Phytochemistry, 1974, Vol 13, pp 1311 to 1318 Pergamon Press Printed in England

INCREASED RNA LABELLING IN BORON-DEFICIENT ROOT-TIP SEGMENTS

K. S. R. CHAPMAN* and J. F. JACKSON
Department of Agricultural Biochemistry, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, South Australia 5064, Australia

(*Received* 19 *November* 1973)

Key Word Index—*Phaseolus aureus*, Leguminosae, mung bean, boron deficiency, RNA metabolism, nucleotides, trace elements

Abstract—The rate of incorporation of radioactive precursors into RNA of root-tip segments has been shown to increase during the early stages of boron deficiency in 6- to 8-day-old *Phaseolus aureus* seedlings This effect is observed well before morphological changes occur, and before both the decrease in level of total RNA and increase in ribonuclease activity can be detected. The nucleotide pool in root tips was too small to measure directly on the rapidly growing seedlings, although it was easily measurable in the slower growing 12- to 15-day-old plants The available indirect evidence suggests, however, that the early effect is not due to changes within the total nucleotide pool

INTRODUCTION

DESPITE a great deal of research on physiological aspects of boron deficiency, the primary site of action of this element is still not known. In contrast to other trace elements, boron has not been demonstrated to be a component or activator of any enzyme system.

Boron deficiency has been shown to affect nucleic acid metabolism. Thus, grossly deficient plants show a reduction in RNA content,[1,2] a lower ATP level,[3] and an increase in ribonuclease activity.[4,5] These effects are recorded only after relatively long periods of growth in deficient medium and therefore may not be good pointers to the principal mode of action of boron. A much earlier effect on RNA metabolism has been demonstrated by Cory, Finch and Hinde[6] and Cory and Finch.[7] They showed that transferring *Vicia faba* seedlings to boron-deficient medium followed by incubation with ^{32}P-inorganic phosphate resulted in an increase in the specific activity of root-tip ^{32}P-RNA over that of control plants maintained in complete medium. The specific activity of the total nucleotide pool was not changed, suggesting an increased rate of RNA synthesis under these conditions.

This paper describes the effects of boron deficiency on nucleic acid metabolism in *P. aureus* seedlings. Incorporation of ^{14}C-uridine and ^{32}P-inorganic phosphate into RNA and acid-soluble nucleotides has been studied in root-tip segments, and levels of ribonuclease and uridine kinase activity determined A distinction is drawn between early and late effects of boron deficiency in interpreting the results.

* Department of Botany, University of Edinburgh, Scotland

[1] ALBERT, L S (1965) *Plant Physiol* **40,** 649
[2] JAWEED, M M and SCOTT, E G (1967) *Proc W Virginia Acad Sci* **39,** 186
[3] SHKOL'NIK, M YA. and MAEVSKAYA, A N (1962) *Dokl Akad Nauk. SSSR* **145,** 222
[4] SHERSTNEV, E A and RAZUMOVA, M V (1965) *Agrochimica* **9,** 348
[5] ABROL, Y. P (1966) *Indian J Biochem* **3,** 263
[6] CORY, S FINCH, L R and HINDE, R W (1966) *Phytochemistry* **5,** 625
[7] CORY, S and FINCH, L R (1967) *Phytochemistry* **6,** 211

RESULTS

Morphological effects

These observations were made in order to put the effects of inadequate boron on nucleic acid metabolism into perspective on a time scale with the better known effects on the morphology of the plant As shown in Fig 1, boron deficiency under the conditions used results in an inhibition of the rate of root elongation after 24 hr and complete cessation after 120 hr of culture Just prior to this (50–100 hr), a thickening of the root tips was observed, which was largely due to an increase in cell size Following this (at about 100 hr), the normally white root tips began to turn brown The usual symptoms of boron deficiency developed in the aerial parts of the plant a long time after the above effects were observed in the root system.

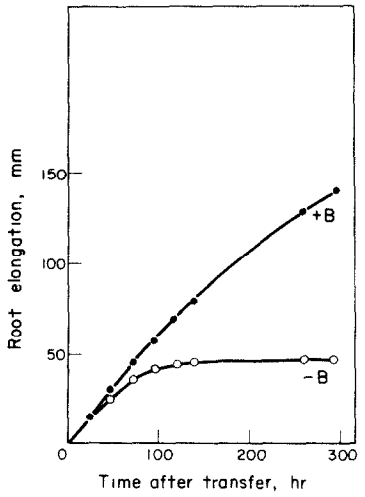

FIG. 1. EFFECT OF BORON DEFICIENCY ON ROOT ELONGATION

Roots were marked with a mixture of finely ground charcoal in lanoline at the time of transfer to deficient medium, and the subsequent elongation measured Measurements from plants transferred to a boron-deficient medium are marked – B, those to a complete medium, + B

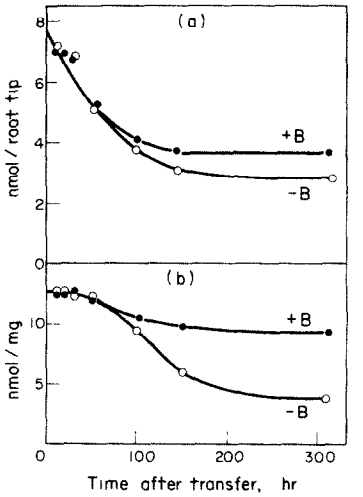

FIG. 2. EFFECT OF BORON DEFICIENCY ON RNA CONTENT OF 5 mm ROOT TIPS

RNA was extracted from 250–300 root tips by the Schmidt–Thannhauser method and determined by (i) absorption at 260 nm, (ii) ribose method and (iii) phosphate method Mean values from the three methods are plotted (a) RNA (as nmol nucleotide content) per root tip (b) RNA (as nmol nucleotide content) per mg (f.w.) of root tips

Nucleic acid content

The RNA content of root tips was found to be decreased by boron deficiency (Fig 2). This effect appeared only after relatively long periods of growth in deficient medium (at about the same stage as morphological symptoms were observed in the root tip) These results are similar to those obtained by Albert[1] and Jaweed and Scott[2] for sunflower root tips. In contrast to RNA, the DNA content was seen to increase, but again only after relatively long periods in the deficient medium. The DNA content per root tip had almost doubled by 200–300 hr following transfer to a boron-deficient medium.

Incorporation of ^{14}C-uridine into RNA and acid-soluble fractions

It is apparent from Fig 3 that, while incorporation of ^{14}C-uridine into the RNA fraction of root-tip segments continues to decline with increasing age after transfer of plants to a

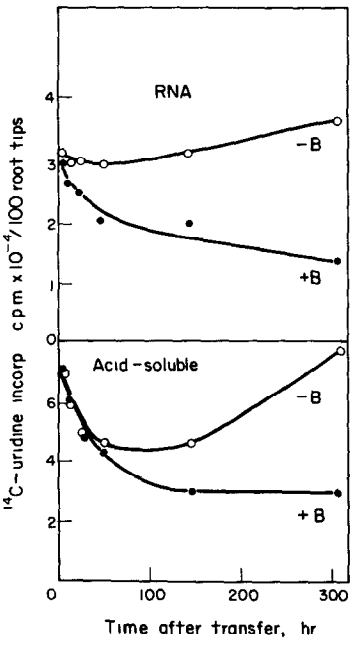

FIG 3 EFFECT OF BORON DEFICIENCY ON UPTAKE AND INCORPORATION OF ^{14}C-URIDINE
100–200 root tips were incubated in ^{14}C-uridine, extracted and separated into RNA and acid-soluble fractions by the Schmidt–Thannhauser method

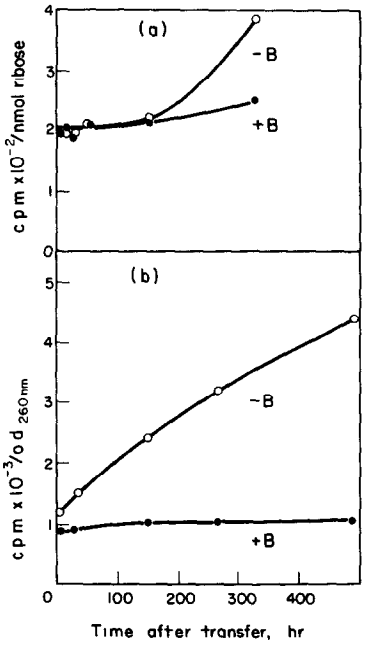

FIG 4 EFFECT OF BORON DEFICIENCY ON THE SPECIFIC ACTIVITY OF RNA AND THE ACID-SOLUBLE FRACTION FOLLOWING ^{14}C-URIDINE INCORPORATION
(a) 100–200 root tips were incubated with ^{14}C-uridine and the acid-soluble fraction extracted by the Schmidt-Thannhauser procedure (b) 400–600 root tips were incubated with ^{14}C-uridine and the RNA extracted by the phenol method

medium containing adequate trace element, transfer to a boron-deficient medium arrests this decline and eventually leads to an increase. Roots from plants removed to the deficient medium have higher incorporation values than their normal counterparts as little as 6 hr after transfer. Hydrolysis of the RNA fraction in 0·3 N KOH followed by separation of the constituent nucleotides on Dowex-1 showed that approx. 40% of the label was incorporated into RNA as the CMP moiety, and that this proportion was not significantly altered by boron deficiency. Incorporation of ^{14}C-uridine into the acid-soluble fraction is also higher when plants transferred to the deficient medium are compared with those transferred to the normal medium (Fig. 3). However, in this case, significantly higher values are not encountered until at least 48 hr after transfer.

The specific activity of the label from ^{14}C-uridine incorporated into RNA and the acid-soluble fraction of root-tip segments is increased by removal of plants to a boron-deficient medium (Fig. 4), while plants transferred to the normal medium give relatively constant specific activities with increasing age. As for total incorporation, the specific activity of RNA increases at a much earlier stage of deficiency than does the acid-soluble fraction, although it should be noted that the measurement of the specific activity of the acid-soluble fraction is based on ribose levels and will be influenced by the reaction of ribose (and other interfering substances) not associated with nucleic acids. The patterns of incorporation of ^{32}Pi are affected by boron deficiency in a similar manner to those for ^{14}C-uridine.

At 24, 48, 144 and 312 hr after transfer to the deficient medium, the increase in specific activity of ^{32}P-RNA in root tips is 53, 69, 100 and 202% respectively.

To test the specificity of the effect of boron deficiency on ^{14}C-uridine incorporation patterns, three other trace element deficiencies were studied. Although inadequate iron, manganese and copper resulted in changes in these patterns, the changes obtained are quite different from those of boron deficiency (Table 1). Thus, manganese and copper deficiencies gave rise to increased uptake into the acid-soluble fraction within the first 48 hr without significantly increased incorporation into the RNA fraction The latter did show a relatively small increase at later stages of deficiency. Despite suggestions that the effects of iron deficiency on growth, cell division and DNA and RNA contents of pea root tips are similar to those of boron deficiency,[8] iron deficiency in *P. aureus* seedlings shows an early inhibition of incorporation into root-tip RNA and no significant effect on uptake into the acid-soluble fraction until the very late stages of deficiency (Table 1)

TABLE 1 EFFECTS OF TRACE ELEMENT DEFICIENCIES ON ^{14}C-URIDINE UPTAKE AND INCORPORATION

Deficient element	Time after transfer to deficient medium (hr) 12	24	48	144	336
		Acid-soluble			
Iron	1	1 21	0·97	0·90	1 85
Manganese	—	1 34	1 42	1 58	1 10
Copper	—	1 08	1 27	1 77	1·33
Boron	1	1 02	1 08	1 57	2 62
		RNA			
Iron	0 75	0 55	0 60	0 62	1 06
Manganese	—	0·98	0 92	1 30	1 56
Copper	—	0 88	1 10	1 32	1 08
Boron	1 07	1 24	1 50	1 52	3 23

Deficient and normal root tips (150 of each) were incubated in ^{14}C-uridine for 4 hr and the acid-soluble and RNA fractions extracted and separated by the Schmidt-Thannhauser method Results are expressed as the ratio —

$$\frac{\text{counts incorporated into 150 deficient root tips}}{\text{counts incorporated into 150 normal root tips}}$$

All samples incorporated at least 10^4 cpm into each fraction

Nucleotides

Root tips from 6- to 8-day-old *P aureus* seedlings used for the present boron deficiency studies were found to contain levels of nucleotides too low to be detected by UV spectrophotometry after chromatography on Dowex-1 or DEAE-Sephadex, even if extracts from as much as 500 mg of root tips were used. That this result is not due to degradation during extraction is shown by the recovery of 77 pmol ^{14}C-UTP after chromatography from 86 pmol ^{14}C-UTP added to the medium used to extract 500 mg root tips. The same extraction procedures yielded appreciable amounts of nucleotides when applied to the root tips of 12- to 15-day-old plants and to mature root tissue (e.g. approx. 100 nmol ATP/g mature root tissue compared with an estimated figure of less than 10 nmol ATP/g tissue for 6- to 8-day-old root tips). Although many have reported on the nucleotide content of roots, there seems to be little information available on changes in the root tip (and thus the meris-

[8] ABBOTT, A J (1972) *New Phytol* **71**, 85

tematic region) during growth and development of the plant. Our results suggest that there is a dramatic increase in the size of the pool as the plant matures, the pool being smallest at the time (6- to 8-day-old plants) of most rapid root growth (Fig. 1). It was considered impractical to attempt to isolate much more than 500 mg of tissue for nucleotide studies since this figure represents approximately 1000 root tips. Hence, it was not feasible to determine the specific radioactivity of the nucleotides following incorporation of ^{14}C-uridine or ^{32}Pi in an attempt to explain increased radioisotope incorporation into RNA in boron-deficient root tips.

It was possible to measure in some cases the incorporation of ^{14}C-uridine and ^{32}Pi into the various nucleotide fractions. At relatively early stages of deficiency (24 and 48 hr) the amount of ^{14}C-uridine incorporated into nucleotides is very small, except for a fraction corresponding to UDPG. Boron deficiency had no significant effect on this pattern. After 144 hr of culture (when the plants are 11 to 12 days old), higher amounts of ^{14}C-uridine are incorporated into nucleotide fractions. As shown in Table 2, boron deficiency did not significantly affect the distribution of ^{14}C-label among the various fractions. With ^{32}Pi,

TABLE 2. DISTRIBUTION OF ^{14}C IN NUCLEOSIDES AND NUCLEOTIDES OF 144 HR DEFICIENT ROOT TIPS FOLLOWING ^{14}C-URIDINE INCORPORATION

Nucleotide or nucleoside	Radioactivity (cpm)		% of Total	
	+B	−B	+B	−B
Nucleosides	7838	8398	74	72 1
CMP	95	57	0·9	0·5
UMP + UDPG	1279	1620	11 1	14
CDP	64	50	0·6	0·4
UDP	293	383	2·8	3 3
CTP	237	188	2 2	1·6
UTP	884	947	8·4	8·1

greater amounts of radioactivity are incorporated into the various nucleotide fractions and, as for ^{14}C-uridine, these increase with the age of the plant. The distribution of radioactivity over the various nucleotides is not significantly altered by boron deficiency; however, by 144 hr in boron-deficient medium, root-tip segments show a general increase in label incorporated into all nucleotides, an effect not shown at 48 hr or earlier. These results are illustrated for the uridine and adenosine nucleotides in Table 3.

TABLE 3. DISTRIBUTION OF ^{32}P BETWEEN ADENOSINE AND URIDINE NUCLEOTIDES

Time after transfer to boron-deficient medium (hr)	Treatment	ADP + UDP		ATP + UTP	
		cpm	% total	cpm	% total
48	+B	39633	38·2	63991	61·8
	−B	33807	38·3	54516	61·7
144	+B	71068	26·5	197996	73·5
	−B	93327	23·9	297604	76·1

Ribonuclease and uridine kinase activities

Boron deficiency leads to a sharp increase in ribonuclease activity, but only after at least 100 hr of growth in deficient medium (Fig. 5). This is the stage at which the RNA level

begins to decline (Fig. 2) and slightly precedes the cessation of root elongation (Fig. 1). It is possible then that the fall in RNA content, a relatively late effect of boron deficiency, is related to increased RNAse activity. The other enzyme tested was uridine kinase; this did show a significant increase, but only after relatively long periods of boron deficiency (and only if calculated on a root-tip basis) (Table 4). This is consistent with the increase in incorporation of ^{14}C-uridine into nucleotides of root tips after relatively long periods of growth in deficient medium (Table 2) and could represent an increase in activity of salvage enzymes at a time when RNA breakdown has increased along with elevated ribonuclease levels.

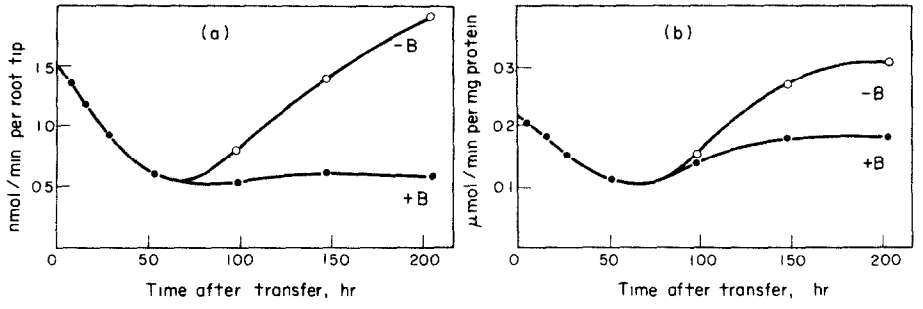

FIG 5 EFFECT OF BORON DEFICIENCY ON RIBONUCLEASE ACTIVITY
100 root tips were harvested for each determination, and the ribonuclease activity of crude extracts determined at pH 5·4 (a) nmol/min/root tip, (b) μmol/min/mg protein

DISCUSSION

The decrease in the level of RNA in root tips reported for other plants subjected to boron deficiency has been confirmed in *P aureus* seedlings. Time-course studies indicated that this only occurs at about the stage when root elongation ceases, i.e. at a relatively late stage of boron deficiency. The lowering of RNA can be correlated with an increase in ribonuclease activity, and these changes are similar to those which occur in maturing and senescing plant tissue.[9,10] It is tempting therefore to attribute these late effects of boron deficiency to an accelerated rate of maturation and senescence in deficient root tips.

TABLE 4 EFFECT OF BORON DEFICIENCY ON URIDINE KINASE ACTIVITY

Time after transfer to boron-deficient medium (hr)	Treatment	pmol UMP produced per hr		
		Protein (mg)	Tissue (mg)	root tip
49	+B	943	14·3	5·18
	−B	1040	16·2	6·24
91	+B	1455	19·9	5·82
	−B	1292	20·4	8·02

During the early stages of boron deficiency before morphological changes become visible (6 to 24 hr after the transfer to the deficient medium), the rate of incorporation of radioactive precursors into the RNA of root-tip segments is increased. That this early in-

[9] MCHALE, J S and DOYLE, L D (1968) *New Phytol* **67**, 505
[10] PHILLIPS, D R and FLETCHER, R A (1969) *Physiol Plant* **22**, 764

crease takes place without any apparent effect on the total precursor pool is suggested by the observation that the total level of radioactivity in the acid-soluble fraction and its distribution between the different nucleotides are not significantly changed until at least 48 hr after transferring plants to deficient medium. The absence of any significant early effect on uridine kinase activity or the distribution of ^{14}C (introduced as ^{14}C-uridine) between UMP and CMP within the newly synthesized RNA also suggests that boron deficiency does not affect the total nucleotide pool. As there is no evidence of altered degradation rates during these early stages of deficiency, the increased incorporation of radioactivity into RNA could be due either to changes in the properties of small specific nucleotide pools or to an increased rate of synthesis of all or certain species of RNA. The properties of the RNA into which increased incorporation of radioactive precursor is observed will be examined in a subsequent paper. It should be noted here, however, that several polyacrylamide-gel electrophoretic fractionations showed the absence of radioactivity in positions corresponding to 16S or 23S RNA, ruling out bacterial contamination as the cause of increased RNA labelling. Another situation leading to increased incorporation of ^{14}C-nucleoside or ^{32}Pi into RNA is that of auxin treatment of intact or excized plant tissue.[11–16] This lends some support to suggestions[2,17] of a possible relationship between hormones and the mechanism of action of boron.

EXPERIMENTAL

Culture of Phaseolus aureus Nutrient solns were prepared using double glass-distilled H_2O which was further distilled in a silica apparatus, passed through a millipore filter (0·2 μm pore size) and stored in sealed plastic containers All equipment was either autoclaved or surface-sterilized with EtOH (or conc. HCl) and exposed to UV light for at least 30 min *P. aureus* seeds were surface-sterilized with EtOH for 1 min and with 0·2% (w/v) sodium hypochlorite for 1 hr, then rinsed three times with sterile H_2O and imbibed overnight The seeds were germinated in the dark on moist blotting paper (previously irradiated with UV light) at 30° for 72 hr in covered containers Seedlings were grown in 10 l black plastic containers from the 3 to 5 cm long radicle stage, as described by Neales [18] Filtered air was bubbled from four silica tubes in each container and the day length maintained at 16 hr. with day and night temps of 25 and 20° respectively Initially all the plants were grown in a complete medium,[19] but after 5 or 6 days, half the plants were placed in a medium deficient in the trace element and the other half in fresh complete medium Each medium was changed every 5 to 6 days thereafter

Incorporation of labelled precursor into excized root tips Excized root tips (each 5 mm) were incubated at 30° for 4 hr in plastic test-tubes (1·35 × 10 cm) in 2 ml of nutrient medium (with or without boron) containing the radioactive precursor, 50 units/ml of Penicillin G and 2% (w/v) sucrose When $^{32}P_1$ was used, the nutrient medium was diluted 4-fold before the incubation to give a sp. act of 3·0 mCi/μmol (500 μCi/ml) for $^{32}P_1$ ^{14}C-Uridine was used at a sp. act of 40 mCi/mmol (1 μCi/ml) After incubation the tips were washed six times with a jet of distilled H_2O before homogenization as described below The rate of incorporation of ^{14}C-uridine into RNA was found to be linear with time up to 4 hr under the conditions used

Nucleic acid extraction and characterization Total nucleic acids were determined, after separation into acid-soluble, RNA and DNA fractions, by the method of Schmidt and Thannhauser[20] as modified by Bonner and Zeevaart [21] For the extraction of nucleotides, root tips were homogenized in 10% TCA (1 ml/250 mg fr. wt) and the mixture shaken at 2° for 15 min After centrifugation for 20 min at 2° and 800 *g*, the pellet was re-extracted with 5% TCA and the combined supernatants were washed ten times with ether to remove TCA The washed supernatant was used for characterization of the nucleotide fraction by chromatography on DEAE-Sephadex

[11] KEY, J. L. and SHANNON, J. C. (1964) *Plant Physiol.* **39,** 360
[12] SACHER, J. A. (1967) *Plant Physiol.* **42,** 1334
[13] TREWAVAS, A. J. (1968) *Arch. Biochem. Biophys.* **123,** 324
[14] KEY, J. L. (1969) *Ann. Rev. Plant Physiol.* **20,** 449
[15] HAISSIG, B. E. (1971) *Bot. Gaz.* **132,** 263
[16] TREWAVAS, A. J. (1968) *Phytochemistry* **7,** 673
[17] COKE, L. and WHITTINGTON, W. J. (1968) *J. Exp. Botany* **19,** 295
[18] NEALES, T. F. (1960) *Aust. J. Biol. Sci.* **13,** 232
[19] HOAGLAND, D. R. and ARNON, D. I. (1950) *Calif. Agric. Exp. Sta. Cir.* 347
[20] SCHMIDT, G. and THANNHAUSER, S. J. (1945) *J. Biol. Chem.* **161,** 83
[21] BONNER, J. and ZEEVAART, J. A. D. (1962) *Plant Physiol.* **37,** 43

or Dowex-1. The acid-insoluble pellet containing RNA was hydrolyzed in 0·3 M KOH for 20 hr at 37°, then neutralized with 0·6 M PCA. After standing at 2° for 15 min, followed by centrifugation at 400 *g* for 5 min, the supernatant was used to estimate the distribution of radioactivity among the bases of RNA. Nucleotides were separated by chromatography on DEAE-Sephadex (A-25), using a column 0·9 × 20 cm. The acid-soluble fraction (diluted five-fold with H_2O to ensure a low salt conc.) was loaded onto the column together with unlabelled marker nucleotides (0·25 μmol each) at a flow rate of 40–50 ml/hr. The column was then washed with water until no UV-absorbing material was present in the eluate. Nucleotides were eluted with a linear gradient of triethylammonium bicarbonate, in which the mixing vessel initially contained 400 ml water and the reservoir 400 ml of the buffer (1 M, pH 8). The flow rate was 40–50 ml/hr. The eluate was monitored automatically at 254 nm with an LKB Uvicord II, and fractions of 4 ml collected. ^{32}P-Containing fractions were assayed by Cerenkov radiation, and ^{14}C by liquid scintillation spectrometry after sorbing a portion of the fraction onto glass-paper. Nucleotides in the alkali-hydrolyzed acid-insoluble fractions were separated by chromatography on Dowex-1. Samples were loaded onto the Dowex-1 column (1·2 × 10 cm) as before, and elution achieved with a two-stage linear gradient system. A 0 to 4 M gradient of formic acid (100 ml) was followed by a gradient of 0–0·5 M ammonium formate in 4 M formic acid (100 ml). The eluate was monitored and fractions collected and radioassayed as before. RNA was extracted from excised root tips (100–400 mg fr. wt) by homogenization at 2° in a mixture of 2 ml of an aq. soln. containing 10 mM phosphate, 50 mM KCl, 10 mM $MgCl_2$, 1·5% (w/v) SDS, 0·2% (w/v) bentonite (pH 7·6) and 2 ml of 90% (v/v) phenol–0·1% (w/v) 8-hydroxyquinoline. The mixture was shaken at 2° for 20 min and centrifuged for 20 min at 1000 *g*. The aq. layer was re-extracted with phenol and bentonite, and the RNA precipitated from the final aq. phase with 2·5 vol. of EtOH overnight (at −15°). The ppt. was washed successively with 70% (v/v) EtOH, 95% (v/v) EtOH, twice with Et_2O, then dried *in vacuo*. RNA-ribose was determined by the method of Schneider,[22] using $CuCl_2$ as catalyst[23] and AMP as the reference standard. The Pi content of RNA fractions was determined by adding a drop of 10% (w/v) $Mg(NO_3)_2$ in EtOH and heating in a test-tube over a strong flame until all the brown fumes had been expelled. When cool, 0·9 ml of HCl was added and the soln. was heated at 100° for 15 min. The soln. was then analyzed for Pi by the method of Chen, Toribara and Warner.[24] DNA was determined as deoxyribose by the method of Burton.[25]

Ribonuclease activity. Ribonuclease activity was determined in crude extracts by the method of Walters and Loring.[26] Extracts were prepared by homogenizing 100 mg fr. wt of root tips with 8 ml of 1 M NaOAc, pH 5·4. Assays were conducted at 37° in 2 ml incubation mixtures containing 0·05 M NaOAc pH 5·4, 4 absorbance units of yeast RNA and 0·1 ml of plant extract.

Uridine kinase activity. PC was used to determine the conversion of ^{14}C-uridine to ^{14}C-UMP. The assay mixture in a total vol. of 0·4 ml contained: 50 μmol Tris-HCl (pH 8·1), 5 μmol $MgCl_2$, 1·5 μmol mercaptoethanol, 15 μmol ATP, 5·6 nmol 2-^{14}C-uridine (4·6 × 10^4 cpm/nmol) and 0·05–0·1 ml of plant extract. The extract was obtained by homogenizing 1 g fr. wt of root tips with 2 ml 50 mM Tris-HCl (pH 8·1)–1 mM glutathione, followed by passage through a French Pressure Cell at 200 kg/cm^2. After incubation at 30° for periods between 30 and 60 min, the reaction was terminated by adding 1·25 ml 10% (w/v) TCA. The supernatant, after centrifugation at 1000 *g*, was removed and extracted twice with Et_2O, and 0·04 ml (containing added uracil, uridine and UMP as markers, 0·1 μmol each) was spotted onto Whatman 3 MM chromatography paper and developed by ascending chromatography in 0·25 M formic acid. The chromatogram was cut into 5 mm strips and the conversion of ^{14}C-uridine to ^{14}C-UMP determined by liquid scintillation spectrometry.

Protein was determined in plant extracts after TCA precipitation by the Folin procedure of Lowry *et al.*,[27] using bovine serum albumin as standard.

[22] SCHNEIDER, W. S. (1957) In *Methods in Enzymology* (COLOWICK, S. P. and KAPLAN, N. O. eds.), Vol. 3, p. 680, Academic Press, New York.
[23] LIN, R. I. and SCHJEIDE, O. A. (1969) *Anal. Biochem.* **27**, 473.
[24] CHEN, P. S., TORIBARA, T. Y. and WARNER, H. (1956) *Anal. Chem.* **28**, 1756.
[25] BURTON, K. (1956) *Biochem. J.* **62**, 315.
[26] WALTERS, T. L. and LORING, H. S. (1966) *J. Biol. Chem.* **241**, 2870.
[27] LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L. and RANDALL, R. J. (1951) *J. Biol. Chem.* **193**, 265.