The calibration model in potassium ion flux non-invasive measurement of plants *in vivo in situ*

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ABSTRACT

SIET (Self-referencing Ion Electrode Technique) provides a novel electrophysiological tool which can non-invasively measure the dynamic influxes and effluxes of ions caused by the diffusion along the concentration gradients *in vivo*. However, in this technique ion fluxes are converted to voltage signals using an ion selective microelectrode at a small amplitude of μV, which is easy to be interfered by the ambient noise. Hence, effective solutions to the suppression of noise and calibration of ion flux measurement system are very important for this method. A K+-selective microelectrode was constructed using liquid ion exchangers (LIX) to investigate ion transport over plant tissue. A standard concentration gradient which simulates plant living cells was produced by an electrode with a certain tip diameter, filled with a solution containing a known K+ concentration in 100 mmol/L. An ion diffusion simulation model was established. This model evaluated the performance of ion flux measurement system in accuracy and reliability by comparing the consistency of the measured value and the predicted curve. K+ fluxes were measured within 25 min at each measuring point of distance 10, 20, 30, 40, 50, 80, and 100 μm from the K+ source, respectively. It can be seen that the K+ fluxes changes little, which indicates that ion flux measurement system has a reliable stability. The study provides a theoretical basis for a new non-invasive ion flux measurement method creation and a new sensors design.

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1. Introduction

SIET, a novel electrophysiological tool, can non-invasively measure the dynamic influxes and effluxes of ions from living biological samples *in vivo in situ* without damaging the organizational structure. These biological samples include a single cell, cellular level, tissues, and even intact plant. Using this technique various intracellular and extracellular ion concentrations, ion fluxes caused by ion exchange will be easy to measure *in situ*. At present, SIET has been more and more widely used in microbiology, medicine, environmental science, molecular biology, and other fields [1–6] to study the relationship among the transmission process, metabolism and other physiological processes. [7–11].

In the ion flux measurement in plants, intracellular and extracellular ion concentrations of plants, and influxes and effluxes of ions from plants are detected by using of ISMEs (Ion Selective Microelectrodes). This kind of biological sensor is pulled from a glass micropipette, filled with pipette electrolyte solution and LIX which converts ion concentration and ion flux to electrical signals. Thus, ion concentration is determined by the electrical signal based on the Nernstian equation.

The voltage gradient over the vibration excursion of the electrode obtained from SIET at μV level which indicates the influxes and effluxes of ions of living plants. It's very easy to be interfered by the ambient noise even submerged. It is important to obtain the weak signal and establish an effective calibration model for non-invasive ion flux measurement which is applied in the plant response to external stimuli. In 1990, Jaffe [12] used a microelectrode with a tip diameter of $\sim 10~\mu m$, filled with high concentration $CaCl_2$ solution, as an artificial standard diffusion source to generate a calcium gradient. The Ca^{2+} concentration and the Ca^{2+} flux were measured along the calcium gradient by the ion flux measurement system. The accuracy, sensitivity and repeatability of the measurement system then were detected by comparing the measurement and theoretical value. Doughty [13] also tested his ion flux measurement using the same method by the detection of Cl^- at different distance of the Cl^- standard source with a certain Cl^- concentration. This method using an artificial standard diffusion source to generate a stable gradient is of high accuracy and easy to repeat. These authors put forward a relational model between the voltage difference signal of microelectrode and the distance from the standard source, but did not take into account the influence of time on their model.

K^+ is one of the main nutrient elements in plants, which involves in the most physiological processes [14] and plays important roles in the process of the electrical signal change when plants respond to external stimuli [15–17]. Matsuoka [15] analyzed the K^+ response to CO_2 stress in *Tradescantia virginiana* L. in the vacuole in leaf cells. Chen [16] measured leaf mesophyll K^+, H^+, and Ca^{2+} fluxes involved in drought-induced decrease in photosynthesis and stomatal closure in soybean. Pottosin [17] studied the difference of the extent of potentiation of the ROS-induced K^+ efflux by polyamines in the salt-sensitive and salt-tolerant barley varieties.

This paper establishes an improved calibration model for K^+ flux measurement system. It not only analyzes relationship between the voltage difference signal of microelectrode and the distance from the standard source, but also analyzes relationship between the voltage difference signal of microelectrode and time. After calibration by this improved model the ion flux measurement system is applied in the measurement of the K^+ flux of maize seedling root.

2. Ion flux measurement system and principle

The ion flux measurement system includes an ion selective microelectrode, a reference electrode, a high impedance microelectrode amplifier, an electric micro manipulator, a faraday cage, an anti-vibration platform, a microscope, and a computer with relevant software as shown in Fig. 1. The Faraday cage is a container made of conductive material that shields external electromagnetic interference. The anti-vibration platform reduces the effect caused by mechanical vibrations. And the voltage signal measured by a microelectrode will be magnified by a high impedance microelectrode amplifier ($>10^{13}~\Omega$), and then output to a computer with ion flux acquisition and calculation software for real-time recording.

An ion selective microelectrode consists of glass microelectrode, Ag/AgCl wire, pipette electrolyte solution and liquid ion exchanger (see Fig. 2) [18]. The relationship of the voltage output E of the microelectrode and the ion concentration C is described using the Nernstian equation Eq. (1):

$$E = k \pm s~\lg C \qquad (1)$$

where E is the measured voltage between microelectrode and reference electrode (mV); C is the ion concentration (mol/L); s is Nernstian slope (mV/dec); k is Nernstian intercept (mV). For a monovalent cation, the theoretical value of Nernstian slope is 59.16 mV/dec at 25 °C. In practice, s is determined by the calibration using two different known concentration. According to the Nernstian equation, there is a significant linear correlation between the ISME's voltage and logarithm of the ion concentration. Then the ion concentration can be calculated based on the ISME's voltage measured.

The ion selective microelectrode is positioned at a small distance (5–10 μm) of the cell, tissue, or organ as shown in Fig. 3. A motor-driven manipulator is used to move the ISME at 2 points over a known distance Δx (10–30 μm, and less than 30 μm) at a low frequency, to measure the voltage signals V_1 and V_2 at each point. Thus the concentration difference ΔC between these 2 points will be calculated by the microelectrode voltage/concentration calibration curve. The ISME moves at a low frequency (routinely no more than 0.5 Hz), which does not affect the distribution of the ion concentration gradient [12,14]. Then the specific ion flux in a certain direction will be obtained from Fick's law:

$$J = D \times \Delta C / \Delta x \qquad (2)$$

where J is the ion flux in the x direction ($pmol/(cm^2~s)$); D is ion/molecule diffusion constant (cm^2/s); $\Delta C/\Delta x$ is ion concentration gradient ($pmol/cm^4$).

3. Materials and methods

K^+ is one of most important ions with a high concentration at 100–200 mmol/L in the higher plant cells, while it is generally dozens of times more than that of extracellular. In fact, K^+ concentration of the environment plants grow in usually maintains 1–10 mmol/L, and even as low as 0.3–0.5 mmol/L [19]. Hence, a plant living cell can be equivalent to a time-continuous point source with a constant K^+ concentration. To determine the performance of ion flux measurement system, we used a microelectrode filled a certain known K^+ concentration as an artificial point source to generate a range of known, steady K^+ concentration gradients without significant voltage gradients.