Development of Portable Dynamic Ion Flux Detecting Equipment

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Abstract: Non-destructive testing of plant organs, tissues, and cells has important implications in studying the immediate physiological status of plants. The portable dynamic ion flux test equipment(PDIFTE) was developed based on Fick′s first law of diffusion and the Nernst equation to achieve the ion flux measurement in pmol•$cm^{-2}s^{-1}$.This equipment integrates micro-imaging, micro-signal processing, automation and control, and biosensor technologies with the original signal acquisition and conditioning module, the motion control module, the macro 3D automatically control platform, micro digital imaging system, electrostatic shielding coating, ion-selective microelectrode, and other components. PDIFTE can detect H^+, K^+, Na^+, Mg^{2+},Ca^{2+}, Cd^{2+},Cl^-, NO_3^-, and NH_4^+. This device can be used in the physiological mechanism research of salt-resistant, drought-resistant, cold-tolerant, heavy metal-resistant, and disease-resistant plants. It can also be used in the research on plant nutrition, ion channel-related gene function, and crop resistant breeding screening.

Keywords: portable dynamic ion flux test equipment(PDIFTE) • ion-selective microelectrodes• liquid ion exchanger (LIX)• ion flux

1 Introduction

Ion absorption is a marker of the normal life activity of plants. Detecting the absorption of external ions is essential in studying the physiology, anti-adversity, disease resistance, and nutrition of plants.

In the past, ion content in plants was detected using atomic absorption spectrophotometer [1], qualitative analysis by detecting the distribution of ions in plants using laser confocal fluorescent probe [2], or using the patch clamp technique to analyze the characteristics of single cell ion channels[3].These static detection methods can be used to analyze the content, distribution, and absorption characteristics of ions in plants, but they cannot achieve lossless, dynamic, and long-term detection.

The development in physics, chemicals, mathematics, and other basic disciplines makes it possible to detect the dynamic absorption/transportation of ions in organisms (transmembrane ion flux). Neuroscientist Lionel F. Jaffe of the US Marine Biological Laboratory put forward the initial model of ion flux detection technique by realizing the ion flux detection outside the cell or organization using Fick's law of diffusion and Nernst equation and the electrical principle of ion/molecule diffusion [4]. Newman of the University of Tasmania investigated the ion flux detection technique equipment and published an article on ion-selective microelectrodes detection of the velocity of H^+ and K^+ in the root tips of corn [5]. In 1995, Smith of the MBL published an article in *Nature* that detailed the detection of transmembrane flux transportation with the ion flux detection technique and reviewed the development of the ion flux detection technique [6]. In 2002, Franklin *et al*. of the University of Massachusetts established the buffer suitable for detecting ions and the research strategy by studying the velocity of H^+ in pollen tube [7]. In 2001, Newman elucidated the significance of ion detection in the gene function research [8]. In 2006, Davies reviewed the achievements of ion flux detection technique in the past years and their application status [9]. In recent years, ion flux detection technique has been used as an important solution for ion transport in many areas of plant research, such as plant physiology[10], plant water stress[11], plant salt stress[12], plant anti-heavy metals stress[13], [14], plant nutrition[15], and other fields[16], [17], [18].With the continuous progress of technology and application of the continuous expansion of the ion flux detection technique, the United States Younger company and the AE company developed ion flux detection techniques for commercial products, such as the Younger model BIO-001B and the AE company model RP-1 ion flux detection technique system. The drawback of the equipment is that it is bulky and unsuitable for handling and field operation, thus limiting the use of the instrument only in the laboratory.

To overcome the shortcomings of the existing dynamic ion current detection equipment and its unsuitability for field operation, we developed the portable dynamic ion flux test equipment (PDIFTE) for laboratory and field environments. This equipment is fitted with a high-performance battery to ensure power supply, which effectively avoids the electrical signal acquisition interference of the alternating current and provides stable signal acquisition performance in the laboratory and field operation. Verified experimental results confirmed the data of PDIFTE and BIO-001B (Younger USA Sci. & Tech. Corp., USA) have very good consistency.

2 Basic Principle of the Dynamic Ion Flux Detection Equipment and System Scheme Design

2.1 Basic Principle of the Dynamic Ion Flux Detection Technique

The principle of the dynamic ion flux detection equipment is based on Fick′s law of diffusion and the Nernst equation. Charged ions in the solution follow the law of diffusion from high concentration to low concentration. With the change in the electrochemical potential from high to low, the gradient can be detected. The diffusion velocity of ions in the solution can also be calculated. Finally, the concentration change rate of ions absorbed or released on the surface of the plant material in the solution can be calculated.

2.2 System Scheme Design

The dynamic ion flux detection equipment adopts the portable scheme design to develop original signal acquisition and modulation module, signal gathering and motion control module, macro 3D automatically controlled platform, micro digital imaging system, electrostatic shielding coating, ion-selective microelectrode design and preparation technique, and carried out the software design and development, finally realize the system integration and application after modulation(Fig. 1).

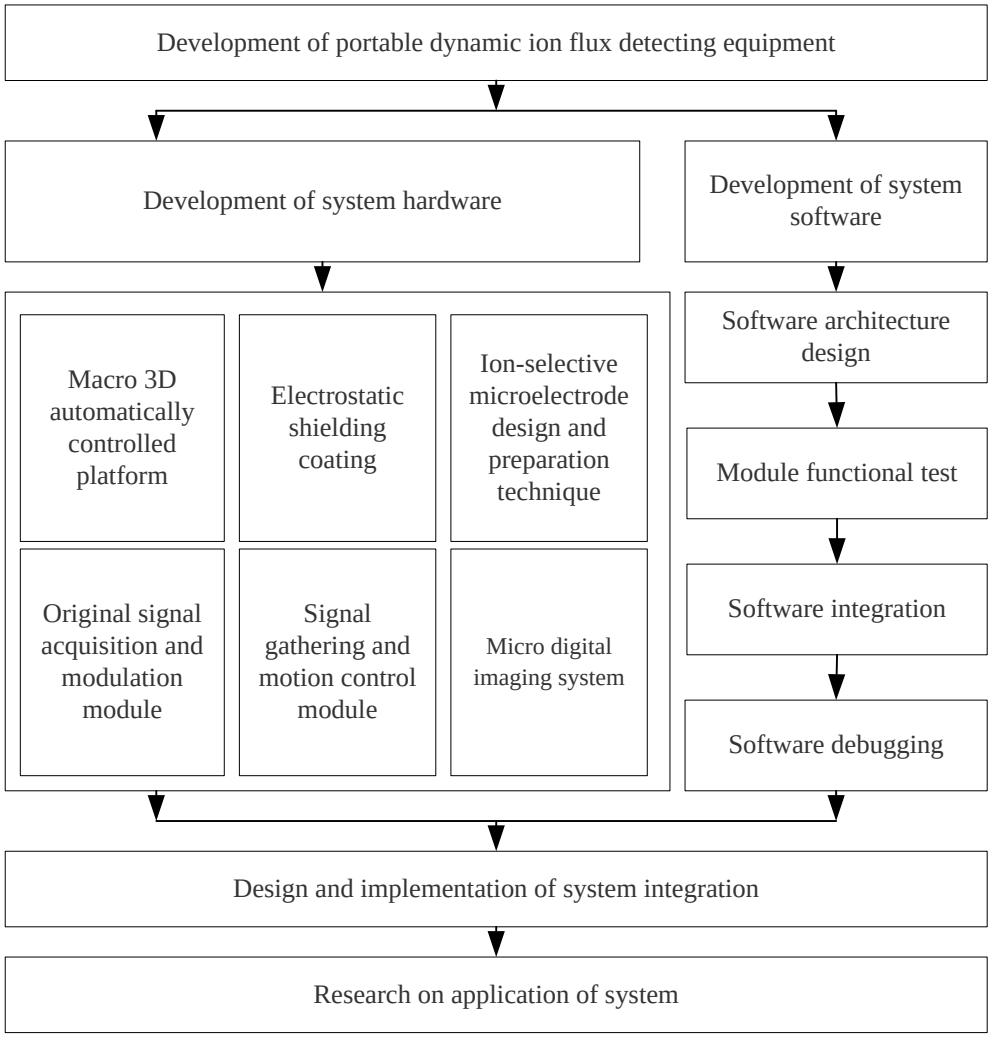

Fig.1. System scheme design sketch

2.3 Hardware Design

2.3.1 System Structure

The dynamic ion flux detection equipment determines the locations of the tested material, reference electrode, and ion-selective microelectrode using a small inverted microscope and high-resolution charge-coupled device (CCD) imaging. It controls the 3D modulation and movement of ion-selective microelectrodes using the high-precision macro 3D automatically controlled platform and the movement of the macro 3D automatically controlled platform using high-performance industrial computer. The system software realizes signal gathering and processing, CCD image presentation, and data analysis(Fig. 2).

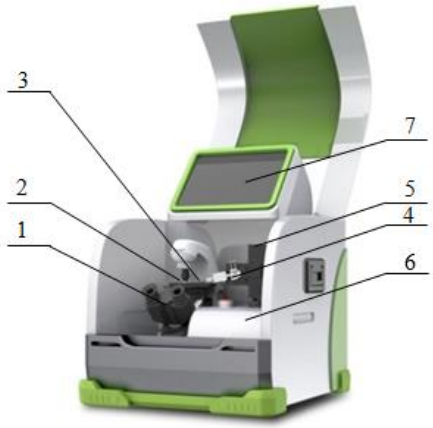

Fig.2. System structure chart: 1. Microscope and CCD imaging module; 2. Detection pond; 3. Ion-selective microelectrode; 4. First signal amplifier; 5. Macro 3D automatically controlled platform; 6. Signal gathering/motion control/data analysis modules; 7. Touchscreen.

2.3.2 Hardware Components and Functions

The microscope and CCD imaging module comprises an inverted biological microscope eyepiece(15X and 20X; objective: 4X, 10X, and 25X). CCD is the colorful picture with the maximum resolution of 1024×768.The sensor is a Sony CCD with an optical size of 1/3 inches and a maximum frame rate of 18FPS.The AD conversion accuracy is 10 digits, and the exposure time is 1/15000s–2s using 12V 1W LED light source.

The detection pond is composed of a petri dish and buffer solution for testing. The components of the buffer solution vary because of the different target ions.

The ion-selective microelectrode has a length of 50 mm, tip diameter of 2–5μm, outer diameter of the terminalof1.5mm, inside diameter of 1.05mm, thickness of pipe wall at 0.225mm, time resolution at 300ms, space resolution at 5μm, and tip-filled liquid ion exchanger (LIX).

The reference electrode comprises an isoplast pipe wall with a diameter of 2mm embedded with Ag/AgCl electrode and filled with 3M KCl with a resistance of 2.7KΩ.

The first signal amplifier comprises an input resistance of the initial signal acquiring module⩾ 10TΩ, with a minimum gain of ×10 anda frequency response of DC～10Hz.

The macro 3D automatically controlled platform has a micromanipulator with a moving precision of0.5μm, moving distance of driver at 1.25″ /2.00″ (30mm/50mm), no-load download speed of 2mm/s, position precision at 0.2% of the moving distance, two-way repeatability at 1.0μm, 400 paces per circle at a distance of1.2598 for each pace.

The signal gathering/motion control/image gathering/data analysis module comprises Intel AtomN2600 low consumption industrial motherboard, 2G industrial INNODISK memory, 500G hardware, 9V–30V input 60W broadband power module, signal processing, data gathering, motion control, and data analysis software developed based on Lab VIEW development environment.

The touchscreen is made of12 inches of industrial capacitance touch screen with a resistance of⩾ 100MΩ/25V(DC).

The electrostatic shielding coating adopts an electrostatic shielding paint with a density of 1.04g/cm^3 and surface resistance of<0.25Ω/cm^2.

2.4 Software Design

2.4.1 System Software Design

The system software interface consists of microscope control module, electrode control module, solution calibration module, data acquisition module, and system configuration module. The system software can control the hardware system, such as themicroscope, CCD camera, and macro 3D automatically controlled platform. It can gather, process, and analyze the electrical signals and finally calculate the ion flux per pmol•cm-2sec-1(Fig. 3).

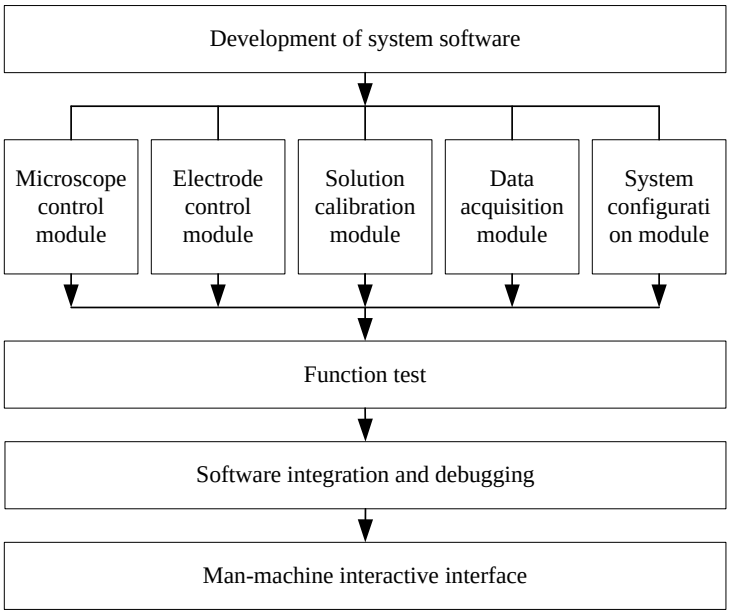

Fig.3. Software design

2.4.2 Hardware Control and Data Gathering of the System

The microscope control module controls the LED light and the automatic focusing. The CCD module is integrated into the microscope and facilitated the real-time imaging, image recording, and management of the detection process using a colorful industrial CCD. The electrode control module of the macro 3D automatically controlled platform facilitates the serial port communication, motion command confirmation, accurate localization, and reciprocating motion of the ion-selective microelectrode. The solution calibration module performs the two-point calibration or the three-point calibration of the ion-selective microelectrode to ensure that the ion-selective microelectrode calibration value has a reasonable slope and intercept. The data acquisition module collect selectrical signals and performs voltage difference processing and ion flux calculation. The system configuration module achieves different language switching and data export(Fig. 4).

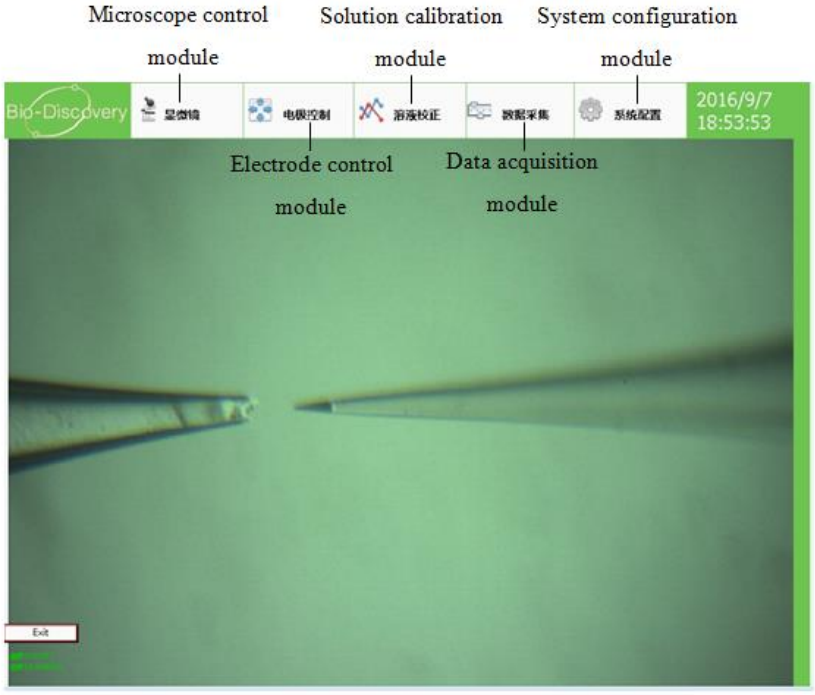

Fig.4. Software interface

3Verification of Results of the Dynamic Ion Current Testing Equipment and Commercial Ion Current Detection Instrument

3.1 Verification Method and Content

The ion source electrode was used as a proven ion-stable release source after the ion source electrode is dissolved by the internal components boiling at 1ml1MKCl and 9ml 0.1% low melting agarose. Devices used to verification the experiment include PDIFTE and a commercialized ion flux detection technique system BIO-001B. K^+ LIX: Potassium ionophore I-cocktail A, Sigma–Aldrich, St.

Louis, MO 63103, USA; backend filled LIX: 100 mM KCl; K^+ test buffer: 0.1mM KCl; and 0.1mM $CaCl_2$, 0.1mM $MgCl_2$, 0.5mM NaCl, 0.2mM Na_2SO_4, and 0.3mM MES are used to detect K^+ and adjust pH 6.0 using KOH and HCl. The constant volume is 200ml; the filling buffer is100 mM KCl; the K^+ standard solution comprises0.1 mM and 1 mM KCl; and the ion-selective microelectrode tip is 2–4 μm in diameter[12].

The filling liquid is pumped into the lumen from the back end of the ion-selective microelectrode and filled the electrode tip. Under micro-operation, the K^+ liquid ion exchanger was injected into the ion-selective microelectrode tip by negative pressure adsorption at a length of about 150μm.Three K^+ ion-selective microelectrodes were made in the same manner. These ion-selective microelectrodes are used for BIO-001B and PDIFTE detection data comparison verification. The K^+ ion-selective microelectrode was calibrated with 0.1 mM and 1 mM KCl.

The K^+ ion source electrode was adhered at the center of the bottom of the detection pond using tape. The detection pond was filled with K^+ test buffer and placed on the inverted microscope 10X objective lens. The tip of the ion source electrode was located in the field of view of the display, and the ion-selective microelectrode movement is driven by the macro 3D automatically controlled platform, which finds the ion-selective microelectrode tip in the display field of view. The ion-selective microelectrode tip was moved to a position 20 μm from the tip of the ion source electrode(Fig. 4).BIO-001B and PDIFTE tested the ion source electrode to release K^+ ion flux at an uninterrupted test time of 5min.

3.2 Validation Results

The results show that DIFTE and BIO-001B continuously detected a stable release of K^+ flux from the K^+ ion source electrode. The data did not show abnormal fluctuations(Fig. 5).The released K^+ net flux detected by DIFTE from the K^+ ion source electrode was 3.50274 pmol•cm^{-2} s^{-1}.The BIO-001B detection of the K^+ ion source electrode showed a released K^+ net flux of 3.498082 pmol•cm^{-2} s^{-1}.A test comparison of the K^+ ions released by the K^+ ion source electrodes at a distance of 20 μm by both devices confirms that DIFTE and BIO-001B can achieve near-consistent data results (Fig. 6).

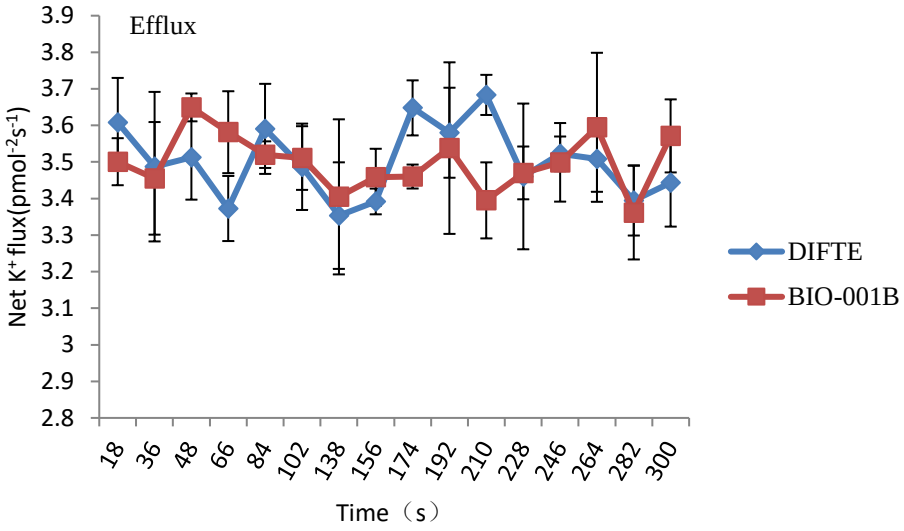

Fig.5. Changes in the stable release of K^+ ion flux detected by DIFTE and BIO-001Bfrom the ion source electrode. The tip of the K^+ ion-selective microelectrode is 20 μm from the ion source electrode tip. The data acquisition of the K^+ ion flux was done continuously for 5 min. Each point represents the mean of three individual K^+ ion-selective microelectrodes detected. The bars represent the standard error of the mean.

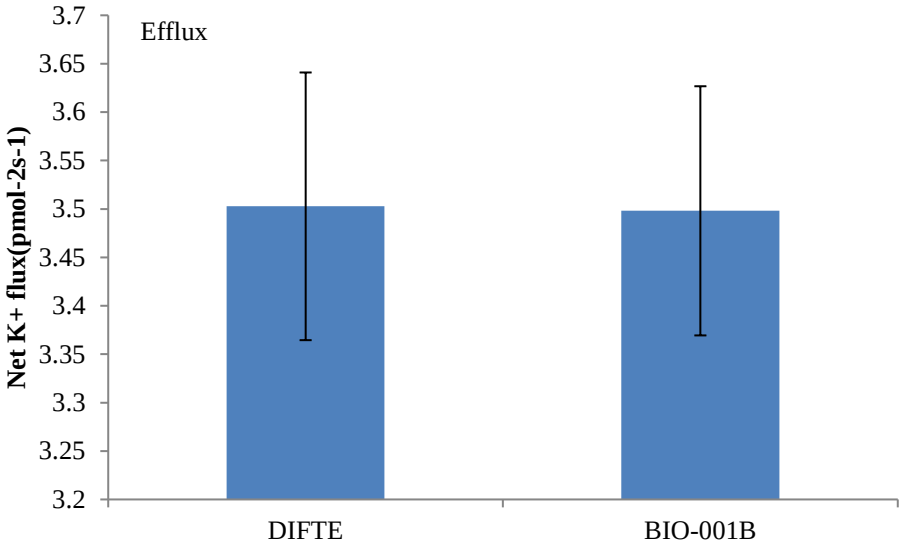

Fig.6. Comparison of the stable release of K^+ net ion flux detected by DIFTE and BIO-001Bfrom the K^+ ion source electrode. The tip of the K^+ ion-selective microelectrode is 20 μm from the ion source electrode tip. Data acquisition of the K^+ ion flux was done continuously for 5 min. Each column represents the mean of three individual K^+ ion-selective microelectrodes detected. The bars represent the standard error of the mean.

4 Results and Discussion

The dynamic ion flux detection equipment solved the weak electrical signal gathering of the modulation technique under complex laboratory and field operation environments. Power to all parts of the system was supplied by battery, thus avoiding the interference of the AC in the signal gathering. The small size and structure satisfy the in-situ, real-time, and non-destructive detection and analysis of plant tissues, organs, and other dynamic ion fluxes in the laboratory and field environments. PDIFTE

can be used in the physiological mechanism research of salt-resistant, drought-resistant, cold-tolerant, heavy metal-resistant, and disease-resistant plants. It can also be used in the research on plant nutrition, ion channel-related gene function, and crop resistant breeding screening. The test result data of PDIFTE and BIO-001B have very good consistency. The signal detected by DIFTE was more stable and can be applied for target material ion fluxdetection.

(**Acknowledgments:** This research was supported by the Postdoctoral Funding from Beijing Academy of Agriculture, Scientific and Technological Innovation Team of Beijing Academy of Agricultural and Forestry Sciences (JNKYT201604, and Fundings from the National Natural Science Foundation of China (Grant No.61571443))

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