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Potential Distribution and Ionic Concentration at the Bean Root Surface of the Growing Tip and Lateral Root Emerging Points

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The electrical potential distribution has been measured precisely around the root surface of the bean sprout *Vigna mungo* (L) Hepper. A large negative potential well was found at the growth portion of the root tip. Also, in the matured region of the root, we found a negative potential well at an unspecified position in spite of the fact that nothing was detected on the smooth surface. A lateral root emerge was found to have initiated after 15–20 hours just at the position corresponding to the potential well. With the expectation that these potentials can be elucidated based on the transport of ions which are released or absorbed by the root as a result of cell activity, we precisely measured the concentrations of major ion species (K^+, H^+, and Cl^-) around the root. The theoretical potential distribution curves obtained by putting all the concentration data into the Henderson's Equation for a liquid junction (diffusion) potential coincided well with the experimental curves.

Key words: Ion concentrations — Lateral root — Potential distribution — Root — *Vigna mungo* (L.) Hepper.

In higher plants, the roots play an important role in their growth. We have found that growth was accelerated by the application of DC or square wave voltage to the roots of bean radicles planted in a culturing bath (Mizuguchi 1994). It is worthwhile to study how the growing intact plant root is related to the potential distribution around the root surface.

Recently, the electrochemical signal detection for the intact higher plants around the root have been investigated. For example, Iwabuchi et al. (1989) observed the electric patterns around growing cress roots. They reported that a change in the electric patterns was brought about by the growth of the root in a given environment. Toko and his coworkers reported on the occurrence of current flow around plant roots (Ezaki 1988, 1990, Souda 1990, Hamada 1992). The current flow picture outside the root was shown by them to be related to growth. Miwa and Kushihashi (1992) reported on the stereoscopic electric current density picture around the root. Their current flow picture was constructed on the basis of the measured spatial pattern of the potential under static conditions with the assumption of the presence of an active ionic flow. They expected a H^+ accumulation in the region of the growing position of the root. However, neither the ionic concentration profile, nor the time dependence of the potential profile appear to have been studied in detail for the root surface during growth. It is interesting to measure directly in the ionic concentration profiles during the growth.

We have measured the potential and ionic concentration distributions around the root tip, and found the presence of a large negative potential well in the growing tip region (Takeuchi 1994). In addition, lateral roots are an important organ for plant growth, since the growth rate of a plant is roughly proportional to the effective surface area of its roots (Troughton 1962). It is important to observe the potential in the lateral roots emerging region. A striking feature of the lateral root is that it makes it possible for us to observe all the changes from the initial period of the germination. The lateral root begins to grow from a lateral root primordia whose metabolic activity during segmentation is expected to extend out of the root surface as an electrochemical signal. The emerging of lateral roots plays an important role in plant growth, and the potential change during the emerging of a lateral root attracted our atten-

tion. We also precisely measured the potential and ionic concentration distributions at the lateral root emerging point. The appearance of a negative potential well which preceded the initiation of the emerging of a lateral root was observed (Watanabe 1994).

In this paper, we report the precise measurement of the potential distribution around the root surface in connection with the measured concentration profile of ions which were generated by the metabolism of the plant. All the potential data could be reasonably explained on the basis of the theoretical equation for a liquid junction (diffusion) potential.

Materials and Methods

Plant materials—Bean seeds (*Vigna mungo* (L.) Hepper) were sterilized with 70% ethanol and 1% NaClO, and cultured for 24 hours in an agar gel medium (0.35%). A seedling having a 1–1.5 cm-length root was transferred to a shallow plastic measurement vessel (50 × 30 × 10 mm) and cultured in sterilized water at 25°C for 24 hours. After 24 hours, when the root had grown to 5–8 cm, the seedling was equilibrated in a 0.1 mM KCl solution in the same bath for 2 hours before the potential measurement.

Potential measurement—The experimental setup for the measurement of the potential distribution and ion concentration near the root surface is shown in Fig. 1. All the potential measurements were performed with an Ag/AgCl reference electrode placed in the measuring bath. A glass micropipette with a tip diameter of 0.7 μm, prepared by drawing a glass tube of 1 mm outer diameter, was filled with saturated KCl solution and used as a potential probe electrode. The potential signal from the microelectrode was fed into an electrometer (Hokuto Denko HE-106) and recorded on a computer. Movement of the microelectrode was accomplished by an oil pressure driver under a stereoscopic microscope at room temperature operated in manual or automatic mode. In automatic mode, the oil pressure driver was connected to a precise step motor which was driven by a computer. Before the potential distribution measurements, the microelectrode was first placed at a position away from the root surface in the bulk solution of the bed, the read value of the electrometer was adjusted to zero, then the probe electrode was moved close to the root surface along the root direction. On the root surface, root hairs were found to grow thick. The root hair length of tip region and mature region were about 100 μm and 200 μm, respectively, which prevented the probe electrode to make close to the surface with the distance less than the root hair length. The potential along the root surface was read by moving the probe along the root surface in the stem direction while keeping a constant distance from the surface.

Potassium ion-selective microelectrode—A potassium ion-selective microelectrode was prepared by coating the inside tip of a glass micropipette with a silicone resin (Toshiba Silicone Co., TSL451-1000) and filling it with liquid potassium ion exchanger (World Precision Instruments, Inc., IE190) at the tip portion, behind which the micropipette was filled with 0.5 M potassium chloride solution. This electrode gave a 50 mV/decade linearity in the pK^+ range of 0–5 (Takeuchi et al. 1994). The potential value read with this electrode is the sum of the potential of the potassium ion concentration and the diffusion potential near the root surface. Hence, for obtaining the pure contribution of K^+, the potential value measured with the glass microelectrode should be subtracted from that measured with the potassium ion-selective microelectrode. The

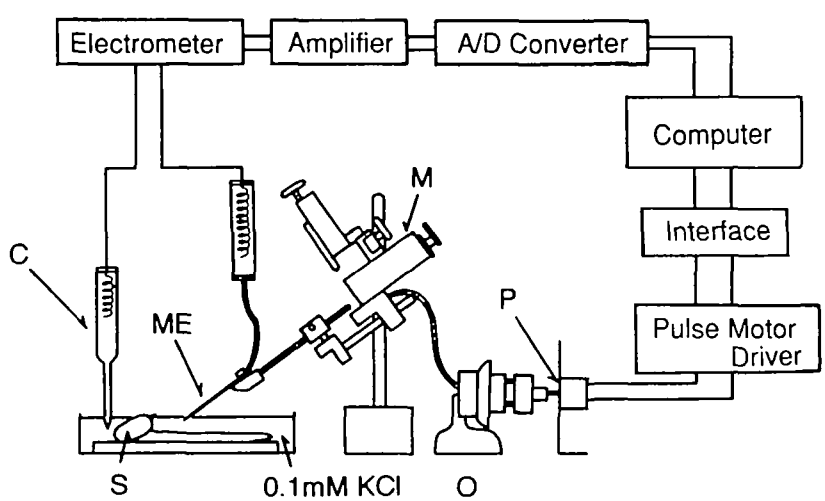

Fig. 1 Experimental setup for the measurement of the potential and ion concentration near the root surface. S=sample bean sprout (about 5 cm-length), C=counter electrode (Ag/AgCl/sat. KCl), ME=glass microelectrode or ion-selective microelectrode, M=micromanipulator, P=pulse motor, O=oil pressure-actuated driver.

potential signal from the electrode was fed to an electrometer (Dia Medical System Co., DPZ-1228S) and recorded on a computer.

Hydrogen ion-selective microelectrode—For pH measurements, a tungsten oxide electrode was prepared as a probe electrode. Tungsten wire (99.95% purity, The Nilaco Co.), whose tip diameter was 0.1 mm, was coated with polyethylene except at the tip. A tungsten oxide layer was formed at the tip by anodizing it in dilute sulfuric acid. This electrode responded well to pH with a 36 mV/pH decade. Since tungsten oxide behaves as an electrochromic material with a reversible electrochemical reaction (Burke 1982, Ohtsuka 1990), it can be utilized for pH measurements. Potential measurements were done in reference to a Ag/AgCl/saturated KCl electrode which was connected to the measuring bath through a salt bridge. The potential signal from the electrode was fed to an electrometer (Dia Medical System Co., DPZ-1228S). We kept in mind that the electrode potential of the electrode might be influenced by the presence of some oxidation-reduction substances in the culturing solution. The pH values given by the tungsten electrode were then examined in the culturing solutions containing several different amount of H^+ by referring to those of a glass pH electrode (Horiba H-7SD). No difference was found between the pH values of the two electrodes.

Results

Potential distribution near the surface of the root tip—The potential distribution along the root direction 100 μm outside the root surface is shown in Fig. 2. The potential measured with a glass micro (pipette) electrode was found to always be a negative value along the root surface. The negative value was relatively small in the matured regions, but it fell to a large negative value at the growing portion of the root tip. This tendency was observed to be more pronounced closer to the root surface.

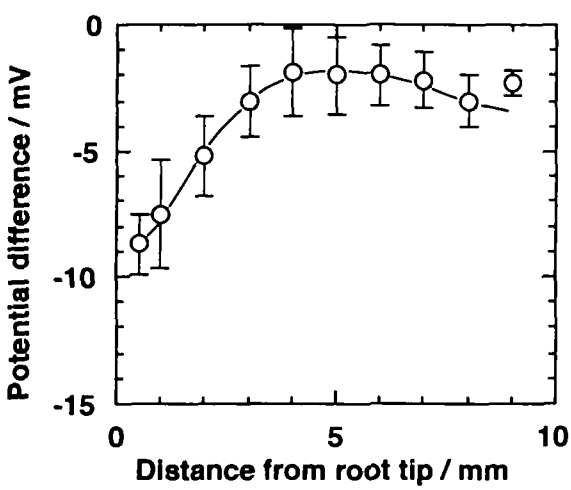

Fig. 2 Potential distribution along the root direction at a distance of 100 μm outside the root surface. Means of 10 seedlings with standard errors (vertical bars) are shown.

Potential changes indicating lateral root emerging—We measured the electrical potential distribution along the root length direction of the matured region where a lateral root was expected to emerge. An example of results is shown in Fig. 3(a). In an unspecified region, we found a negative potential well in spite of the fact that nothing was found under the microscope on the smooth surface. As seen in Fig. 3(a), the well was deeper at locations closer to the surface. After about 10 hours, we found a lateral root emerging at the position just corresponding to that of the potential well. Potential distribution curve obtained with the averaged values of several potential curves is shown in Fig. 3(b). Such a phenomenon was found not occasionally, but always for lateral root emerging whenever the seedling was actively growing. Therefore, this potential well is really a preindication of the emerging of a lateral root. The well could be observed even at the side part of the emerge, indicating a stereoscopic potential distribution (shown in Fig. 3(c)). This well begins to appear 15–20 hours before the emerging, and grows gradually until the time of the emerging. The root cross-section was made in the region corresponding to negative potential well, and we observed the section through a microscope. These results are shown in Fig. 4. The depth of the potential well was found to be in good relation to the growth level of the lateral root primordia. The deeper the potential well, the greater the growth level of the lateral root.

Distribution of ionic concentrations—The qualitative feature of the proton concentration around the root could be obtained by setting a living seedling in a 3 mm-thick agar gel layer of a culturing solution containing bromocresol purple as a pH indicator (shown in Fig. 5). A dye color change from purple to yellow was observed very clearly in the surface region of the root, indicating that the H^+ concentration became, in general, higher than that of the bulk. The concentration, however, appears lower in the root tip region. This was ascertained by detailed pH measurements with a pH measuring electrode made of tungsten oxide. By using the tungsten oxide electrode, the H^+ concentration profile was measured in the root tip region (shown in Fig. 6(b)). The H^+ concentration of the root tip was observed to be lower than that of the bulk solution. Parallel K^+ concentration measurements were also performed in the region with a K^+ ion-selective microelectrode. The concentration distribution of K^+ near the root surface thus obtained is shown in Fig. 6(a), where the ionic concentration is shown to be higher near the root tip in contrast to the H^+ concentration.

Similar tendencies of the ion concentrations were found also in the lateral root emerging region. In the grown region, the H^+ concentration was always higher than that

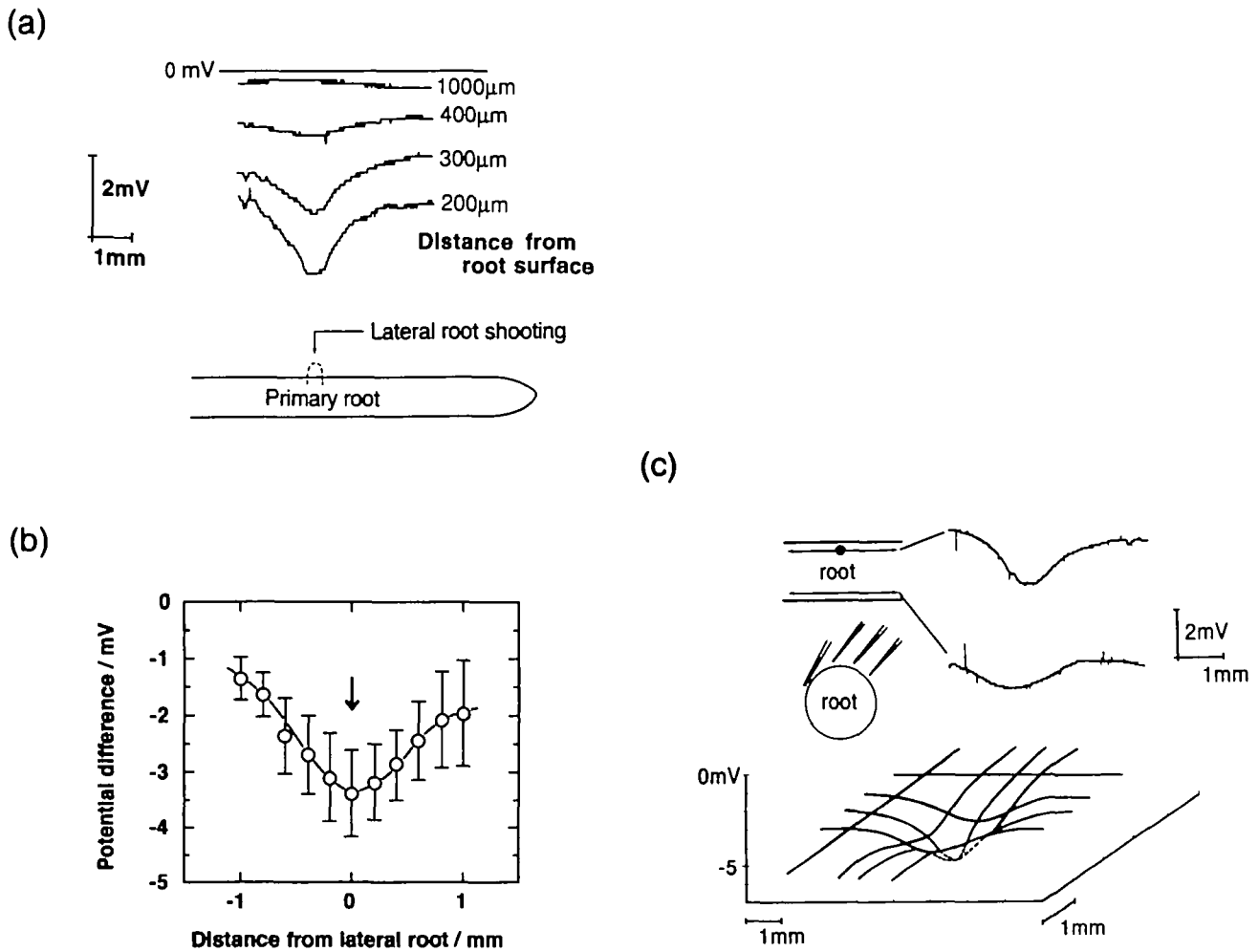

Fig. 3 (a) Potential distribution along the root direction near the root surface at varied distances. The lateral root will emerge in the region corresponding to the negative potential well. (b) Potential distribution curve obtained with the averaged values along the root direction at the distance 200 μm outside the root surface. Means of 10 seedlings with standard errors (vertical bars) are shown. (c) Stereoscopic potential distribution.

of the bulk (Fig. 5). By moving the tungsten oxide electrode along the root direction, the H^+ concentration profile along the root surface could be obtained in the region of the electric potential well which indicates lateral root emerging. The results are shown in Fig. 7(b). The H^+ concentration was found to be the lowest at the lateral root emerging point. In contrast, the K^+ concentration was found to be higher in the region of the potential well as shown in Fig. 7(a).

We must also consider anions for charge balance in the measuring solution. The largest anion quantity in the solution is Cl^-. We measured the Cl^- concentration distribution near the root surface using a AgCl layer-coated Ag probe electrode (Ag/AgCl electrode). It was found that the concentration of Cl^- is uniform throughout the solution, even near the root surface.

Discussion

Whenever the plant was active, negative potential wells were always observed at the root tip and in the lateral root emerging region. The range of the negative potential wells was -4 to -15 mV. This fact implies that negative potential wells are strongly related to the growth of the seedling. Especially, there is a close relationship between the depth of the negative potential well and the growth level of the lateral root primordia as shown in Fig. 4. Blakely et al. reported that the lateral root started to develop about 8 hours after the formation of the lateral root primordia inside the primary root. The lateral root emerged outside the primary root surface about 20 hours later. In the present study, a negative potential well was observed outside the primary root as soon as the lateral root started

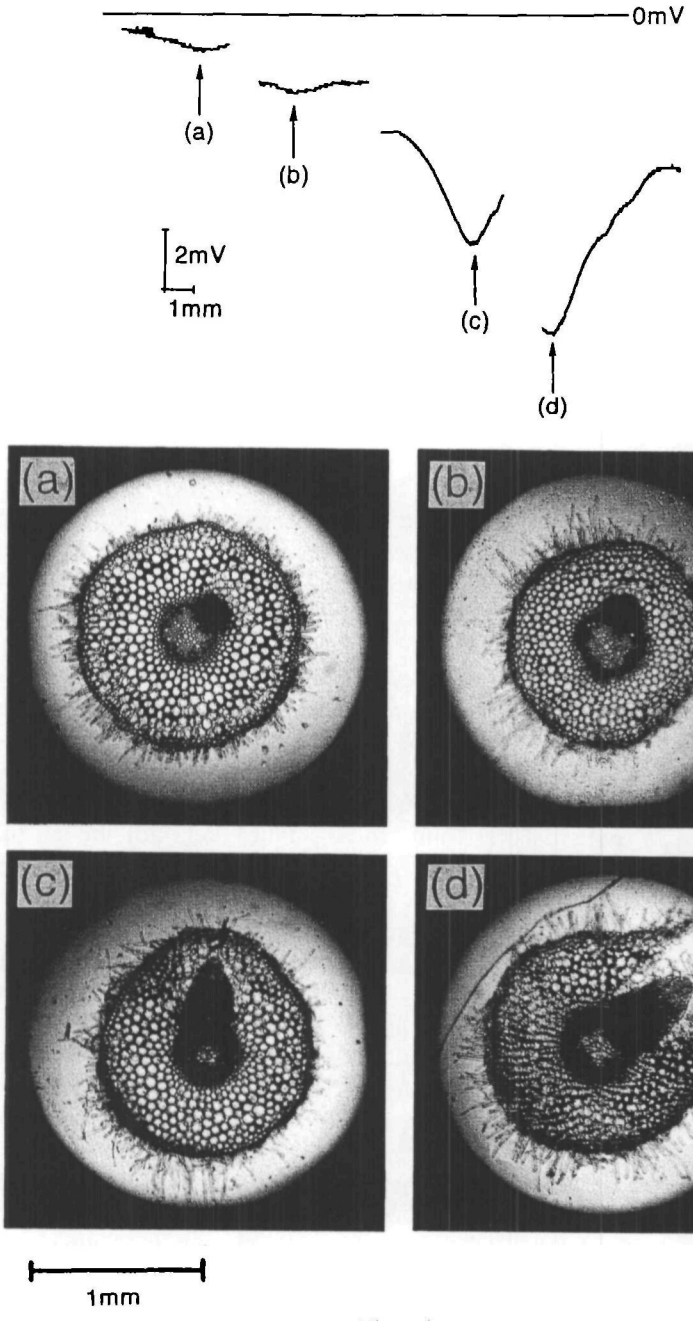

Fig. 4

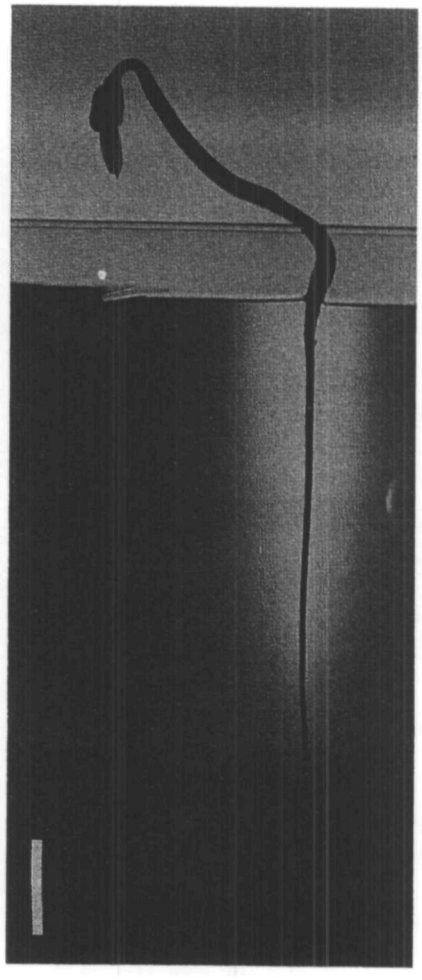

Fig. 5

Fig. 4 Microscopic photographs of the root cross-section in the region of the lateral root primordia and the negative potential well just corresponding to the position of the lateral root primordia. The potential distribution was measured along the root direction at a distance of 200 μm outside the root surface, and the arrow indicates the lateral root emerging position. The time sequences from (a) to (d) of the potential curves correspond to those of the microscopic photographs.

Fig. 5 H^+ distribution around the root revealed by the color change of a pH indicator (bromocresol purple). The yellow color surrounding the mature root corresponds to a low pH value. This color is less intense around the elongation region and the root tip, indicating a higher pH. Bar = 1 cm.

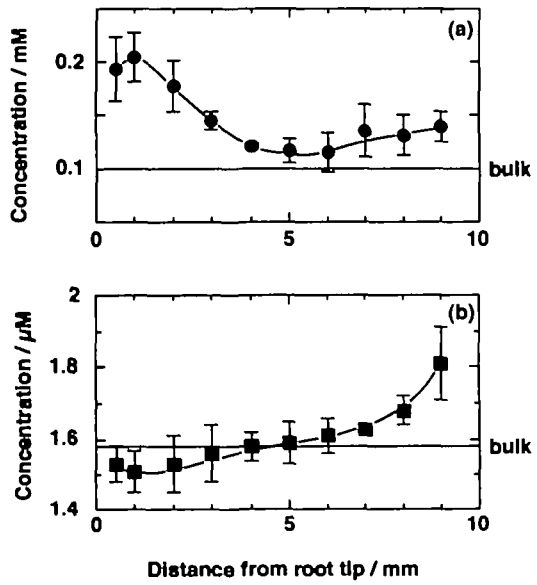

Fig. 6 K^+ concentration (a) and H^+ concentration (b) along the root direction at the root tip. These were measured at a distance of 100 μm outside the root surface. Means of 10 seedlings with standard errors (vertical bars) are shown.

to grow from the primordia. This fact was pointed out by Hamada et al. (1992), too. We confirmed the starting cell division of lateral root primordia in the primary root by microscopic observation. The time of starting of the cell division was found just correspond to that of ion concentration change.

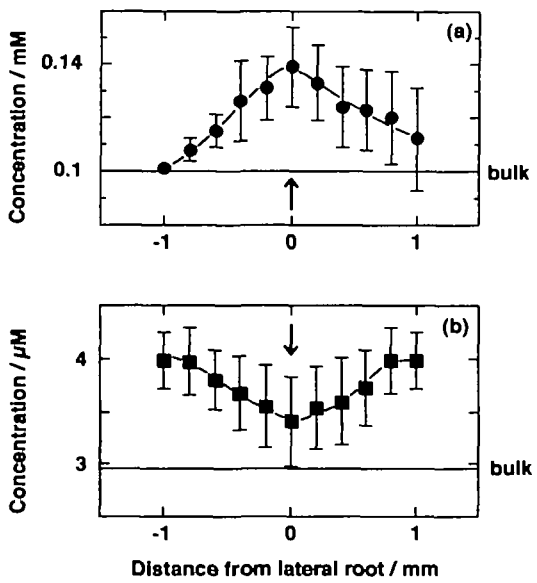

Fig. 7 K^+ concentration (a) and H^+ concentration (b) along the root direction in the mature region of the root (lateral root emerging portion). These were measured at a distance of 200 μm outside the root surface. The arrow indicates the lateral root emerging position. Means of 10 seedlings with standard errors (vertical bars) are shown.

We intended to make clear what factors are the main origin of the negative potential well. There is a possibility that the potential well is a result of penetrating out of electric field of some membrane potential inside the root. Generally, the electric field decays very rapidly, i.e., being inversely proportional to the square of distance, so that it is unlikely to expect the presence of observable potential field even away from the root surface. On the other hand, in the growing cell, uptake or extrusion of various substances are expected to occur by the action of ion pump and so on. As a result, the substance concentration of root surface may differ from the bulk concentration.

Therefore, we may most likely attribute the cause of the potential difference to a diffusion potential. A diffusion potential is caused by the contact of two solution phases which have different ionic concentrations. The magnitude of the diffusion potential depends on the ionic concentration difference between the two solution phases. A smaller potential difference is observed for a smaller concentration difference.

Such a tendency was observed when we measured the potential distribution around the root surface in potassium chloride solutions of various concentrations. When the concentration of the bulk solution was larger (closer to ion concentration near the root surface), the value of the negative potential was found to be smaller (data not shown; to be published elsewhere). On the other hand, with a fixed solution concentration, when the probe electrode was moved from the bulk toward the root surface, the negative potential value became larger. These facts suggest that the negative potential is caused by an ionic concentration difference caused by the uptake and extrusion of ionic species in the root. These results may be most likely elucidated on the basis of a liquid junction (diffusion) potential based on an ionic concentration difference generated by the plant's metabolism.

A potential change thus caused by different in ionic concentrations can be described quantitatively by the so-called liquid junction potential due to a difference in ion concentration. In general, a potential difference, $\delta\Phi$, caused by an ion concentration difference between two solution phases, I (root surface region) and II (bulk), which are in contact with each other, is expressed quantitatively by Henderson's Equation (1) for a liquid junction potential (Henderson 1907).

$$\delta\Phi = \Phi^{I} - \Phi^{II} = \frac{\sum_i u_i/z_i(C_i^{I} - C_i^{II})}{\sum_i u_i(C_i^{I} - C_i^{II})} \frac{RT}{F} \ln \frac{\Sigma u_i C_i^{II}}{\Sigma u_i C_i^{I}} \qquad (1)$$

where C_i is the concentration of i species; u_i, the mobility; and z_i, the charge of the ion.

The measured ionic concentration distributions over

the root surface are given in Figs. 6 and 7. At the root tip, the K^+ concentration was higher than that of the bulk solution. In contrast, a low H^+ concentration was observed at the root tip. On the other hand, the Cl^- concentration was nearly unchanged throughout the region. Similar ionic concentration distributions were observed in the lateral root emerging region.

The high concentration of K^+ ion at the root tip is probably due to the release of K^+ from the active tissue near the tip. This release of the cation causes a spontaneous absorption of H^+ ion into the tissue which enables the cell wall to loosen, resulting in the decreased H^+ concentration. Necessity of H^+ ions was reported on the cause cell wall loosening in the elongation region (Rayle and Cleland 1970, Hayashi 1989). Such a H^+ ion concentration near the root tip is well compensated with the supply of H^+ from the root hairs present near the region which is supported by the experimental results. The most active uptake of H^+ is expected to occur at the root tip as well as the lateral root primordia inside the primary root.

In the present case, the major ion species contributing to $\delta\Phi$ are considered to be K^+, H^+, and the counter anions. For the calculation of the junction potential by taking the charge balance into consideration, it is necessary to consider the concentrations of the anion species. We determined the concentration of Cl^-, which was found to be nearly uniform throughout the region. The contribution of an other anion should thus be considered from the electroneutrality among K^+, H^+, and the ions. For an anion which is released from the tissue accompanied by large quantities of K^+, it is most plausible to consider a large organic anion like galacturonic acid or glucuronic acid. These organic anions are contained in the hemicellulose which constitutes the cell wall (Wareing and Rhillips 1981). Such organic anions may possibly be released during cell wall loosening. We are now attempting to confirm the presence of this organic acid by HPLC. Our results will be reported elsewhere.

For our calculations, we evaluated the mobilities of the ion species (u_i) from the published data (Landolt-Börnstein 1959). The mobilities of K^+, H^+, and Cl^- are 73, 349, and 76 mho cm^2, respectively. We also evaluated the mobility of the organic anion. The mobilities of two organic acid anions similar to galacturonic acid ($C_6H_9O_7^-$), benzoic acid ion ($C_7H_5O_2^-$) and oxybenzoic acid ion ($C_7H_5O_3^-$), are 32 and 35 mho cm^2, respectively. We thus estimated the mobility of galacturonic acid at about 30 mho cm^2.

By putting the K^+, H^+, Cl^-, and organic anion concentrations thus evaluated into eq. (1), we could calculate the potential near the root surface. The results are shown in Fig. 8. As a first order approximation, the coincidence between the theoretical curves and the experimental curves is sufficient. Therefore, the potential distribution near the root surface may be attributed to a junction potential based on an ionic concentration gradient.

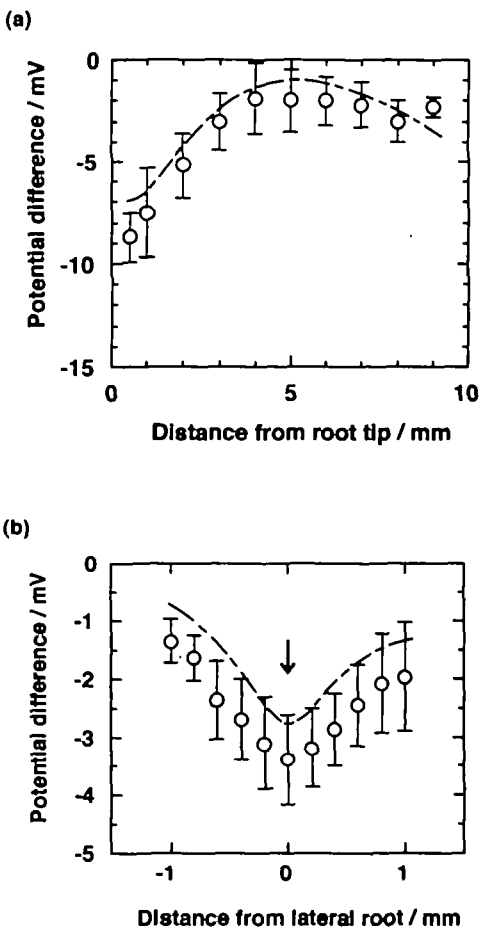

Fig. 8 Comparison between theoretical and experimental potential distribution curves along the root direction at the root tip (a) and lateral root emerging point (b). ○, experimental potential results; –·–, theoretical potential distribution curves.

We are now extending our observations to other kind of ion species also for some other kinds of plant seedlings.

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