nucleotides, or both, is altered by boron deprivation.

We were particularly interested in the observation (30) that plants growing in the absence of boron could be protected from developing boron-deficiency symptoms by adding a hydrolysate of yeast RNA to the nutrient solution. Work in our laboratory (2, 15, 16), and elsewhere (4, 5) tested the effects of both purine and pyrimidine bases on plant growth to determine which component(s) of the RNA hydrolysate afforded this protection. Intact plants and isolated organs cultured in the absence of boron were protected to varying degrees from developing boron deficiency symptoms when pyrimidine bases were added to the medium.

This result was taken as evidence that the state of boron deficiency may, in fact, be a case of pyrimidine starvation. Such an interpretation was supported by the observations that both barbituric acid and 6-azauracil, known inhibitors of pyrimidine biosynthesis (13, 25, 26), produced symptoms identical with those of boron deprivation (2, 5, 16). Various investigators (5, 19–21, 36) have suggested that boron deficiency results in impaired *de novo* biosynthesis of pyrimidine nucleotides. Such impairment could occur through either a loss in amount of one or more enzymes or enhanced sensitivity of the *de novo* pathway to end product inhibition. Starvation for pyrimidine nucleotides could also result from an inability of boron-deficient plants to salvage or reutilize pyrimidine bases or nucleosides, or from an acceleration of pyrimidine catabolism.

In this communication, we report the results of a comparison of the capacity of roots from boron-sufficient (+B)[3] and boron-deficient (−B) squash plants (*Cucurbita pepo* L., cv. Early Prolific straightneck) to synthesize uridine nucleotides *de novo*. We also include an examination of the possibility that boron deprivation interferes with the mechanism regulating the *de novo* pathway through end product inhibition. In addition, since catabolism opposes *de novo* biosynthesis, we also assessed the capacity of +B and −B roots to catabolize uridine and uracil. Finally, we measured salvage activity for the reutilization of pyrimidines during these two states of boron nutrition.

[3] Abbreviations: +B, 0.1 mg boron/l hydroponic culture solution, boron-sufficient; −B, boron withheld from the hydroponic culture solution, boron-deficient; ΣUMP, sum of total uridine nucleotides converted to UMP by acid hydrolysis.

MATERIALS AND METHODS

Chemicals. All radiolabeled chemicals were purchased from New England Nuclear. Mineral salts for Shive's nutrient solution were of analytical reagent quality from Fisher Scientific Co. All other chemicals were from Sigma.

Plant Materials. Summer squash seeds (*Cucurbita pepo* L., cv. Early Prolific Straightneck), supplied through the courtesy of the Joseph Harris Co., Inc., were imbibed in distilled H_2O for 24 h at room temperature. They were then spread evenly between two sheets of paper toweling placed in a plastic box (33 × 23 × 9.5 cm), and moistened with 7 ml H_2O. The covered box was placed in a growth chamber where the seeds germinated in the dark for 48 h at 31°C. After germination, the terminal 5 mm of the primary root was pinched off to promote lateral root formation, and the seedlings were inserted into holes in the lid of a polyurethane breadbox (38 × 14 × 13 cm, 5.5 L) covered with aluminum foil and containing Shive's nutrient solution. The box was transferred to a growth chamber where the plants were hydroponically cultured in aerated solution at 31°C under continuous illumination of 1500 ft-c. After 3 days, plants of uniform appearance were selected for treatment. The lateral roots of several plants were marked with India ink 10 mm from the tip to determine the rate of root elongation. The plants were then transferred to soft glass (boron-free) jars (1.8 L) containing either complete Shive's nutrient solution (0.1 mg boron/liter) (+B) or modified Shive's nutrient solution with the boron omitted (−B). The plants were transferred to a growth chamber (this was designated as T_0) and allowed to grow for specified periods in aerated solutions at 31°C under continuous illumination of 1500 ft-c. At the end of the treatment period, net elongation was determined and the terminal 5-mm or 3-cm segment of the root was excised and used as described below.

Determination of Cell Number. Root segments were harvested and immersed in 2.0 ml 5% chromic acid (w/v) in 1 N HCl and stored at 4°C. Immediately before counting, the cells were separated by repeatedly forcing the sample through a No. 22 needle. A sample was withdrawn and placed in a Levy-Houser deep well (0.2 mm) counting chamber with a Fuchs-Rosenthal grid. The number of cells in an area 0.2 × 1 × 1 mm counted at × 400. Cell number was determined for four samples for each root and two roots were examined in each experiment.

Measurement of *de Novo* Biosynthesis, Salvage and Catabolism of Uridine Nucleotides. Metabolic activities were assessed routinely in the intact cells of 500-mg samples of terminal 3-cm root segments excised from +B and −B squash plants. The activity of the *de novo* pathway of uridine nucleotide biosynthesis was assessed by measuring the incorporation of $NaH^{14}CO_3$ (10 mM, 6600 dpm/nmol) into ΣUMP as described previously (22). The capacity for salvaging preformed pyrimidine bases and nucleosides was assessed by measuring the incorporation of $[^{14}C_2]$uracil (5 mM, 860 dpm/nmol) and $[^{14}C_2]$uridine (5 mM, 140 dpm/nmol) into ΣUMP, with the total uridine nucleotides being converted to UMP by acid hydrolysis and isolated with carrier UMP as in measurements of *de novo* activity. Catabolic activity was measured simultaneously with salvage activity by determining the amount of $^{14}CO_2$ generated ✓ from ✓ the ✓ degradation ✓ of ✓ $[^{14}C_2]$uracil ✓ or ✓ $[^{14}C_2]$-uridine; the $^{14}CO_2$ was released upon acidification and trapped in 20% KOH on a filter paper wick in a plastic center well suspended from the stopper which sealed the reaction flask. The amount of radioisotope contained in the samples was measured in a Nuclear-Chicago model 300 liquid scintillation spectrometer.

RESULTS

Effects of Boron Deprivation on Root Growth. One of the earliest symptoms of boron deficiency is inhibition of root elongation. When 5-day-old squash plants hydroponically cultured in boron-sufficient (+B) medium are transferred to a boron-deficient

(−B) medium, a measurable reduction in the rate of root elongation occurs within 6 h (Fig. 1). By 18 h boron deprivation, root elongation has essentially stopped. Metabolic studies routinely employed root segments excised from 6-day-old squash plants which had been transferred to boron-deficient medium for the last 30 h culture. Root elongation was approximately 6 mm during this entire 30-h period of boron deprivation, with over half the growth occurring during the first 6 h. By comparison, root elongation in +B plants was 44 mm during the same period. Cessation in root growth in −B plants was accompanied by other visible changes: (a) increase in the diameter and weight per unit length of lateral root apices; (b) initiation of lateral root primordia almost to the root tip which develops a lateral hook; and (c) browning of the terminal root tissue. Apical root segments excised from plants deprived of boron for 24 and 30 h weighed 30% more than equivalent root segments from +B plants of the same age (Table I). This increase in weight was not due to an increase in cell number (Table I).

The viability of the −B plants was assessed by testing their ability to resume root elongation at a rate equal to that of the +B plants of the same age when transferred to +B nutrient solution. The rate of root elongation in plants deprived of boron for 24 and 30 h equaled that of +B plants within 24 h following transfer to +B medium (Fig. 2).

De Novo Biosynthesis of Uridine Nucleotides in +B and −B Roots. We previously demonstrated the occurrence of the complete orotic acid pathway in boron-sufficient squash roots (22), provided evidence that end product inhibition functions as a mechanism regulating the activity of the *de novo* pathway in this tissue (22), and characterized the activity of this pathway in roots excised from 2-day-old and 6-day-old plants (22, 23). The activity of the orotate pathway in 6-day-old roots was approximately 2% of that observed in 2-day-old roots. There is indirect evidence that the reduced activity in older plants is due, at least in part, to a greater pool size of end product inhibitors of the pathway (23). This evidence includes (a) the insensitivity of incorporation of
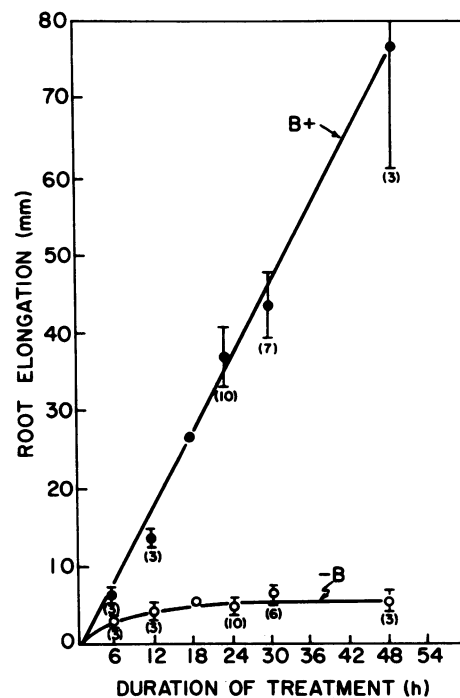

FIG. 1. Root elongation in mm ± SE with number of observations given in parentheses in 5-day-old squash plants transferred to +B nutrient solution (0.1 mg boron/l) (●) and to −B nutrient solution (boron withheld) (○).

Table I. *Fresh Weight and Cell Number for 5-mm and 3-cm Terminal Root Segments Excised from +B and −B Squash Plants*

Plants were 5 days old when treatment started; +B (0.1 mg boron/1), −B (boron withheld). Each value is the average ± SE of four separate experiments for which five different aliquots each containing 10 root segments were weighed. For each assay of cell number, four determinations were made at × 400 on four separate samples of each root, and the average of these 16 cell counts constituted the cell count for that assay. Each result reported above is the average value of several such assays ± SE with the number of such assays given in parentheses.

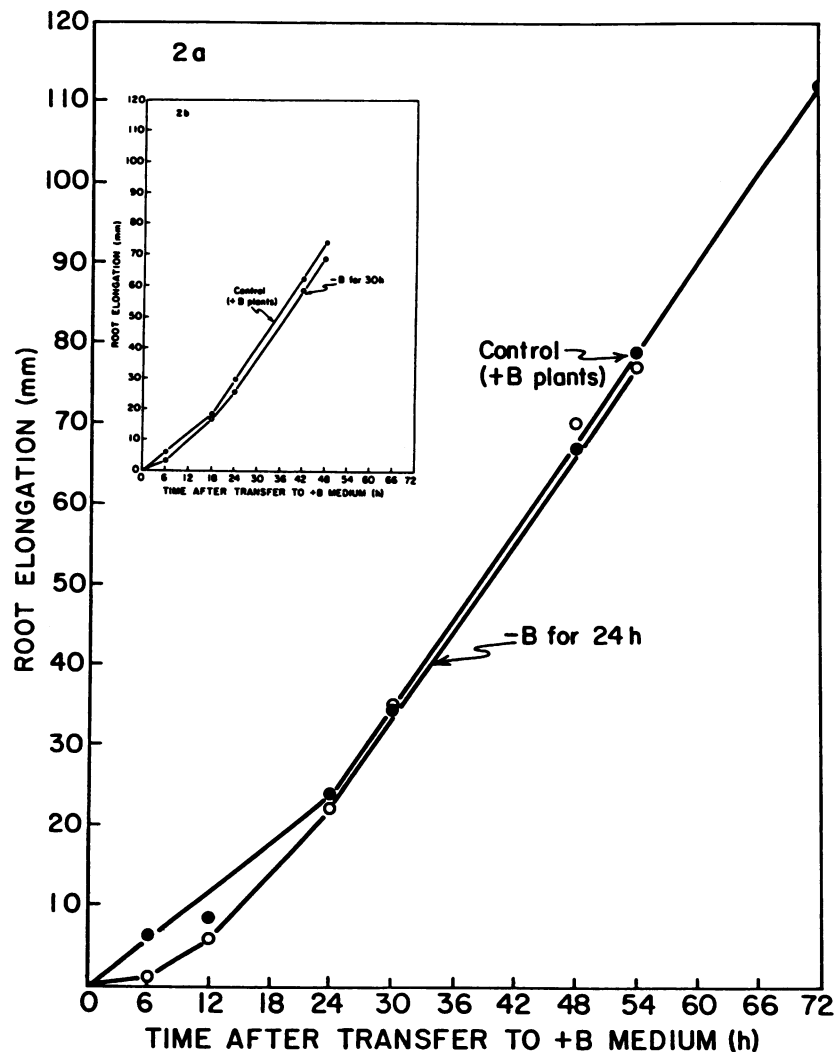| Time	Treatment Condition	Weight at Segment Length		Cell number ± SE × 10^{-4} at Segment Length	
		5 mm	3 cm	5 mm	3 cm
h		*mg/10 root segments ± SE*			
24	+B	7.0 ± 0.3 (4)	46.8 ± 4.2 (4)	6.4 ± 0.8 (6)	8.7 ± 0.6 (5)
24	−B	9.3 ± 0.3 (4)	61.5 ± 3.6 (4)	6.3 ± 0.8 (6)	7.6 ± 1.2 (6)
30	+B	7.3 ± 0.5 (4)	47.7 ± 1.9 (4)	6.7 ± 0.2 (4)	9.1 ± 0.9 (5)
30	−B	10.6 ± 0.9 (4)	63.5 ± 2.1 (4)	4.3 ± 0.3 (4)	9.5 ± 1.1 (6)

FIG. 2. Recovery from boron deprivation. Five-day-old squash plants were transferred to +B nutrient solution (0.1 mg boron/1) designated as control (●) and to −B nutrient solution (boron withheld) (○). At the end of 24 h (2a) and at the end of 30 h (2b), both +B and −B plants were transferred to fresh nutrient solution containing 0.1 mg boron/1.

NaH^{14}CO$_3$ into ΣUMP to exogenous uridine in the older plants, while such additions reduce the incorporation in 2-day-old roots by 80%; (b) the greater stimulation on a percentage basis of *de novo* activity in older plants by adding sugars which presumably deplete end product inhibitor(s) during the synthesis of pyrimidine nucleotide-sugars (only results with exogenous sucrose are re- ported here); and (c) the antagonism of the effect of added sugars by exogenous uridine (Table II). While verification of our hypothesis regarding the mechanism by which various sugars stimulate pyrimidine biosynthesis is part of a future study, the observation with added sucrose is useful to the present study in a comparison of the capacity of +B and −B plants to make uridine

Table II. *Influence of Sucrose and Uridine on Incorporation of $NaH^{14}CO_3$ into ΣUMP*

Sucrose was added to the preincubation and incubation mixtures at a final concentration of 100 mM in each case. Uridine, when added, was added to the incubation mixture only at a final concentration of 0.5 mM. $NaH^{14}CO_3$ was provided in the incubation mixture at a concentration of 10 mM and at a specific radioactivity of 1650 dpm/nmol and 6600 dpm/nmol for experiments employing 2-day-old and 6-day-old roots, respectively. When the results for more than two experiments are reported, the values are shown as averages ± SE with the number of observations given in parentheses.

Additions	2-day-old roots	6-day-old roots
	nmol $NaH^{14}CO_3$ incorporated into $\Sigma UMP/g$ tissue·3 h	
Control	144 ± 10 (15)	2.4 ± 0.1 (11)
Uridine	24 ± 4 (8)	2.6 ± 0.2 (3)
Sucrose	209, 162	19.0 ± 2.0 (5)
Sucrose and uridine	61, 41	4.0 ± 2.0 (4)

Table III. *Sensitivity of Orotate Pathway in Intact Cells of Terminal 3-cm Root Segments from +B and −B Squash Plants to Uridine and Sucrose*

Plants were 5 days old at the initiation of the 30-h treatment period; +B (0.1 mg boron/1) and −B (boron withheld). Sucrose was added to the preincubation and incubation mixtures at the concentrations indicated. Uridine, when added, was added to the incubation mixture only, at a final concentration of 0.5 mM. $NaH^{14}CO_3$ was provided in the incubation mixture at a final concentration of 10 mM and at a specific radioactivity of 6600 dpm/nmol. The data represent the pooled results of three separate experiments.

Additions	+B		−B	
	−Uridine	+Uridine	−Uridine	+Uridine
	nmol $NaH^{14}CO_3$ incorporated into $\Sigma UMP/g$ tissue·3h[a]			
Control	100	101	100	99
Sucrose, 100 mM	748	175	890	193
Sucrose, 10 mM	278	170	305	190

[a] Expressed as a percentage of the +B, −uridine control.

nucleotides.

The basal level of pyrimidine biosynthesis was not altered by 30 h of boron deprivation; incorporation of $NaH^{14}CO_3$ into ΣUMP was 2.3 ± 0.2 and 2.1 ± 0.1 nmol/g tissue (average value ± SE; n = 6) during the 3-h incubation of terminal 3-cm root segments from +B and −B plants, respectively. In addition, pyrimidine biosynthesis was accelerated to a comparable extent by sucrose in both states of boron nutrition, and uridine was equally effective in antagonizing the action of sucrose in both cases (Table III). These responses and the additional observation that exogenous uridine alone is without effect on pyrimidine biosynthesis in both boron-deficient and boron-sufficient roots, provide evidence that the orotate pathway and its regulation are not impaired during boron deprivation. In addition, they suggest that the level of the pyrimidine end product responsible for the feedback inhibition is similar in both states of boron nutrition.

Salvage of Uracil and Uridine in +B and −B Roots. It was established previously that plants utilize exogenously supplied pyrimidine bases and nucleosides for nucleotide biosynthesis (27). In this study, incorporation of [$^{14}C_2$]uracil and [$^{14}C_2$]uridine into ΣUMP in intact cells of terminal 3-cm root segments excised from −B plants was essentially the same as that observed for roots from +B control plants (Table IV).

Catabolism of Uracil and Uridine in +B and −B Roots. Available evidence indicates that plants and mammals catabolize pyrimidines similarly, via the dihydrouracil catabolic pathway (28,

Table IV. *Salvage and Catabolism of Pyrimidines in Intact Cells of Terminal 3-cm Root Segments Excised from +B and −B Squash Plants*

Plants were 5 days old at the initiation of the 30-h treatment period; +B (0.1 mg boron/1) and −B (boron withheld). With the exception of the salvage and catabolism of [$^{14}C_2$]uridine, all results are reported as averages ± SE with the number of observations given in parentheses.

Treatment	+B	−B
	Salvage	
nmol [$^{14}C_2$]uracil incorporated into $\Sigma UMP/g$ tissue·3 h		
	7.7 ± 0.5 (3)	7.9 ± 0.6 (3)
nmol [$^{14}C_2$]uridine incorporated into $\Sigma UMP/g$ tissue·3 h		
Exp 1	84	100
Exp 2	72	66
	Catabolism	
nmol $^{14}C_2$ generated from [$^{14}C_2$]uracil/g tissue·3 h		
	442 ± 26 (3)	502 ± 39 (3)
nmol $^{14}CO_2$ generated from [$^{14}C_2$]uridine/g tissue·3 h		
Exp 1	738	1045
Exp 2	512	672

37). The occurrence of this pathway in the intact cells of squash roots was indicated by generation of $^{14}CO_2$ from [$^{14}C_2$]uracil and [$^{14}C_2$]uridine. The generation of $^{14}CO_2$ from either [$^{14}C_2$]uracil or [$^{14}C_2$]uridine was only slightly elevated by boron deprivation (Table IV).

We also examined the possibility that changes in pyrimidine metabolism resulting from different states of boron nutrition may have been obscured by our using 500-mg samples of terminal 3-cm root segments. Available evidence demonstrates that boron is essential for the normal functioning of root apical meristems (1, 3, 6, 9, 15, 16, 24, 31). However, little is known about the sensitivity of root cells in the elongation and differentiation zones to boron deprivation. One study addressed this question. Using squash roots, Cohen and Lepper (9) confirmed that withholding boron for 24 h resulted in cessation of mitosis in the apical meristem (8, 15), but found that cell elongation and differentiation in squash roots were not altered by boron deficiency for up to 72 h. Thus, withholding boron for 30 h may result in impaired pyrimidine metabolism only in the root apex. This alteration could be masked in our assays employing 3-cm terminal root segments by 2.5 cm tissue which may still be capable of normal pyrimidine metabolism. In addition, since 3-cm root segments from −B plants weigh more, but have approximately the same number of cells as +B root segments (Table I), fewer cells are contained in the assays employing 500 mg 3-cm root segments from −B plants. We tested the possibility that these variables might have obscured changes in pyrimidine metabolism brought about by boron deprivation by repeating the assays with a fixed number of root tips (250) comprising only the apical 5 mm. While the bulk of activity was, indeed, in the apical 5 mm (*e.g.* de novo synthesis was 4 times greater per mm and 6 times greater per mg apical root tissue than subapical tissue), our findings were consistent with those obtained with 500-mg samples of terminal 3-cm root segments (Table V). There was no evidence of impairment of pyrimidine biosynthesis in either the apical 5-mm root tips or in the following subapical 2.5-cm segments resulting from boron deprivation. In addition, the following factors were taken as evidence that the regulation of the orotate pathway through end product inhibition also was not altered in the apical 5-mm root segments from boron-deficient plants: (a) the activity of the orotate pathway was insensitive to the addition of 0.5 mM uridine; (b) the pathway's activity was stimulated 13-fold by the addition of 100 mM sucrose; and (c) this

Table V. *Pyrimidine Metabolism in Intact Cells of Apical 5-mm Root Segments and the Corresponding Subapical 2.5-cm Root Segments Excised from +B and −B Squash Plants*

Plants were 5 days old at the start of the treatment; +B (0.1 mg boron/1) and −B (boron withheld); $NaH^{14}CO_3$ (10 mM, 8000 dpm/nmol); $[^{14}C_2]$uracil (5 mM, 1250 dpm/nmol).

Time	Treatment Condition	Segment Size and Type		
		5-mm root tips		2.5-cm segments subapical to root tips in Exp. 2
		Exp. 1	Exp. 2	
		Biosynthesis *de novo*		
		nmol $NaH^{14}CO_3$ incorporated into $\Sigma UMP/250$ root segments·3 h		
h				
24	+B	0.8	1.2	1.3
	−B	1.2	1.6	1.4
30	+B	1.1	0.8	1.1
	−B	1.6	1.8	1.4
		Salvage		
		nmol $[^{14}C_2]$uracil incorporated into $\Sigma UMP/250$ root segments·3 h		
24	+B	5.4	7.9	6.6
	−B	4.7	7.6	6.3
30	+B	5.3	6.9	7.0
	−B	5.0	6.4	5.9
		Catabolism		
		nmol $^{14}CO_2$ generated from $[^{14}C_2]$uracil/250 root segments·3 h		
24	+B	234	269	470
	−B	340	256	495
30	+B	215	235	454
	−B	331	301	569

stimulation was reduced to 1.5-fold when uridine was included in the incubation mixture with sucrose (data not shown).

DISCUSSION

Withholding boron from 5-day-old squash plants for only 18 h results in the cessation of root elongation. This inhibition of root elongation correlates temporally with the cessation of mitosis and a loss of capacity to synthesize DNA in the root apex (8, 15, 19). Cessation of elongation resulting from boron deficiency can be partially overcome in roots (16) and cotton fibers (5) by the addition of various pyrimidine nucleosides and bases. Symptoms characteristic of boron deficiency can also be induced by the addition of 6-azauracil or barbituric acid, known inhibitors of pyrimidine biosynthesis (13, 25, 26). These observations strongly suggested to us (19, 21) and others (5, 20, 36) that boron plays a fundamental role in pyrimidine metabolism. The hypothesis that boron is essential for maintaining adequate levels of pyrimidine nucleotides unifies several of the roles for boron reported in the literature. The ability of pyrimidine bases to protect plants cultured in the absence of boron from developing the symptoms of boron deprivation and the effectiveness of 6-azauracil in mimicking the symptoms of boron deficiency have been observed in dividing cells without concomitant maturation (root meristem cells of intact tomato and squash plants [2, 15, 16]) and in elongating cells which do not undergo cell division (*in vitro* cotton fiber development [4, 5]). Boron deprivation has also been shown to influence carbohydrate metabolism. Dugger and co-workers (5, 10, 36) have provided evidence that boron deprivation results in reduced UDP-glucose formation. This would result in reduced sucrose synthesis (10), increased starch formation (11), and inter-

ference with normal cell wall formation (12, 17, 18, 33–35), all of which typify boron deficiency. Since pyrimidine nucleotides are involved directly in the biosynthesis of UDP-glucose and other nucleotide sugars, the hypothesis that boron is essential to the maintenance of adequate levels of pyrimidine nucleotides also unifies the seemingly disparate roles for boron in nucleic acid biosynthesis and carbohydrate metabolism.

The results of this study provide evidence that there is no impairment of pyrimidine synthesis, salvage or catabolism under conditions of boron deprivation. These results argue against the hypothesis that −B plants are starved for uridine nucleotides collectively, but leave open the possibility that boron may be essential for maintaining adequate levels of one or more specific pyrimidine nucleotide species. The present study shows that boron deprivation does not impair the activity of the orotate pathway for the *de novo* biosynthesis of uridine nucleotides, at least not in the intact cells of roots excised from 5-day-old squash plants deprived of boron for 30 h. Nor did boron deprivation interfere with the mechanism regulating the activity of the *de novo* pathway through end product inhibition. Whether boron deprivation leads to an impairment of the utilization of uridine nucleotides for the synthesis of cytidine or thymidine nucleotides or an alteration in the interconversion of nucleotides for the provision of adequate levels of pyrimidine mono-, di-, and triphosphates remains to be examined.

In two separate experiments, the incorporation of $NaH^{14}CO_3$ into ΣUMP was stimulated 7-fold by adding 0.1 M sucrose; stimulation of pyrimidine biosynthesis by added sucrose is consistent with release of the orotate pathway from end product inhibition as the end product inhibitor is depleted during the formation of pyrimidine nucleotide-sugars. It may be significant that sucrose was as effective in stimulating *de novo* activity in +B roots as in −B roots. This observation provides indirect evidence that depletion of feedback inhibitors through the synthesis or pyrimidine nucleotide-sugars occurs at a similar rate in both states of boron nutrition and provides further evidence that the capacity of −B roots to synthesize pyrimidine nucleotides *de novo* is not impaired. However, stimulation of *de novo* uridine pyrimidine biosynthesis by sugars must not be taken as evidence that the formation of all species of pyrimidine nucleotide-sugars is normal in −B tissue. Indeed, reports that UDP-glucose formation is reduced under boron-deficient conditions (5, 10, 36) underscore the need to identify the pyrimidine nucleotide-sugars formed when roots from +B and −B plants are provided with sucrose.

Withholding boron from 5-day-old squash plants for 24 or 30 h had no effect on the capacity of roots to salvage uracil or uridine, and the catabolism of these two compounds was either unaffected or only slightly increased. Whether a slight increase in catabolism would cause significant perturbations in the pool size of specific pyrimidine nucleotides is not known, but emphasizes the need to determine the levels of the various pyrimidine nucleotides available during these two states of boron nutrition.

Thus, two possibilities remain to be investigated: (a) whether the interconversions of UMP, UDP, UTP, and UDP-glucose or the synthesis of the ribonucleotides and deoxyribonucleotides of cytidine or thymidine are impaired under conditions of boron deficiency, and (b) whether the utilization of a particular pyrimidine nucleotide in a specific metabolic process might be impaired under conditions of boron deficiency.

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