enough to hold back the smallest bacteria, and the distance l is at least 100 times greater than that of the cell wall's pores.

Summary

1. Physiology texts state that the "transpirational pull" initiates the ascent of sap by means of a diffusion of liquid water from the top of the vessels in the leaf to the evaporating surface of the mesophyll cells. This concept is shown to be impossible because under commonly found conditions, the diffusion rate of the water vapor may be 600,000 times that of the liquid water.

2. The objection of Curtis and Clark that it would require a pressure of 100,000 atms to produce a flow through the microcapillaries of the cell wall is also shown to be in error. Actually, less than 0.5×10^{-8} atms would be needed.

3. A consistent and physically sound theory of the ascent of sap is possible only if it is assumed that surface tension forces initiate the rise—i.e., that the adhesive (imbibitional) forces are increased at the surface due to evaporation and this causes the rise of the whole column due to cohesion between the water molecules.

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EFFECT OF RIBONUCLEASE ON SALT ABSORPTION BY EXCISED MUNG BEAN ROOTS[1]

T. TANADA

Soil and Water Conservation Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland

The transport of ions into plant root cells is generally believed to be by means of ion-binding carrier compounds. Several types of compounds have been proposed as carriers, but none has won universal support (6). Recently, from observations on the effect of ribonuclease on absorption of calcium by Elodea cells and from cytological evidence, Lansing and Rosenthal (4) have suggested that ribonucleic acid may function as an ion-binding carrier compound during salt absorption. The results reported here lend support to their proposal.

In this study, mung bean (*Phaseolus aureus*) were germinated and grown at 25° C with roots in an aerated 10^{-4} M $CaCl_2$ solution which was changed daily. The first centimeter of root tip was excised from 3-day-old seedlings. Fifty root tips were placed in a beaker, washed, and treated with a solution of crystalline ribonuclease (100 μgm/ml) for short periods at 25° C. The enzyme was preheated at 70° C for 20 minutes before use. Control roots were similarly treated but without the enzyme. After enzymic treatment the roots were washed and placed in a 10^{-4} M solution of either RbCl with Rb^{86} or KH_2PO_4 with P^{32}. The activities of the solutions were less than 5 μc/l. In some cases the solutions contained $Ca(NO_3)_2$ of 10^{-3} M. The roots were allowed to absorb Rb or phosphate for 30 min at 25° C under vigorous aeration. After the absorption period, the roots were washed several times with inactive salt solution of 10^{-2} M and water and dried at 100° C. The radioactivity of the roots was determined in the conventional manner.

Typical results showing the effect of ribonuclease on Rb and phosphate absorption are presented in table I. The data are the means of duplicate determinations. The experiment has been repeated several times with similar results. The data indicate that there was a very marked effect of the enzyme on Rb absorption by mung bean roots. The enzymic effect was apparently influenced strongly by the presence of Ca in the absorption medium. In the absence of Ca in the absorption medium, the ribonuclease pretreatment enhanced the uptake of Rb by roots. The absorption was linear up to one hour. Rb uptake in the

[1] Received January 6, 1956.

TABLE I

EFFECT OF RIBONUCLEASE ON RUBIDIUM AND PHOSPHATE ABSORPTION BY EXCISED MUNG BEAN ROOTS

PRETREATMENT TIME WITH ENZYME	CALCIUM IN ABSORPTION MEDIUM	ABSORPTION	
		RUBIDIUM	PHOSPHATE
min	*M*	*cps*	*cps*
No pretreatment	0	8.4	4.2
10	0	14.2	3.6
20	0	19.6	2.7
No pretreatment	10^{-3}	20.4	13.4
10	10^{-3}	8.0	9.3
20	10^{-3}	4.2	8.3

presence of Ca, however, was depressed by the pretreatment. Similar results were obtained with 10^{-3} M $MgSO_4$; but $NaNO_3$ of 10^{-3} M had little or no effect. These results indicate an interaction between the effects of ribonuclease and polyvalent cations. The absorption of phosphate, on the other hand, was less affected by the enzyme and was decreased both in the absence and presence of Ca by the pretreatment. The depressing effect of Ca on Rb absorption by enzyme-treated roots is interesting in view of previous reports (7, 8) that Ca enhances salt uptake by normal roots. This can also be seen in the data in table I which show a marked stimulation by Ca of both Rb and phosphate absorption by control roots.

From the results presented in table I, it appears that ribonucleic acid, or more probably a ribonucleoprotein, could be involved in ion transport during salt absorption by plant roots. Lansing and Rosenthal (4) have cited several chemical and cytological evidences supporting the role of ribonucleic acid as an ion-binding carrier compound. Tanada (7) has recently shown that Rb absorption by excised mung bean roots is markedly affected by ultraviolet radiation of 2537 Å, a wavelength which is strongly absorbed by nucleic acids. It is realized, however, that all these evidences are indirect in nature; but they could be utilized for obtaining direct evidence on the identity of ion transport compounds.

Additional support for the contention of Lansing and Rosenthal (4) that some ribonucleic acid is localized on cell surfaces was obtained by repeating their technique of staining with toluidine blue after ribonuclease treatment. According to Heller and Kopac (2), toluidine blue is specific for ribonucleic acid. In this investigation, excised mung bean roots were pretreated for 20 min with a ribonuclease solution (100 μgm/ml). The roots were washed and placed with control roots in a toluidine blue solution of 10 ppm for 5 min. The stained roots were then washed with water. It was easily apparent that the enzyme-treated roots stained less than control roots. Microscopic observation showed some staining localized near the surfaces of cells. Incidentally, Lundegårdh has commented on the presence of nucleotides in cell surfaces (5). It is possible that the action of ribonuclease might not be localized only at the surface of cells. Recent investigations by Kaufmann and Das (3) and by Brachet (1), using longer time of treatment or greater concentration of enzyme, suggest that ribonuclease can enter into cells. These results, however, do not exclude some ribonuclease action near the surfaces as it enters into the cell.

If the effects of ribonuclease reported in this paper were due to degradation of ribonucleic acids near the external surfaces of root cells, there is a possibility that some of the degraded products of the nucleic acids might diffuse outward into the surrounding medium. With the object of exploring this possibility, after the usual enzymic treatment of roots the ribonuclease solution was analyzed spectrophotometrically in the ultraviolet with a Beckman DU spectrophotometer. The solution showed two absorption maxima with the largest in the 250 to 260 mμ region and the other in the 290 to 300 mμ region. The absorption in the wavelength region of 250 to 260 mμ could be due to purine- and pyrimidine-containing compounds formed from the degradation of nucleic acids.

In order to determine whether the effects of ribonuclease reported here were specific for the enzyme, some of the experiments were repeated using crystalline desoxyribonuclease (100 μgm/ml). The results indicated that up to 20 min of pretreatment, desoxyribonuclease had no effect on the absorption of Rb and on the staining by toluidine blue of excised mung bean roots.

SUMMARY

The effects of crystalline ribonuclease on Rb and phosphate absorption and on staining by toluidine blue of excised mung bean roots were investigated. The enzymic pretreatment increased the uptake of Rb in the absence of Ca and Mg. In the presence of Ca and Mg, treated roots absorbed markedly less Rb. Phosphate absorption was decreased both in the absence and presence of Ca by the ribonuclease treatment. Root cells pretreated with the enzyme showed less staining with toluidine blue than untreated cells. Desoxyribonuclease had no effect on Rb uptake and on staining by toluidine blue of excised roots. Ribonuclease solutions which were in contact with excised roots showed two absorption maxima in the ultraviolet, one at 250 to 260 mμ and the other at 290 to 300 mμ. These results have been interpreted to suggest the involvement of a ribonucleoprotein in ion absorption by mung bean roots.

The writer wishes to express his appreciation to Dr. A. W. Specht of the Horticultural Crops Research Branch for his assistance with the ultraviolet absorption measurements.

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KINETIN AND AUXIN ACTIVITY[1]

R. S. DEROPP
RESEARCH DIVISION, AMERICAN CYANAMID COMPANY,
PEARL RIVER, NEW YORK

Kinetin (6-furfurylaminopurine) has been shown to increase the rate of cell division in tobacco wound callus tissue when used in conjunction with 2 μgm/ml of indoleacetic acid (IAA) (2, 3). The present experiments were undertaken to determine the effect of kinetin on the responses of fragments of sunflower hypocotyl to IAA previously described (1).

The sunflower hypocotyl test for multiple auxin activity (1) was employed because this test demonstrates not only the stem-elongation inducing activity of an auxin but also its growth promoting (in the sense of increase in dry weight) and root initiating activity. All these activities have been shown to be correlated with auxin concentration, point of application of the auxin and position on the hypocotyl from which the fragment was excised. Sterile sunflower embryos were germinated on agar in darknes as described previously (1). When three days old they were removed and the 5-mm fragment immediately below the cotyledon (apical) and the fragment immediately above the root (basal) were removed. The fragments were transferred to sucrose mineral agar to which IAA and synthetic kinetin had been added before autoclaving in the concentrations shown in the tables. Stem elongation was studied using apical fragments placed upright with their apical ends in contact with the agar and cultured in complete darkness for 3 days. Weight increase and root production by apical fragments were studied by placing the fragment with the basal end on the agar and culturing it in darkness for 14 days. Weight increase and root production in the basal fragments were studied by placing the basal 5 mm with its apical end on the agar and culturing it for 14 days. At the end of the growth period, length, fresh and dry weights and number of roots on each fragment were determined. Average values obtained are shown in tables I, II and III. Ten replicates were used in each experiment. The means are subject to a standard error of ± 10 %. The following conclusions can be drawn from these figures:

1. The elongation induced in apical sunflower fragments by IAA (0.1 μgm/ml) applied to the apical end of the fragment was completely suppressed by 1.0

[1] Received January 10, 1956.

TABLE I

AVERAGE LENGTH OF APICAL HYPOCOTYL FRAGMENT AFTER 3 DAYS' CULTURE WITH APICAL END ON SM AGAR ± IAA (0.1 μGM/ML) + KINETIN (MEANS OF 10 ESTIMATES)

IAA	KINETIN CONC (μGM/ML)			
	1.0	0.1	0.01	0
	mm	*mm*	*mm*	*mm*
Present	10.0	12.8	13.0	16.2
Absent	9.7	11.0	11.0	11.1

TABLE II

FRESH AND DRY WEIGHTS OF APICAL FRAGMENTS AFTER 14 DAYS' CULTURE WITH BASAL END ON SM AGAR ± IAA (1 μGM/ML) + KINETIN (MEANS OF 10 ESTIMATES)

IAA	KINETIN CONC (μGM/ML)							
	1.0		0.1		0.01		0	
	FRESH WT	DRY WT	FRESH WT	DRY WT	FRESH WT	DRY WT	FRESH WT	DRY WT
	mg	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*	*mg*
Present ...	228	14	208	14	259	16	245	13
Absent ...	99	6	121	7	59	3	53	3