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Short communication

Responses of *Bruguiera gymnorrhiza* to saltwater monitored by miniature electrodes

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Abstract

We measured the voltage between two Ag/AgCl electrodes, one inserted into the seedling of the salt-tolerant plant *Bruguiera gymnorrhiza* and the other into the vermiculite in which the seedling was potted. Four seedlings were placed in different environments, in saltwater or pure water, with light or alternating light/dark conditions. We have found that (1) the voltage profiles showed periodical oscillatory behavior; (2) seedlings in saltwater showed higher voltage compared to the ones in pure water; (3) in the light environment, the voltage was higher compared to the one in the dark environment; (4) in the dark environment, a voltage wave was hardly observable; and (5) electrodes inserted into the propagule cortex, stem cortex, and petiole showed different voltage wave amplitudes. The voltage profiles will provide an effective way to evaluate the movement of salt water inside the salt-tolerant plant.

Keywords: *Bruguiera gymnorrhiza*; *Vigna angularis*; Safranin; Ag/AgCl electrode

1. Introduction

Desertification of farmable land is one of the most serious world-wide problems. Desert soil often contains concentrated salt, and the plants growing in such an environment suffer from salt stress. The effect of salinity on plant growth has been reported [1], and studies on ion transport in the xylem [2,3] are important to investigate the mechanism of salt stress. Microelectrode probes have often been used for this purpose, i.e., for ion transport measurement [4–8], for membrane potential measurement [9–12], or activities of ions [13–15]. For the purpose of ion stress measurement in the field, a battery-driven, manually operated system is desirable. We have previously reported the applicability of the concentration cell-based electromotive force (emf) method in monitoring the salt permeation process of *Vigna angularis* [16]. In the previous report, we proved that in vivo emf measurement is possible using two Ag/AgCl electrodes, one inserted into the pith cavity of *V. angularis* and the other into the saline surrounding the root. When the concentration cell-based emf method is used, the emf profiles show characteristic peaks that give information on the salt stress to the plant [17]. *V. angularis* wilts in about an hour in the saline environment. It is desirable, therefore, to use some salt-tolerant plants for the evaluation of salt stress for a longer period.

In this work, we inserted a Ag/AgCl electrode into several parts of *Bruguiera gymnorrhiza*, known as a salt-tolerant mangrove plant found in tropical and subtropical forests. *B. gymnorrhiza* is reported to have a salt filtration structure in the root, and transpiration is thought to be the main driving force in absorbing water from the sea water [18]. Sodium chloride concentration inside the xylem sap of this plant is reported to be from one thousand [19] to one hundred [20] times lower than that in sea water. This concentration difference is expected to show, according to the concentration cell model, a voltage of several hundreds

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of millivolts when two Ag/AgCl electrodes are inserted into the plant and into the sea water surrounding the root. Generally, the plant lives in brackish water regions, so the sodium chloride concentration around the root changes daily because of the tides. It is therefore conceivable that the sodium chloride concentration in xylem or phloem sap changes during a day. Another factor that may affect the sodium chloride concentration in the sap is transpiration. Plants generally open stomata during the daytime to release oxygen and absorb carbon dioxide for photosynthesis. Evaporation of the water inside the space in the spongy tissue drives the absorption of water at the root. It is reasonable to assume that the sodium chloride concentration inside the plant is controlled by light and saline around the root. We report here the voltage profile obtained from a Ag/AgCl electrode inserted into the cortex or petiole of *B. gymnorrhiza*. The effect of light on the profile is also examined.

2. Materials and methods

2.1. *B. gymnorrhiza*

Germination and growth of the *B. gymnorrhiza* seedlings were conducted in a room with controlled humidity (70%) and temperature (25 °C). The propagules (seeds) of *B. gymnorrhiza* were potted among vermiculites soaked in tap water until the measurement. Lighting was controlled to alternate between 12 h of light and 12 h of dark. After 7 months, when the seedlings became ca. 40 cm in height above the pot, water was exchanged for saline containing 400 mM of sodium chloride. After the plants' adaptation to the saline for 8 weeks, the electrodes were inserted and the measurement started.

2.2. Electrodes

Silver wires (50 mm in length, covered with Ag/AgCl for 35 mm, 200 μm in diameter) were purchased from TOA-DKK Company (Tokyo, Japan). Holes were drilled manually into the stem and a petiole of the *B. gymnorrhiza*. Electrodes were inserted into the holes and were fixed and covered with sealing films. Inserted positions were evaluated using safranin staining. Safranin is known as a cationic biological stain for plants and can stain a plant structure, such as xylem, which is rich in lignin. When 1% (w/w) safranin (Wako Pure Chemical Co., Osaka, Japan) solution is added to vermiculite, the plant absorbs the stain through the root, and the xylem is stained red. We found that the xylem of *B. gymnorrhiza* forms a very hard concentric circular structure (Fig. 1 (a)). Microscopic images of the stained part strongly suggest that the structure contains vessels (Fig. 1 (b),(c)). While drilling the stem of *B. gymnorrhiza* manually, one could feel the tip of the drill hit the hard layer. Holes were drilled, therefore, for the inserted electrodes to have as much contact with the hard layer as possible. Thus, the electrodes were inserted into the cortex surrounding the xylem and possibly contacted the xylem sap, because the cortex is known to conduct water and ions to and from the xylem. Electrodes were also inserted into the petioles following the procedure previously reported [21]. Here, the electrodes are thought to have made contact with the xylem sap.

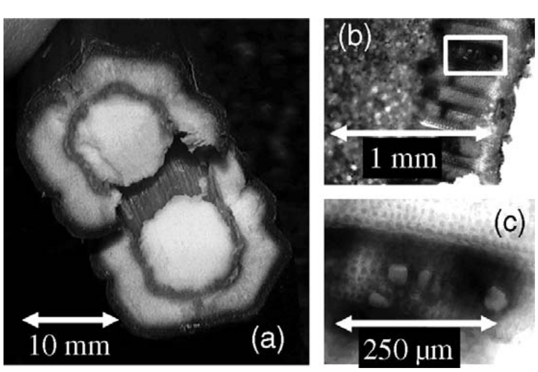

Fig. 1. Images of *B. gymnorrhiza* cross section. (a): Concentric stained structure, (b): Microscopic image of the staned structure, (c) Magnified image of the enclosed part in (b).

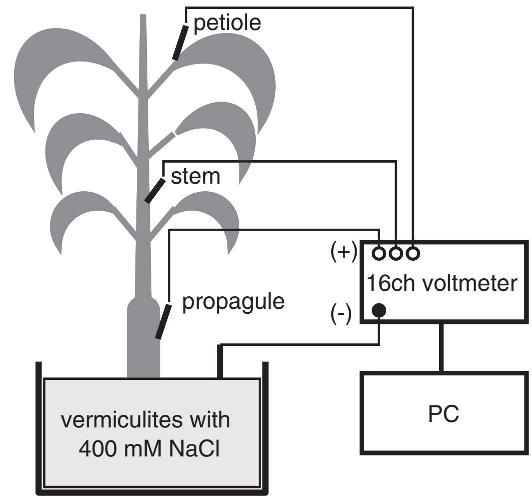

Fig. 2. Schematic illustration of the measurement system. Three Ag/AgCl electrodes were inserted to propagule, stem, and petiole of the plant. Ag/AgCl electrodes connected to negative terminals were inserted among vermiculites that were soaked with 400 mM NaCl.

2.3. Equipment

Ag/AgCl electrodes were connected to a 16 ch voltmeter with an input impedance of >1 MΩ (NR-250, Keyence Corporation, Osaka, Japan). Justification of the system was reported in the previous work [16]. Three electrodes were inserted into the plant as illustrated in Fig. 2. All the measurements were performed at 28 °C, and the humidity was controlled at 70%.

3. Results and discussion

3.1. Effect of electrode position on voltage profile

Three holes were drilled manually in one seedling. The lowest hole was drilled in the propagule (seed) part, the middle hole in the stem, and the highest in the petiole. Measurement started soon after the electrode insertion. Before the measurement, the effect of light on the voltage differences was examined using a bare Ag/AgCl electrode. The effect was negligible (data not shown). The seedling was placed under a 12 h light/12 h dark condition throughout the measurement. In three voltage profiles, periodical waves were observed (Fig. 3). Peaks were observed at different times of the day for the three profiles, and the wave amplitude was highest in the profile from the petiole electrode.

Comparison of the two voltage profiles after the 4th day, from the propagule and from the stem, indicates that the chloride concentration is higher in the propagule than in the stem. This fact agrees with the result from a measurement of collected sap using a conventional method (data not shown). Voltage differences between each pair of electrodes (petiole–stem, stem–propagule, and petiole–propagule) were measured using a galvanostat, and the results strongly agreed with the voltage differences calculated from the curves in Fig. 3. Such results were obtained from 5 seedlings out of 5 that had healthy green leaves.

In our experiment we used a voltmeter with an input impedance of 1 MΩ (not infinite), and the resistances between the two electrodes were as follows: ca. 30 kΩ (between vermiculite and propagule), ca. 180 kΩ (between vermiculite and stem), and ca. 500 kΩ (between vermiculite and petiole). Such a resistance might be one factor in determining the voltage. Some current therefore flowed through the circuit. Although this means that the precise value of the voltage cannot be argued because of the large resistance compared to the input impedance, we showed in our previous report [21] that a similar periodical voltage behavior is measured using a voltmeter with the input impedance of 10 GΩ. When we focus on the field monitoring of the periodic behavior, therefore, our method is useful because of its simplicity. The system can then be regarded as the NaCl concentration cell with transference [22] as follows: Ag|AgCl|NaCl(c_1)|NaCl(c_2)|AgCl|Ag where c_1 and c_2 represent the chloride concentration in the saline and the xylem, respectively. In such a cell, the potential E is calculated by the equation

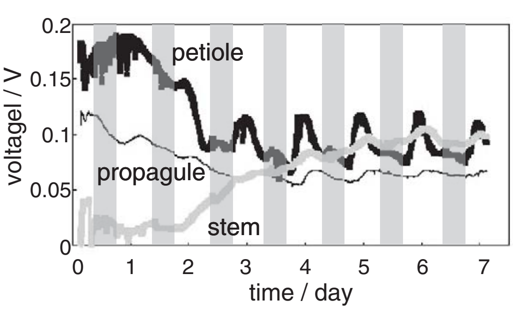

Fig. 3. Voltage profiles obtained from the electrodes inserted to petiole, stem and propagule. Grey bands represent the dark period.

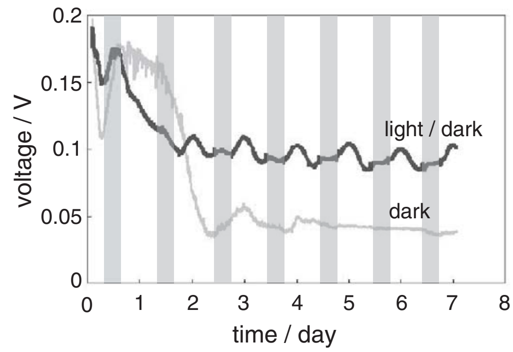

Fig. 4. Voltage profiles obtained from two seedlings, one in light/dark condition, and the other in dark condition. Electrodes were inserted at the stems. Grey bands represent the dark period.

$$E = -0.11830 t_+ \log \frac{a_2}{a_1}$$

where t_+ represents the transference number of sodium ion and a_1, a_2 represent the activities of ions in the saline and the xylem, respectively. To know the main reason of the voltage we therefore need to know such parameters as transference number and activity. In the case where $t_+=0.5$ then we can say that we measured Nernst potential. Evaluation of the transference number is now being conducted by the author. Consideration of junction potential is also necessary. When two solutions with different NaCl concentrations are connected with a salt bridge, there is no junction potential [22]. If the root contains saturated NaCl, the root might function as the salt bridge. Roots of mangrove plants are reported to have low selectivity between NaCl and water and to have a low permeability [23]. NaCl concentration in the root is therefore nearly identical with that in the surrounding saline, and ionic conductance in the root is expected. The reason for the voltage is now under investigation by the author, but the significance of our findings is that our simple measurement system can evaluate the response of the plant to salt and light environment.

3.2. Effect of darkness on voltage profile

Two seedlings, one placed in a dark box and the other placed in the 12 h light/12 h dark environment, were prepared for the measurement. One Ag/AgCl electrode was inserted into the propagule of each seedling. The voltage profile from the seedling in the light/dark environment showed periodical behavior from the second day (Fig. 4). The seedling in the dark environment, on the other hand, showed a different profile. Two peaks appeared on the third and fourth day, but none afterwards. Such behavior was observed in 3 healthy seedlings out of 3. Finally, the voltage value was higher in the plants grown in the light/dark environment compared to the plants grown in the dark environment. Illumination causes stomata to open and increase transpiration. Stomata openings are thought to be necessary for the exchange of gas (oxygen, carbon dioxide, and water vapor). It is therefore reasonable to think that in a light environment, water absorption is activated by transpiration and results in a lower chloride ion concentration (=higher voltage), assuming that in mangrove plants, there is some filtration system to absorb water from saline [18]. The fact that the seedling in the dark environment showed two peaks may be due to the periodic activity of the plant. The seedlings used in the experiment grew in the circadian light/dark environment, so the plant cell might have "remembered the custom." The exact reason for the periodic behavior in the voltage profile has not yet been proven, but our system enabled the in vivo estimation of chloride ions in the *B. gymnorrhiza* cortex. This method is suitable for field monitoring because it can be performed using a battery-powered voltage logger.

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