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Short Communication

Membrane Electrical Potentials in the Cortex and Stele of Corn Roots[1]

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This paper reports the results of experiments which are of interest concerning the relationship of root cortex and stelar transmembrane electrical potentials to ion transport into the xylem vessels. Specifically, the electrical potential difference between cells in the cortex and stele of corn root segments and the external solution is reported. It has been suggested (6, 10) that if cells of the cortex or stele participate in ionic delivery to the vessels—as with the symplast theory (1, 5), for which there is considerable supporting evidence (11)—then knowledge of the PD[2] between cortical and stelar parencyhma cells and the xylem vessels would be important. Previous studies of this nature have considered only the PD between the xylem exudate and the external solution (2–4, 14, 15). The results reported here are also of interest regarding the theory that inner root cells leak ions into the vessels (11, 12). Leaky inner root cells should be at a lower (less negative) potential than outer cortical cells, and the PD of the inner cells should change dramatically in response to changes of external ion concentration; PD measurements are, therefore, reported at two concentrations of external K^+.

MATERIALS AND METHODS

Seeds of *Zea mays* L., var. Golden Bantam, were germinated for 2 days in vermiculite moistened with nutrient solution of the following composition: 1.0 mM KCl, 1.0 mM NaCl, 1.0 mM $Ca(NO_3)_2$, 0.25 mM $MgSO_4$, and NaH_2PO_4 and Na_2HPO_4 in sufficient quantities to make the total Na^+ concentration 2.0 mM and buffer the solution at pH 5.5 to 5.7. In experiments with 0.1 mM K^+ the nutrient solution contained 1.9 mM NaCl, making the sum of K^+ and Na^+ 3.0 mM and Cl^- 2.0 mM as in the 1.0 mM K^+ nutrient solution. After 2 to 3 days in vermiculite, the seedlings were placed in Plexiglas supports and suspended over a large volume of aerated nutrient solution for 2 more days. All aspects of the experiments described were performed at 22 ± 1 C.

Cell electrical potentials were determined by inserting a glass microcapillary electrode (tip diameter 0.5 to 1.0 μ and resistance 10–15 megohms) into the cell and measuring the potential between it and a similar electrode in the external solution; microcapillaries were inserted with a micromanipulator. To permit microscopic observation of this operation, tissue was held in a Plexiglas perfusion chamber of the type described by Etherton (7), which allowed change in the composition of the constantly flowing solution without disturbing the preparation. By cutting root segments in half lengthwise and placing the resulting inner flat surface against the cover glass on the upper face of the perfusion chamber, microcapillaries could be inserted into the ends of exposed cells at various positions between the stele and the epidermis. For measurement of stelar parenchyma cell PD values, it was necessary to separate steles from the cortex to expose cells for microcapillary insertion. Steles were excised as described by Laties and Budd (12). After excision stelar segments were pretreated in nutrient solution for 3 hr; microcapillary insertions were made either into the ends or, in a few experiments, the sides of exposed parenchyma cells. The microcapillaries were pulled and filled with 3 M KCl essentially as described by Etherton (7, 8). The electrolyte in the microcapillaries was connected to the measuring circuit with Ag-AgCl wire electrodes. The measuring circuit consisted of a high input impedance amplifier and a voltage calibrator connected to an oscilloscope.

RESULTS AND DISCUSSION

Vacuolar electrical potentials given in millivolts for epidermal cells and for parenchymatous cells located at different positions in the cortex or stele are shown in Table I for external K^+ at 0.1 and 1.0 mM (other salts as described above). For these determinations freshly excised root segments (or steles) were pretreated for 3 hr in 0.1 mM K^+ nutrient solution. Segments were then mounted in the perfusion chamber, and PD values were measured in 0.1 mM K^+ nutrient solution. Following this, with the microcapillary in a single cell, the tissue was perfused with 1.0 mM K^+ nutrient solution. Readings were recorded after the change in concentration when the PD had stabilized, usually a matter of several minutes. Table I shows that the PD becomes less negative when external K^+ is increased from 0.1 to 1.0 mM and that the extent of depolarization in each case is considerably less than the 58 mv change predicted for an ideal potassium cell by the Nernst equation. Similar results with changes in external K^+ have been reported in other studies with higher plants (7, 9) and with algae (17) and fungi (16). The small K^+-dependent depolarization of the PD reported here may be a consequence of the presence of Ca^{2+} in the bathing medium (16, 17). In several experiments after PD measurements were made in 1.0 mM K^+, the perfusing solution was changed to the original 0.1 mM K^+. The PD in every case returned essentially to the original value in 0.1 mM K^+, with a hyperpolarization of about 5 mv in some cells. It may also be seen from Table I that epidermal cell PD values in both 0.1 and 1.0 mM K^+ solution are about 10 mv less negative than PD values of cells in the

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[2] Abbreviation: PD: potential difference.

Table I. *Vacuolar Electrical Potential of Corn Root Cells as a Function of External K^+ Concentration*

Values given are the means ± standard deviation for epidermal cells (column 2), cortical cells approximately midway between the epidermis and the stele (column 3), cortical cells close to the stele (column 4), and stelar cells close but not adjacent to the xylem vessels (column 5). The numbers in parentheses are the number of cell potentials measured for a given cell location.

External K^+	Electrical Potential			
	Epidermal cells	Middle cortical cells	Inner cortical cells	Stelar cells
mM	*mv*			
0.1	−112 ± 3(20)	−122 ± 4(8)	−119 ± 5(16)	−118 ± 10(12)
1.0	−98 ± 5(20)	−109 ± 4(8)	−106 ± 3(14)	−104 ± 6(9)

cortex and stele. In most experiments PD values of stelar parenchyma cells were only several mv lower than those of cortex cells.

The similarity of the vacuolar PD values for epidermal cells and those in the cortex and stele (Table I) suggests that stelar or inner cortical cells are not especially leaky for ions. That the inner root cells are not leaky is further substantiated by the data on changing K^+ in the bathing medium from 0.1 to 1.0 mM; cell membranes with high K^+ permeability would be expected to depolarize to a considerable extent with an increase in external K^+, which was clearly not the case in these experiments. If the inner cortical or stelar parenchymatous cells should have leaky plasmalemmas, as believed by Laties and co-workers (*cf.* 11), then the vacuolar PD values for the inner root cells shown in Table I may represent tonoplast PD values. Attempts to separate the plasmalemma and tonoplast PD values in this study were unsuccessful. However, even if the plasmalemma of inner root cells is leaky, the PD values recorded are high enough to indicate that the tonoplast is not leaky and that ions are accumulated in the vacuole. Thus, the results presented here are in accordance with those of Läuchli *et al.* (13) and Yu and Kramer (18, 19), who showed that stelar parenchymatous cells are capable of accumulating ions.

The data in this paper suggest that the gradients of electrical potential between the vessels and inner root cells are of sufficient magnitude to significantly affect ion movement into the vessels. For example, from the previously reported xylem exudate PD of −30 to −50 mv for corn roots bathed in 1 mM K^+ solution (6), it may be seen that the algebraic difference of PD between the stelar parenchymatous cells and the xylem exudate is 54 to 74 mv (parenchyma negative). Whereas it proved impossible to measure PD values of cells directly adjacent to the vessels, it seems unlikely that these cells would have PD values significantly different from those of other cells in the stele. A paper to follow will characterize the electrochemical gradients and ion fluxes between root parenchyma and the xylem vessels.

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