Use of X-ray microanalysis for study of cation distribution in potassium deficient pumpkin roots

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Abstract

Ice slices of root tissues were investigated by X-ray microanalysis. It is shown that the cytoplasm of the meristematic and differentiated cells of potassium deficient roots maintains a high potassium level. The vacuoles of various root cells loose more K and accumulate more Na and Mg than does the cytoplasm.

Key words: potassium deficiency, K, Na, Mg distribution, X-ray microanalysis

INTRODUCTION

It is known that various physiological processes — synthesis of some organic substances, energy conversion, transport of ions and nonelectrolytes — differ in their sensitivity to potassium deficiency. The reason for this may be different requirements for potassium in some enzymatic reactions. On the other hand, a sharp difference in the degree of potassium deficiency may exist between functionally diverse cells and cell compartments. Variations in potassium concentration may induce changes in the level of other cations in a cell.

The influence of potassium deficiency on the content of chemical elements in pumpkin seedling root tips was investigated. It is known that in a root there are zones of cells at different stages of development (root cap, meristematic zone, zone of elongation, absorbing zone and translocating zone) which have different physiological functions. The functional roles of cells located along the radius of the root (epidermis, cortex, endoderm, different stellar cells) are also diverse. The distribution of elements in cells was investigated using a local X-ray microprobe technique which makes it possible to evaluate the native distribution of elements in cells with the highest possible degree of approximation.

MATERIALS AND METHODS

The investigation was carried out on the seedling roots of *Cucurbita pepo* L. Seeds were germinated in the dark on filter paper for 5-6 days and then transferred to dilute Knop's solution containing (mM): $\text{Ca}(\text{NO}_3)_2$ — 1.2; MgSO_4 — 0.5; KCl — 0.5; KH_2PO_4 — 0.5. The seedlings were kept for 2-3 days in light. The solutions were changed every day. In the experiments with potassium deficiency, potassium salts were substituted with equivalent amounts of sodium salts. Zones rich in meristematic cells (0-2 mm from the apex) and two zones of differentiated tissues (located at a distance of 5-7 mm and 10-15 mm from the apex) were selected. These root segments were dried and then ashed with concentrated HNO_3 . The cation content was measured by flame photometry after transforming the salts into chloride form.

For X-ray microanalysis, segments of roots 4-7 mm in length were frozen by immersion in liquid propane cooled with liquid N_2 . Sections were cut with steel knives. The angle of the knife was about 40° . The sections were cut using a Porter Blum cryoultramicrotome. Slice thickness and temperature of slicing varied depending on the degree of cell differentiation in the sample: 1-2 μm thick at -40° to -60°C for the meristematic zone and 10-12 μm at -25°C for differentiated zones with large unstructured vacuoles. Slices were mounted inside the cryochamber on a copper holder between two alluminized collodium films. The holder with the specimens was then transferred to the cryostage of a JSM U3 scanning electron microscope fitted with a wavelength spectrometer. The cryostage was cooled with liquid N_2 to approximately -100°C .

The cell structure was observed in secondary or transmitted electrons. The distribution of elements was detected with diffraction spectrometer at the characteristic X-ray radiation wavelength of specific element (K_α line). The analysis was carried out at an accelerating voltage of 25 kV and beam current of 10-20 μA . X-ray radiation was registered either along the line chosen on the imagination of the section (Figs. 2b and 3) or from the whole surface of the section (Fig. 1d). In some cases half-quantitative measurement of the radiation of an area in counts per minute was carried out.

RESULTS AND DISCUSSION

Sections of the highest quality were prepared using well structured meristematic cells. In this case sections 1-2 μm thick with good preservation of intracellular structure were obtained with series of sections even possible. Figure 1 presents several pictures of this zone. One can see the good preservation of root cap cells (Fig. 1a), all the cells preserved their contents (Figs. 1c, 2a). Nuclei, granules of storage substances (Fig. 1b, c), small vacuoles (Fig. 2b) are well visible in such cells.

It is more difficult to get sections of tissues which consist of cells having large unstructured vacuoles. Vacuoles often break out from the slices. But if the slices

Fig. 1. a — Frozen-hydrated cryosection of pumpkin root tip. There is a compact central group of cells surrounded by cells submerged in slime, x500; b — a group of cells from the meristematic zone, nuclei, starch granules are visible in these cells, x2600; c — a group of meristematic cells, x600; d — potassium distribution in this section

PLATE II

Fig. 2. a — Frozen-hydrated cryosection from the elongation zone, x500; b — part of esemithin (2μm) section, epidermis and cortical cells from the elongation zone and potassium distribution along the white line in photo, x2000

PLATE III

Fig. 3. Thick ($10\mu\text{m}$) frozen-hydrated cryosection of differentiated pumpkin root, potassium and magnesium distribution along the black line in photo, $\times 420$

are placed between two collodium films, the vacuoles are fixed on these films. It thus becomes possible to analyse their content. At the same time the shrinkage and concentrating of vacuole contents because of ice crystal formation often occur. That is why it is difficult to draw conclusions about the distribution of elements inside the vacuole and more correct to measure only the bulk vacuole content. Good preservation of intracellular structures was achieved due to preparation of thicker slices — up to 10-12 μm (Fig. 3).

The structure of fully hydrated sections is invisible in the electron microscope. But such sections gradually become lyophylized and then one can see the cell structure. This process is not accompanied by visible changes in element distribution: potassium distribution analysis in a fully hydrated section and after its lyophylization does not reveal any differences.

The pattern of cation distribution along the longitudinal axis of primary roots carried out on cut segments was the same as described earlier (Scott et al. 1968, Kravina 1974). Meristematic cells of K-seedling roots accumulated K, Na, Ca and Mg in the highest concentrations. The concentrations of these cations decreased in the basal direction (Table 1).

Table 1

Cation content in root segments of pumpkin seedlings grown in different nutrient solutions for 2 days

Distance from root tip, mm	Nutrient solutions	Cation content, $\mu\text{moles/g}$ fresh weight			
		K	Na	Mg	Ca
0-2	+K	93.6	7.6	21.0	10.3
	-K	89.6	7.9	30.2	9.2
5-7	+K	110.3	6.1	11.6	4.9
	-K	49.9	16.3	27.9	6.3
10-15	+K	63.9	5.2	11.7	7.1
	-K	21.2	21.6	39.5	4.5

X-ray microanalysis was used to study potassium distribution in separate cells of different root zones. The potassium distribution between the cells in the meristematic zone depended weakly on the K concentration in the growth medium. The root tip was surrounded by cells of the root cap. They usually contained less potassium than meristematic cells. The potassium content in the cells enveloped by slime differed significantly. There occurred cells with the potassium concentration equal to that in the cells of the tip root as well as the cells with lower content of the potassium. This probably depended on the extent of their degradation. All meristematic cells contained equal high potassium concentrations. The Ca distribution was opposite to that of K: its content

increased in the direction of the periphery. Magnesium and sodium were distributed more uniformly.

Figure 1c shows a section of a potassium-deficient root tip. Meristematic cells are located in its centre. Surrounding them are a few layers of root cap cells, covered by a slime envelope. The K distribution over the area of this section is shown in Fig. 1d. It is obvious that the distribution of potassium practically uniform in all meristematic cells. The K level in root cap cells was somewhat lower.

In Table 1 one can see that the meristematic cells of plants grown without K maintained high K and low Na levels. At the same time the Mg level increased.

There are contradictory data in literature concerning the distribution of elements among cells of the differentiated zone of the root. For example, different species under varying growth conditions may have a stele potassium content which is higher, lower or equal to that cortex (Läuchli et al. 1971, Chino 1981).

In our experiments, the distribution of potassium between stellar and cortical cells was dependent on the K content in the growth solution. When seedlings were grown in the K-solution, the content of this element in the stele and cortex cells was the same and equalled 114 and 98 counts per min respectively, according to the results of point analysis.

Under potassium deficiency, the bulk K content in the cells of the differentiated zone decreased 2-3 times. At the same time, Na and Mg are accumulated (Table 1). In the case, a distinction in elemental distribution between cortex and stellar cells was manifested. Figure 3 presents a section of the differentiating part of the root and the K distribution along the line running across the centre of root shown at this slice. It is possible to see that the stele contains more K and less Mg than the cortex. Recording X-ray radiation impulses from the reduced raster of stele and cortex gave similar results: 460 and 180 counts per min for potassium and 80 and 140 counts per min for Mg, respectively.

Mg accumulation is a characteristic feature of K-deficient plants. These cations presumably interact during transport across cell membranes and mutually inhibit one another's absorption (Leggett and Gilbert 1969). In light of the fact that Mg accumulation also takes place in meristematic cells, the maintaining of a constant intracellular level of K indicates that this process is a result of the absence of potassium in the medium and does not occur due to a decrease in intracellular concentration.

It is known that stellar cells are richer in cytoplasm than highly vacuolated cortex cells (Läuchli et al. 1977). This is why one of the reasons for the notable K reduction in the cortex cells may lie in preferable reduction of its concentration in the vacuole as compared with cytoplasm. This suggestion is in agreement with the idea that initial alteration of ion composition occurs in vacuoles while the cytoplasm maintains its homeostasis (Leigh and Wyn Jones 1984).

In some differentiated cells it was possible to measure the X-ray counts (counts per min) in the regions of vacuoles and these of cytoplasm. Evidence was obtained to support the idea that potassium depletion first occurs in vacuoles. At the same time the potassium content in the cytoplasm was relatively high (2-3 times more than in vacuole).

Thus, the ability to maintain a high potassium level in K^+ deficient cells is characteristic for meristematic and stellar cells and for the cytoplasm of root cortex cells. The vacuoles of various root cells lose more potassium and accumulate more Na and Mg than cytoplasm. The maintenance of a high potassium level in the cytoplasm as compared with vacuoles may be a general property of plant cells (Pitman et al. 1981, Leigh et al. 1986). In this case, the initial stages of K deficiency are probably connected not with changes in some potassium-dependent metabolic reactions taking place in the cytoplasm but rather with processes affected by the presence of potassium in the growth medium (cell surface properties, membrane potential) or in vacuoles (osmoregulatory reactions).

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*Badanie metodą mikroanalizy w promieniach X rozdziału kationów w korzeniach
dyni hodowlanej w deficycie potasu*

Streszczenie

Przeprowadzono mikroanalizę w promieniach X skrawków mrożeniowych tkanek korzeni. Wykazano, że w korzeniach roślin hodowlanych w deficycie potasu cytoplazma komórek merystematycznych i różnicujących się zawiera wysoki poziom potasu. Wakuole różnych komórek korzeni tracą więcej potasu i gromadzą więcej sodu i magnezu niż cytoplazma tych komórek.