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Short communication

Study of salt permeation process into *Vigna angularis* using Ag/AgCl electrodes

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Abstract

In this work we report a novel method that can monitor in vivo the salt permeation process into a plant *Vigna angularis* using two Ag/AgCl electrodes. The method is based on the electromotive force (emf) measurement using two Ag/AgCl electrodes, one inserted into the *V. angularis* pith cavity and the other placed into saline ([NaCl] = 1 M) surrounding the roots. Temporary change of emf ranging from 0 to ca. 0.115 V was measured in vivo using the system, and the possible physiological phenomenon during the period was discussed.

Keywords: Electromotive force; Pith cavity; *Vigna angularis*

1. Introduction

Global deserting is a serious problem, and we have to find a way to grow crops to support growing population under this circumstance. Deserting soil often contains highly concentrated salt. An easy method to evaluate the salt stress to plants is therefore expected, since plants suffer from the severe stress caused by salinity. Electric potential measurements have been widely performed for plants. For example, effect of salinity on the growth of a plant was evaluated using a specific ion electrode [1]. Ion-selective microelectrodes are used for pH [2] or sodium ion concentration [3] measurement in plant body. Such microelectrode methods have advantages that they enable lower or noninvasive approach to the plant. These methods, however, require careful preparation of the electrodes having complicated structure. Also, they require reference electrode and a voltmeter with input impedance of 10^{12}–10^{15} Ω. Insertion of microelectrodes often require micromanipulator [4]. Here we report the applicability of the concentration cell-based electromotive force (emf) method to the salt permeation process monitoring of *Vigna angularis*. Unlike ion-selective electrode method, ours use two Ag/AgCl electrodes directly placed into sap and into saline. A fine Ag/AgCl electrode with the diameter of 200 μm was inserted into the pith cavity of *V. angularis*. Time course of the emf between two Ag/AgCl electrodes, one inserted into the pith cavity and the other placed into the solution surrounding roots, was measured.

2. Experimental

Three-week-old seedlings of *V. angularis* on the agar gel were used. One of the two Ag/AgCl electrodes was inserted vertically into the pith cavity of the stem after a pin hole was opened for the entrance of the electrode, and the other was placed into the solution around the roots (Fig. 1a). This arrangement is electrochemically equivalent with the electrolyte concentration cell Ag | AgCl | NaCl(c_1) | NaCl(c_2) | AgCl | Ag, where c_1 and c_2 are the activities of ions which in this case can be approximated as the concentration of NaCl inside and outside the plant, respectively. Saline solution was prepared by dissolving the sodium chloride using Milli-Q® quality water. The diameter and the length of the Ag/AgCl electrodes used in the measurements were 200 μm and 35 mm, respectively. Emfs were measured using a 16 ch voltmeter with input impedance of > 1 MΩ (NR-250, Keyence, Osaka, Japan). All the measurements were performed at 25 °C. Accuracy of our emf method was tested using standard NaCl solution and the tube with ceramic disk with typical

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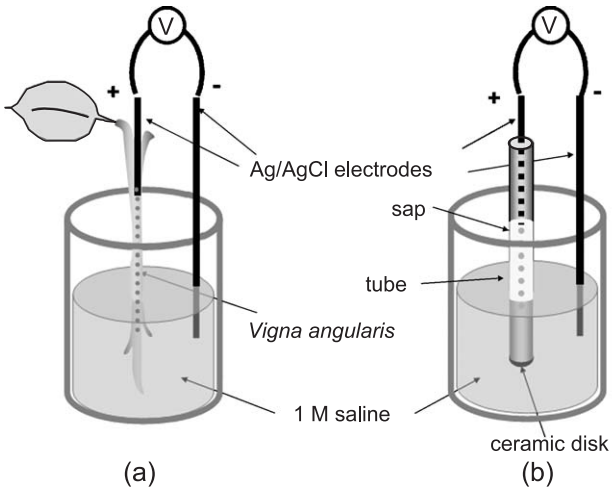

Fig. 1. Schematic illustration of the emf measurement system. Emf measurement using *V. angularis* stem (a) and ceramic disk tube (b).

resistance of < 1 MΩ (EE008, Cypress Systems, Lawrence, Kansas), and then the sap collected from inside the pith cavity was injected into the tube. For this test emf was measured and the Cl^- concentration in the sap was calculated using Nernst equation.

3. Results and discussion

Our emf method proved to be accurate for the chloride ion concentration ranging 1–1000 mM when tested using standard NaCl solution and the tube with ceramic disk (data not shown). A pith cavity was observed in the center of the stem. Such cavity was not seen in the younger seedling (Fig. 2a) and it grew larger with time (Fig. 2b indicated by an arrow), and finally became ca. 300 μm in diameter (Fig. 2c). Such cavity formation is often found in plants. [5] Xylem and phloem were found in a concentric circle arrangement with vascular cambium. The cavity was filled with sap, and when the Cl^- concentration of the liquid was measured, the value (ca. 1.5 mM) was in good agreement with the one in xylem sap [6]. The origin of the cavity sap is unknown. An Ag/AgCl electrode was inserted into this open space for ca. 20 mm (Fig. 2d–f). When making a pinhole, we carefully avoided to destroy the vascular bundle. As a result, the pinhole was made between the two bundles, but the inserted electrode may contacted electrically with xylem or phloem sap. In other experiment, when two electrodes were inserted at two points with different height (5 and 10 cm from the root), lower electrode showed peak earlier than the other. This fact suggests that the electrode signal reflected some chemical change that rose upward the cavity.

As shown in Fig. 3b, emf of ceramic disk tube gradually increased and finally reached the equilibrium value (ca. 0.115 V). Emf of *V. angularis* showed apparently different curve (Fig. 3a). As a result, emf was measured successfully using the configuration illustrated in Fig. 1a, unaffected by significant electric noise. This indicates that the tissue showed a good ionic conductance for the emf measurement. In this case, emf finally approached 0 V because of the diffusion of the surrounding NaCl solution into the pith cavity. The emf interestingly showed increasing tendency and a peak appeared (at ca. 2500 s) before the monotonous decrease.

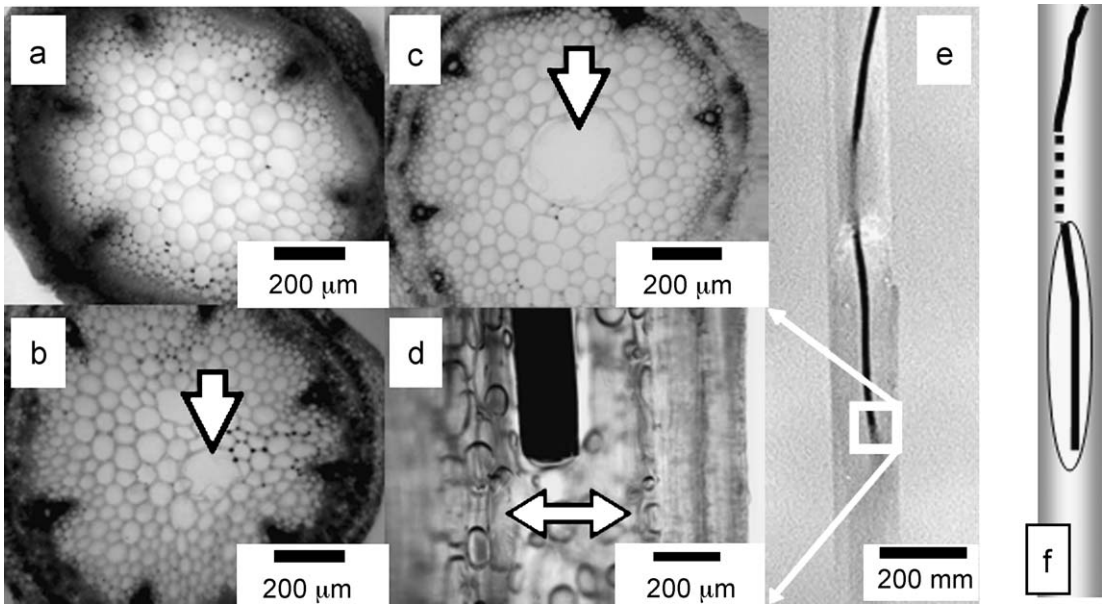

Fig. 2. Pith cavity found in the cross section of *V. angularis* stem. Young seedling (ca. 2 weeks after germination) with no cavity (a). Small cavity found in the pith (2–3 weeks) (b). Large pith cavity found in 3-week-old seedling (c). Magnified image of the vertical cross section of the stem; inserted electrode tip is located in the pith cavity (cavity is indicated by an arrow) (d). Electrode inserted from the pinhole; part of the stem is removed to expose the electrode (e). Schematic illustration of the electrode inserted into the pith cavity (f).

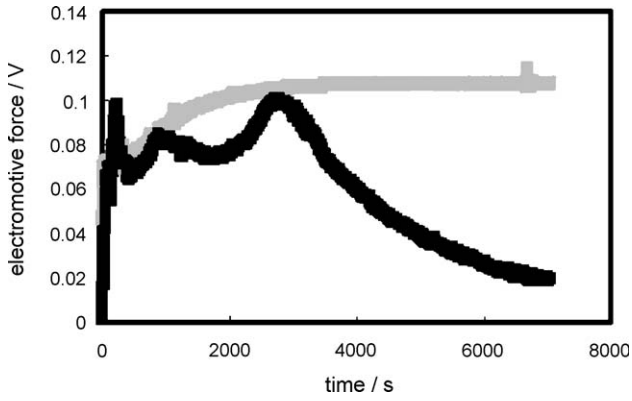

Fig. 3. Emf time courses obtained from measurements using *V. angularis* stem (▬) and ceramic disk tube (▬). Sampling rate 1 s; temperature 25 °C.

The plant immediately wilted after the emf reached the peak because of the loss of water absorbency. Such tendency was observed in all the seedlings ($n = 5$) tried, although the time to observe the peak emf differed. What was happening during this period is unknown, but Cl^- diffusion into the pith cavity was somehow suppressed until the emf peak appeared, or the transference numbers of ions were changed through the process. Presumably the osmotic pressure inside the stem might have decreased critically and that lead the plant to be withered. A voltmeter with 16 ch theoretically enables measurement of multiple points at the same time. This is an advantage for the multi-point measurement in a single plant body, although the input impedance is far smaller compared to the one reported [4]. Unlike ion-selective electrode, Ag/AgCl electrode can easily be fabricated in a smaller diameter suitable for inserting into smaller space. This method might be effective in that the permeation process can be measured using a low-cost voltmeter.

4. Conclusions

Emf measurement using two Ag/AgCl electrodes was applied to the in vivo salt permeation process monitoring. In this specific case, the system possibly could monitor the osmotic stress process from the surrounding saline. This system might be applied to the in vivo evaluation of the physiological plant stress from electrochemical point of view.

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