Anisotropy and nonlinear properties of electrochemical circuits in leaves of Aloe vera L.

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1. Introduction

A monocot Aloe vera (L.) is a member of the Asphodelaceae (Liliaceae) family with crassulacean acid metabolism (CAM) and has been used for thousands of years in medicine, cosmetics, and as an ornamental plant. The natural habitats of Aloe vera are the subtropical parts of the world and it is considered to be intolerant of low temperatures [1]. The succulent, non-fibrous leaves of the Aloe vera grow from the base in the rosette pattern. In Aloe vera, stomata are open at night and closed during the day [2]. CO₂ acquired by Aloe vera at night is temporarily stored as malic and other organic acids, and is decarboxylated the following day to provide CO₂ for fixation in the Benson–Calvin cycle behind closed stomata. Electron microscopy shows two distinct parts of the Aloe vera L. leaf: outer green rind and inner clear pulp, with vascular bundles located in the pulp and adjacent to the green rind [3].

Electrical phenomena in plants have attracted researchers since the eighteenth century [4–8]. The cells of many biological organs generate electric potentials that result in the flow of electric currents [9]. Electrical impulses may also arise as a result of stimulation. Once initiated, these impulses can propagate to adjacent excitable cells. The change in transmembrane potential creates a wave of depolarization which affects the adjoining, resting membrane [10]. Electrical signals can propagate along the plasma membrane on short distances in plasmodesmata [11] and on long distances in conductive bundles [12,13].

Monitoring action potentials in higher plants represents a promising method to investigate intracellular and intercellular communication during environmental changes. For example, heat stress induces high speed action potentials in Aloe vera [14].

The most frequently used methods for the evaluation of electrical circuits in plants are patch clamps, electrochemical impedance measurement, and electric charge stimulation. The patch clamp method can be used to study electrical characteristics of individual ionic channels in a biological membrane in vitro. The electrochemical impedance method measures static electrical parameters, such as resistance and capacitance, at high frequency alternative currents (AC). However, different electrochemical circuits can have the same electrochemical impedance [15]. The description of equivalent electrical circuits based on electrochemical impedance AC measurements is based on the researcher’s intuition and can lead to various mistakes. Moreover, this method cannot characterize dynamic changes and events, such as ion channel opening and closing.

The Charge Stimulation Method (CSM) [16–19] was used to estimate, with high precision, the amount of electrical energy necessary to induce a response. This method permits direct in vivo evaluation of the simplest electrical circuits in a cluster of cells or in a single cell.

When a function generator is used to apply pulses with a given potential, it generates a potential of zero volts when the pulse is not being applied. For example, when the generator applies a 100 mV pulse lasting for 1 s, the function generator will generate a 0 mV output before...
and after the pulse. This means that the native plant potential (~20 mV) will be forced to zero during periods of no stimulation. Therefore, in order to estimate the plant’s response after stimulation, we have to effectively “disconnect” the function generator from the plant and monitor the plant’s electrical response. This feature is not available with standard function generators. We propose the use of the charge stimulation method [12,13,16–19] that allows delivery of electric charge and disconnection from the plant. The charge is delivered from a capacitor that is charged at a given potential. When a capacitor with capacitance $C$ is connected to the source with potential voltage $U$, the total capacitor charge is $Q = CU$, which allows precise regulation of the amount of charge during stimulation by using different capacitors and applying various voltages. A mechanical or electronic switch can instantaneously connect the charged capacitor to the plant and induce a response for a given stimulation period and disconnect the capacitor to monitor the plant’s response.

Experimentation with electrical stimulation of plants requires precise control of plant electrical parameters. Our primary objective was to determine precisely the amount of electric charge that generates a given biological effect [17]. Therefore, we implemented a custom stimulator that allows precise timing of the stimulation (i.e. number of pulses and their duration) and stimulation voltage.

The purpose of this study is to evaluate the electrical responses of Aloe vera induced by electrostimulation in real time. The information gained from this study can be used to elucidate the effects of electrostimulation on higher plants and to observe intracellular and intercellular communication in plants. Equivalent electrical schemes of biologically closed electrical circuits were then evaluated inside the Aloe vera leaf.

2. Materials and methods

2.1. Electrodes

All measurements were conducted in the laboratory at 21 °C inside a Faraday cage mounted on a vibration-stabilized table. Ag/AgCl electrodes were prepared in the dark from Teflon coated silver wires (A-M Systems, Inc., Sequim, WA, USA) by electrolysis in 0.05 M KCl aqueous solution [10]. The anode was high-purity silver wire and the cathode was a platinum plate. Electrical current in the electrolytic cell was limited to 1 mA/cm² of the anode surface. Stabilization of electrodes was accomplished by placing two Ag/AgCl electrodes in 0.05 M KCl solution for 24 h and connecting a short circuit between them. The electrode with the positive and negative potentials are always considered as the measuring and the reference electrode, respectively. The resistance between two Ag/AgCl electrodes that are 2 cm apart in 0.1 M KCl solution was found to be 10 kΩ. The response time of Ag/AgCl electrodes was less than 0.1 µs. Plants were allowed to rest after electrode insertion.

2.2. Plant electrostimulation

PXI (PCI eXtensions for Instrumentation) is a rugged PC-based platform that offers a high-performance solution for measurement and automation systems. PXI combines the Peripheral Component Interconnect electrical bus with specialized synchronization buses and key software features. PXI also adds mechanical, electrical, and software features that define complete systems for test, measurement, and data acquisition. A NI-PXI-4071 digital multimeter (National Instruments, Austin, TX, USA), connected to 0.2 mm thick nonpolarizable reversible Ag/AgCl electrodes, was used to record the digital data. A NI-PXI-4110 DC Power Supply (National Instruments) or electrical battery was the

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**Fig. 1.** (a): Time dependence of electrical discharge in the Aloe vera leaf between two Ag/AgCl electrodes connected to a charged capacitor. These results were reproduced 7 times. (b): Time dependence of electrical discharge in Aloe vera’s leaf between two Ag/AgCl electrodes connected to a charged capacitor in logarithmic coordinates. (c): Normalized presentation of time dependence of electrical discharge in the Aloe vera leaf between two Ag/AgCl electrodes connected to a charged capacitor. $U$ is the capacitor voltage and $U_0$ is the initial voltage in volts.

**Fig. 2.** Electrical equivalent schemes of a capacitor discharge in plant tissue. Abbreviations: $C_1$ — charged capacitor from voltage source $U_0$; $C_2$ — capacitance of plant tissue; $R$ — resistance, $D_1$ and $D_2$ — diodes as a model of voltage gated ion channels.
The 47 μF charged stimulation method (CSM) was then used in all experiments for electrostimulation of Aloe vera.

The primary objective of our experiments was to precisely determine the conditions of the charged electrical stimulation that generate a given biological effect [16,17]. We implemented two types of electrostimulation: a manual switch and a custom made specific controller. Manual stimulation is convenient for a single stimulation as it does not require additional equipment. It was implemented using a double pole double throw (DPDT) switch to connect the known capacitor to the voltage source during charging and then to the plant during plant stimulation to induce a response. However, manual switching does not allow precise control of timing of the stimulation. Therefore, we designed and implemented a custom plant stimulator to allow multiple stimulations with precise timing and voltage during stimulation. The plant stimulator is a battery powered portable device controlled by a low-power microcontroller MSP430F1611 (Texas Instruments, Texas, USA). A specialized PC program allows flexible configuration of the controller and communicates with the controller through optically isolated USB interface. During each stimulation cycle, the controller charges capacitor with predefined voltage using integrated digital to analog (DA) converter of the microcontroller. A dual integrated analog switch controlled by the microcontroller connects the capacitor to DA converter during charging and to the plant during stimulation, allowing stimulation with microsecond resolution.
and location of electrodes along leaf are shown. Leaves. The average humidity was 40%. Irradiance was 700 μmol m⁻² s⁻¹. All experiments were performed on healthy adult specimens under light conditions.

3. Results

3.1. Electrostimulation of a leaf by a charged capacitor

Following insertion of the electrodes, the plants were allowed to rest until a stable potential difference with amplitude up to 20 mV was obtained between Ag/AgCl electrodes located perpendicular or parallel to the conductive bundles.

Fig. 1a shows the time dependencies of a capacitor discharge between Ag/AgCl electrodes located perpendicular to conductive bundles on top and bottom of the Aloe vera's leaf at different initial voltages on the charged capacitor. Polarity of electrodes does not influence the kinetics of a capacitor discharge.

If a capacitor of capacitance C with initial voltage $U_0$ is discharged during time $t$ through a resistor $R$, the voltage at time $t$ is

$$U(t) = U_0 e^{-t/\tau},$$

where $\tau = RC$ denotes the time constant. Eq. (1) in logarithmic form reads:

$$\log_{10} U(t) = \log_{10} U_0 - t / 2.3 \tau.$$  

The time constant $\tau$ can be determined from the slope of this linear function.

Fig. 1b shows time dependencies of the capacitor discharge in logarithmic coordinates. All time dependencies of the capacitor discharge at voltages from 0.25 V to 2.0 V are linear according to Eq. (2) and the equivalent electrical circuit is shown in Fig. 2a.

Normalized presentation of time dependence of electrical discharge in Aloe vera's leaf between two Ag/AgCl electrodes connected to a charged capacitor in logarithmic coordinates. (c): Normalized presentation of time dependence of electrical discharge in the Aloe vera leaf on polarity of electrodes (Figs. 4a–5a).

The volume of soil was 2.0 L. Aloe vera plants had 25–35 cm leaves. The average humidity was 40%. Irradiance was 700–800 μmol photons m⁻² s⁻¹. All experiments were performed on healthy adult specimens under light conditions.

Aloe vera L. was grown in clay pots. Fifty plants were exposed to a 12:12 h light/dark photoperiod (Environmental Corporation, USA) at 22 °C. The volume of soil was 2.0 L. Aloe vera plants had 25–35 cm leaves. The average humidity was 40%. Irradiance was 700–800 μmol photons m⁻² s⁻¹. All experiments were performed on healthy adult specimens under light conditions.

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For each stimulation, the capacitor was connected to electrodes in the plant until complete discharge and then disconnected from the electrodes. The following experiments with different conditions were performed at least 5 min later, although there was no noticeable difference in response for different time periods between stimulations. Voltage in the plant was measured between experiments, but no additional effects were detected between experiments. Electrodes remained in the plant between experiments.

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resistance \( R = 277.0 \text{s/47} \mu \text{F} = 5.89 \text{M}\Omega \) between electrodes in a leaf does not depend on applied voltages.

Time dependence of electrical discharge in the Aloe vera leaf between two Ag/AgCl electrodes connected to a charged capacitor located along the leaf, parallel to the conductive bundles, is shown in Figs. 4 and 5. If the applied potential has an amplitude higher than 1 V, there is a strong deviation in logarithmic coordinates from the Eq. [2] predictions (Figs. 4b and 5b). The deviation of a capacitor discharge from a linear dependence in logarithmic coordinates can be described by the equivalent electrical schemes shown in Fig. 2b and 2c [19]. If the capacitor discharge is represented by two exponential functions and does not depend on polarity of electrodes in the plant tissue, the deviation from linear dependence can be described by Fig. 2b. Our previous work has demonstrated typical values of capacitance and resistance in the model for Mimosa pudica [19]. If the response changes with the polarity of stimulation, a rectifier based model represented in Fig. 2c must be used. Kinetics of a capacitor discharge depends on the polarity of electrodes in the Aloe vera leaf (Figs. 4c and 5c). Fig. 6 shows the difference in the kinetics of a capacitor discharge as a function of the polarity of stimulation as represented in Fig. 4a and 5a. Dependence of a capacitor discharge on the polarity of electrodes in the Aloe vera leaf, shown in Figs. 4–6, can be explained by a change in resistivity with applied potential due to opening of ion channels, which can be modeled by diodes in Fig. 2c. Opening of voltage gated channels induce the effect of electrical rectification shown in Fig. 6. We found similar rectification effects in the Venus flytrap [18] and Mimosa pudica [20]. We modeled a voltage gated channels using silicon rectifier diode and reproduced experimental dependencies of a capacitor discharge in plant tissue [18].

We measured synchronously both voltage and electrical current during a capacitor discharge in the Aloe vera leaf (Figs. 4a, 5a and 7) using the experimental setup shown in Fig. 8a. Discharge kinetics, measured as time dependence of electrical current and of voltage, are similar. Figs. 7b and 7d show the normalized presentation of the time dependence of electrical discharge in the Aloe vera leaf between two Ag/AgCl electrodes connected to a charged capacitor.

Measuring the current synchronous with the voltage during discharge of capacitor, as represented in Fig. 8a, provides useful information on dependence of input resistance \( R \) between Ag/AgCl electrodes in the Aloe vera leaf on the initial voltage of the charged capacitor \( U_0 \). Fig. 8b shows dependence of initial resistance between electrodes in the pulp of the Aloe vera leaf. If the amplitude of applied potential does not exceed 1 V, the input resistance is about 120 kΩ; at higher voltages the resistance drops by half (Fig. 8b). We hypothesize that this decrease can be caused by the fast opening of voltage gated ion channels. The same dependence can be seen as resistance \( R(t) = U(t)/I(t) \), represented in Fig. 8c. Slowly increasing resistance in time could be explained by different time constants of voltage and current change, as it can be seen in Figs. 4a and 7a.

There is a significant difference in kinetics of discharge between night and day. During the night, the discharge is significantly faster than during the day. We are currently investigating this phenomenon.

4. Discussion

Bioelectrochemical circuits operate in all plants including the Aloe vera. The activation of biologically closed circuits with voltage gated ion channels can lead to various mechanical, hydrodynamical, physiological, biochemical, and biophysical responses. Different environmental stimuli evoke specific responses in living cells that have the capacity to transmit a signal to the responding region. In contrast to chemical signals such as hormones, electrical signals are able to rapidly transmit information over long distances [21]. Pathways for long distance electrical signal transduction may include capillary systems of conductive bundles and plasmodesmata.
Electrodes in the cells or in a single cell. Using this method we discovered strong electrical direct in vivo evaluation of the simplest electrical circuits in a cluster of parenchyma cells facilitates long distance signaling. Low degree of electrical coupling in a lateral direction, caused by membrane appears to play a role in this process as well. Moreover, the structures of the sieve tube components are unique throughout the whole plant. The sieve tube system appears to possess extend between organs. These low resistance bridges are found signaling network between cells [11]. Low resistance connections vary in species indicating that plasmodesmata are relays in the avoidance of electrical phenomena for improvement of agricultural technology. These reasons provide significant basis to the importance of further profound investigations of electrical phenomena in plants.

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References