SHORT COMMUNICATIONS

THE SITE OF PHOSPHORUS ACCUMULATION IN MAIZE ROOTS*

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Introduction

Crossett and Loughman (1966) reported an examination of the absorption and translocation of ^{32}P by barley seedlings. It was concluded that after absorption ^{32}P was either retained permanently in the root as orthophosphate or was translocated after mixing with only a small proportion of the total phosphorus content of the root.

This communication reports the results of an attempt to locate the site of ^{32}P accumulation in maize roots using an autoradiographic technique. Maize was chosen instead of the barley used in earlier work because it was possible to obtain autoradiographs which had better resolution. Maize roots had two main advantages: firstly, a larger cross-sectional area gave a greater separation of the structures to be resolved; and secondly, since shattering did not occur so readily, thinner sections could be prepared.

Methods

(i) *Seedling Production.*—Maize seeds, *Zea mays* (L.) cv. White Horse Tooth, were surface-sterilized by immersion for 1 min in 1% mercuric chloride solution, rinsed, and finally soaked in distilled water for 24 hr. The seeds were then germinated between moist filter paper for 36 hr before being transferred to stainless steel mesh which was supported above a 1-litre tank of nutrient solution. The concentration of the various salts in the nutrient solution (pH 5·5) were as follows: KH_2PO_4 1 μM, $CaSO_4$ 2 mM, KNO_3 6 mM, $NaNO_3$ 2 mM, $MgSO_4$ 2 mM, $Ca(NO_3)_2$ 1 mM. The seedlings were grown under a bank of eight Philips TL80 Daylight fluorescent tubes. The temperature varied between 20 and 25°C.

(ii) *Preparation of Labelled Material.*—Seedlings were used 6 days after the start of germination. At this time the primary roots were about 6 cm long. Four seedlings of matching appearance were selected and each placed on a glass support with its roots dipping into a beaker containing 1 litre of ^{32}P-labelled nutrient solution. The chemical composition of the nutrient solution was identical to that used to grow the seedlings, except for the addition of ^{32}P. The amount of ^{32}P added to each beaker was chosen so that, given equal and constant rates of phosphorus absorption, seedlings would contain approximately equal amounts of radioactivity when used to prepare autoradiographs. The duration of ^{32}P absorption, and ^{32}P concentrations used, are shown in the following tabulation:

Series	A	B	C	D
^{32}P concentration in nutrient solution (mCi/l)	10·0	2·5	2·5	0·6
Duration of ^{32}P absorption (hr)	0·5	2·0	2·0	8·0

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During ^{32}P absorption the nutrient solutions were stirred but not aerated because of the risk of formation of a radioactive aerosol. At the end of the ^{32}P absorption period seedlings were removed, rinsed twice in distilled water, and blotted dry. The seedling which was used to prepare the autoradiographs in series C was rinsed in inactive nutrient solution and transferred to a beaker of inactive nutrient solution where it remained for 6 hr before processing.

(iii) *Preparation of Autoradiographs.*—The technique used in this investigation has already been described in detail (Crossett 1967). It was adopted to avoid any risk of redistribution of radioactivity during processing. At the end of each experimental treatment roots were frozen quickly and sections 10 μ thick were cut from them using a cryostat microtome operating at $-20°C$. Sections were mounted on the autoradiographic film without allowing them to thaw, and exposed for 1 week at a temperature of $-20°C$. Groups of 15 sections were cut at 1-mm intervals from the apical 5-mm section of each root. Within each group of 15 sections, five were used to prepare autoradiographs, five were mounted on slides for microscopic examination, and the remainder were placed on aluminium planchets for subsequent determination of radioactivity using an end-window Geiger–Müller tube and scaler.

(iv) *Analysis of Autoradiographs.*—A recording microdensitometer* was used to measure variations in the optical density of a strip, 10 μ wide, across the diameter of each autoradiograph. The use of this instrument, the resolution of the autoradiographs, and the precautions required to ensure that the densitometer trace is equivalent to the radioactivity of the specimens have already been discussed (Crossett 1967).

Typical microdensitometer traces are shown in Figure 1. It was not possible to make a statistical analysis of the autoradiographs because losses during preparation were considerable, and in two cases only one of the five replicates remained at the end of the procedure. The main causes of loss were: firstly, low microtome knife temperatures which caused shattering and a complete loss of cell contents; and secondly, the separation of the autoradiographic emulsion from its supporting coverslip during development.

The graduations on the centre line of each of the series of traces shown in Figure 1 are in proportion to the specific activity of the appropriate nutrient solution. The traces are therefore on a common scale relative to the centre line graduations.

Results

(i) *Accumulation of ^{32}P.*—The radioactivity of each group of sections is shown in Table 1. Three conclusions may be drawn from the data:

(1) ^{32}P accumulation increased with distance from the apex of the root.

(2) Within series of groups cut at similar positions from the various roots ^{32}P was accumulated at an approximately constant rate.

(3) There was no massive loss of ^{32}P from the root after transfer to an inactive solution.

In the stained and mounted preparations vascular tissues were fully differentiated, even in the sections which were cut closest to the root apex. The main difference between apical and basal sections was that the cortical cells of the former had a much higher ratio of cytoplasm to vacuole.

(ii) *Distribution of ^{32}P in the Autoradiographs.*—The relative distribution of radioactivity across diameters of the root (shown in Fig. 1) changed markedly with time, changes being greatest in sections taken farthest from the root apex. As the

* Supplied by Joyce-Loebl, Gateshead, England.

TABLE 1

RADIOACTIVITY OF TRANSVERSE SECTIONS CUT FROM FROZEN MAIZE ROOTS AFTER ABSORPTION OF ^{32}P

The measured radioactivity values (counts/section/sec) have been multiplied by factors of 4 (series B and C) and 16·6 (series D) to compensate for variation in the specific activity of the nutrient solutions used [see Methods(ii)]. Sections used were 10 μ thick

Distance from Root Apex (mm)	Mean Radioactivity for Sections of Roots from Series: A	B	C	D
1	1·37(±0·21)	4·72(±0·81)	5·16(±0·73)	21·76(±1·82)
2	2·05(±0·17)	7·26(±0·71)	9·40(±0·76)	25·12(±1·41)
3	2·17(±0·19)	8·12(±0·77)	9·00(±0·91)	39·36(±1·78)
4	3·04(±0·25)	8·20(±0·86)	9·32(±0·87)	42·08(±1·97)
5	3·26(±0·22)	8·28(±0·59)	10·92(±0·64)	53·32(±2·41)

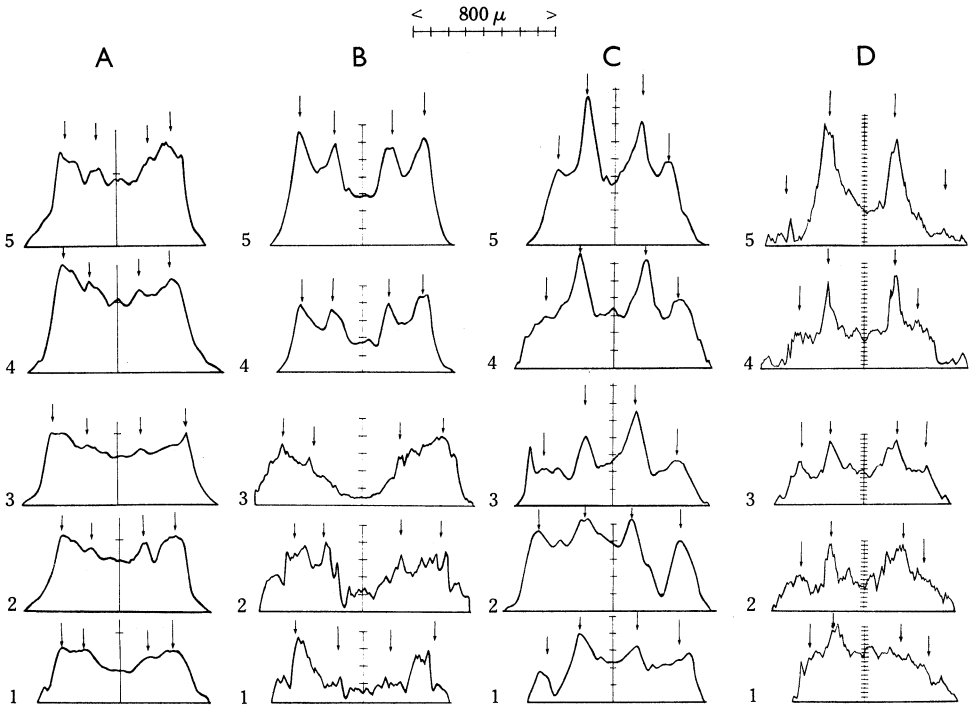

Fig. 1.—Recorder traces showing the variation in image intensity on diameters of the various autoradiographs. The treatments given to each of the roots A, B, C, and D are described in the text and the number beside each trace represents the distance, in millimetres, of the transverse section from the root apex. The graduations on the centre line of each trace are in proportion to the specific activity of the nutrient solution that was used and are therefore arbitrary units of phosphorus accumulation. The inner pair of vertical arrows indicate the point on the trace corresponding to the phloem while the outer pair correspond to the mid-cortex. The very sharp "peak" on the extreme left of trace 3C corresponds to a bacterial colony.

duration of ^{32}P absorption increased, the optical density "peaks" separated by 0·28–0·38 diameters increased relative to those separated by 0·68–0·75 diameters. A similar trend was apparent in a comparison between the basal sections cut from the root which had absorbed ^{32}P for 2 hr and those cut from the root which had absorbed ^{32}P for 2 hr and had then been transferred to an inactive solution.

Although the autoradiographs of sections cut close to the root apex show a less well-defined pattern of ^{32}P distribution a similar trend was apparent, the stele becoming more prominent with increasing duration of ^{32}P absorption.

It has been impossible to estimate the resolution of the autoradiographs because early experiments with roots which had been equilibrated with tritiated water showed that 95% of water sublimed from tissue sections within 15 min. It was therefore probable that the thickness of the sections during exposure was very different from the nominal 10 μ. (For a given photographic emulsion, isotope, and tissue, resolution depends both upon the thickness of the emulsion, and that of the section, as well as the intimacy of contact between the two — see Perry 1964.) It is therefore impossible to make any detailed interpretation of the fine structure shown in the optical density traces.

A comparison of the distribution of radioactivity with the stained tissue sections showed that the inner pair of optical density peaks corresponded to the phloem region of the stele while the outer pair corresponds to the cortex.

Discussion

The simplest explanation of the increasing prominence of the inner pair of optical density peaks is that as the duration of ^{32}P absorption increases the cortical cells tend toward isotopic equilibrium while net phosphorus accumulation (or a very much slower equilibration) occurs in the phloem region of the stele. There are two pieces of evidence which support this hypothesis. Phloem tissue has been found to have a high affinity for phosphorus — Bieleski (1966) found that the phloem of both celery and apple accumulated ^{32}P-labelled phosphate more readily than the surrounding parenchyma. The ratio, ^{32}P in cortex/^{32}P in stele, decreases with duration of ^{32}P-labelled orthophosphate absorption. Yu and Kramer (1967) measured the radioactivity contained in the cortex and stele of maize roots dissected after varying periods of ^{32}P absorption. It was found that after 4 hr of ^{32}P absorption the radioactivity of the cortex was greater than that of the stele, but after an 8-hr absorption period the two values were equal.

The present results are difficult to relate to the one result reported earlier (Crossett 1967). In the earlier experiment ^{32}P was in the mid-cortex, but greatest amounts were observed at the endodermis and in the xylem vessels; ^{32}P was absent from the phloem. It is impossible to decide whether the difference between distributions was due to the shorter ^{32}P absorption period (15 min) or the different nutrient solution (10^{-5}M $CaSO_4$) that was used to grow seedlings for the earlier investigation.

The present findings have one important implication. The penetration of the endodermis has been cited as a process which limits the movement of ions from the environment to the xylem, e.g. Weigl and Lüttge (1962). If maize roots are physio-

logically similar to those of barley, in that the bulk of phosphorus in the root is not available for translocation (Crossett and Loughman 1966), the present results do not justify the assignment of any special role to the endodermis. This conclusion is based upon the fact that the long term accumulation of ^{32}P occurs inside the endodermis and thus must also be preceded by endodermal penetration.

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