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Electrotonic potentials in *Aloe vera* L.: Effects of intercellular and external electrodes arrangement

Alexander G. Volkov [a,*], Eunice K. Nyasani [a], Clayton Tuckett [a], Jessenia M. Scott [a], Mariah M.Z. Jackson [a], Esther A. Greeman [a], Ariane S. Greenidge [a], Devin O. Cohen [a], Maia I. Volkova [a], Yuri B. Shtessel [b]

[a] *Department of Chemistry, Oakwood University, 7000 Adventist Blvd., Huntsville, AL 35896, USA*
[b] *Department of Electrical and Computer Engineering, University of Alabama in Huntsville, Huntsville, AL 35899, USA*

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Electrostimulation of plants can induce plant movements, activation of ion channels, ion transport, gene expression, enzymatic systems activation, electrical signaling, plant-cell damage, enhanced wound healing, and influence plant growth. Here we found that electrical networks in plant tissues have electrical differentiators. The amplitude of electrical responses decreases along a leaf and increases by decreasing the distance between polarizing Pt-electrodes. Intercellular Ag/AgCl electrodes inserted in a leaf and extracellular Ag/AgCl electrodes attached to the leaf surface were used to detect the electrotonic potential propagation along a leaf of *Aloe vera*. There is a difference in duration and amplitude of electrical potentials measured by electrodes inserted in a leaf and those attached to a leaf's surface. If the external reference electrode is located in the soil near the root, it changes the amplitude and duration of electrotonic potentials due to existence of additional resistance, capacitance, ion channels and ion pumps in the root. The information gained from this study can be used to elucidate extracellular and intercellular communication in the form of electrical signals within plants.

1. Introduction

1.1. Electrostimulation of plants

The bioelectrochemical phenomena in plants have attracted researchers since the eighteenth century [1]. The cells of many biological organs generate electrical potentials that result in the flow of electric currents [2]. Electrostimulation of plants can induce activation of ion channels and ion transport [3–10], gene expression [11–13], enzymatic systems activation [14,15], electrical signaling [3–6,8–10,16–22], plant movements [5–9,21,23–25], enhanced wound healing [26], plant-cell damage [27] and influence a plant growth [20,28–30]. The electrostimulation by bipolar sinusoidal or triangular periodic waves induce electrical responses in plants, fruits, roots and seeds with fingerprints of generic memristors [21,31–37].

The electrotonic potential in plants was recently discovered [38,39]. Electrostimulation of electrical circuits in the Venus flytrap, *Aloe vera*, *Arabidopsis thaliana*, *Mimosa pudica*, apple fruits and potato tubers induce electrotonic potentials with amplitude exponentially decreasing along a leaf or a stem [35,36,38,39]. In the electrical stimulation of the Venus flytrap, the lower leaf induces electrotonic signals within the entire plant. The trap closes if the stimulating voltage of the lower leaf is above the threshold level of 4.4 V [39]. Electrical responses in the Venus flytrap were analyzed and reproduced in the discrete electrical circuit [39]. In small neurons, exponentially decreasing electrical potentials are referred to as electrotonic potentials [40]. Electrotonic potentials can induce action potentials in plants [39], small neurons, and dendrites [41].

Recently, we analyzed anisotropy and nonlinear properties of electrochemical circuits in the leaves of *Aloe vera* [22]. There is a strong electrical anisotropy of the *Aloe vera* leaf. In the direction across the conductive bundle, the behavior of the system is completely passive and linear like in a regular electric circuit with a constant resistance. Conductance parallel to vascular bundles are two orders of magnitude higher than the perpendicular direction [22].

Electrical signals can propagate along the plasma membrane on long distances in vascular bundles, and on short distances in plasmodesmata and protoxylem [3,4]. There are many publications in literature about so called variation [42] or systemic [43] potentials in plants with amplitude decreasing with distance. At the same time, there is no information in literature about variation potentials dependence on neither the distance of propagation nor mechanism of their generation.

Using the synchronous electrostimulation of the *Aloe vera* leaf from two different points, we studied collisions of propagating electrotonic potentials [38]. These electrical potentials can propagate either with a positive or negative pole in front of them. If a signal with a positive

Abbreviations: *C*, capacitance; *DAQ*, data acquisition; PXI, PCI extensions for instrumentation; (*Ref*), reference electrode; *R*, resistance; TEACl, tetraethylammonium chloride; *V*, voltage; V_{in}, input voltage; λ, the length of electrotonic potential.

* Corresponding author.
E-mail address: agvolkov@yahoo.com (A.G. Volkov).

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front collides with a negative one, they will amplify each other. However, if two electrical signals with positive fronts collide, they will partially annihilate each other.

1.2. Intercellular, surface and external electrodes in plant electrophysiology

Electrode arrangement while monitoring the transduction of electrical signals in plants is of great importance. Various electrode locations in a leaf, stem, root, and soil can be used to study multiple electrical circuits in the electrical networks of plants and trees.

An ideal reference electrode should be reversible and reproducible. Ag/AgCl electrodes can be prepared by electrodeposition of AgCl on a silver wire or plate [44].

There are a few different experimental methods to measure electrical signal propagation in plants. Reversible Ag/AgCl electrodes can be inserted into plant tissue (Schematic 1) or attached to the surface of a plant for measuring the intercellular or surface potentials. Amplitude and duration of electrical signals along the surface of a leaf or a stem depends on the type of contact between the attached electrodes and the plant's surface. Ksenzhek et al. [45] used small drops of honey mixed with KCl to establish the electrical contact with low resistance between the surface of a leaf and the Ag/AgCl electrode. Mousavi et al. [46] used silver wire modified with HCl without electrodeposition as a quasi-reversible surface electrode in a drop of KCl in agar. Favre et al. [47,48] used a piece of cotton imbibed with an electrophysiological solution. Propagation of electrical signals inside plants can induce electrical signals between surface electrodes attached to a leaf. In many publications, the external reference Ag/AgCl electrode was inserted in the soil [17,49–51], compost [47,48] or aqueous buffing solution [20,52]. Relationships between results such as amplitude, duration, and speed of electrical signal propagation are obtained using inserted electrodes, attached to a leaf, or external electrodes in a soil are unknown.

Despite the vast amounts of accumulated information concerning electric effects in plants, their physiological and bioelectrochemical mechanisms remain poorly understood. Further investigation could provide information on the outlook of possible uses of these phenomena for improvement of agricultural technologies. These reasons provide significant basis for the importance of further profound investigations of electrical phenomena in plants, fruits and trees [21].

The purpose of this study is to evaluate the electrical signal transduction in plants induced by electrostimulation using intercellular or external electrodes for measuring electrical responses in a plant.

2. Materials and methods

2.1. Plants

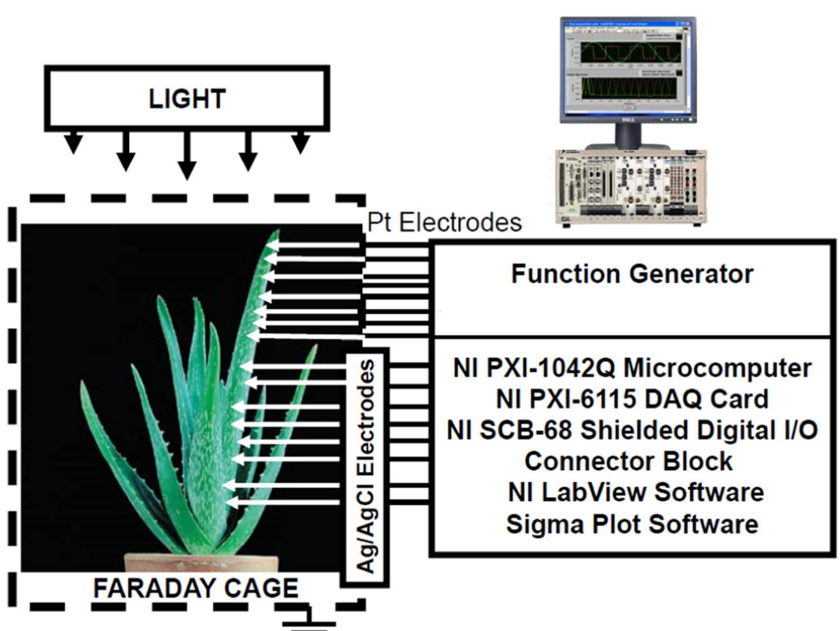
Fifty *Aloe vera* L. plants were grown in clay pots. Plants were exposed to a 12:12 h light/ dark photoperiod at 21 °C. *Aloe vera* plants had 20–35 cm leaves. Volume of soil was 1.0 L. The average air humidity was 40%. Irradiance was 700–800 μmol photons m^{-2} s^{-1} PAR at plant level. All experiments were performed on healthy adult specimens.

2.2. Electrodes

All measurements were conducted in the laboratory at 21 °C inside a Faraday cage mounted on a vibration-stabilized table. Teflon coated silver wires (*A-M Systems, Inc., Sequim, WA, USA*) with a diameter of 0.2 mm were used for preparation of non-polarizable electrodes. Reversible Ag/AgCl electrodes were prepared in the dark by electrodeposition of AgCl on 5 mm long silver wire tip without Teflon coating in a 0.1 M KCl aqueous solution. The anode was a high-purity silver wire and the cathode was a platinum plate. Electrical current in the electrolytic cell was limited to 1 mA/cm^2 of the anode surface. Stabilization of electrodes was accomplished by placing two Ag/AgCl electrodes in a 0.1 M KCl solution for 24 h and connecting a short circuit between them. The response time of Ag/AgCl electrodes was less than 0.1 μs. Identical Ag/AgCl electrodes were used as working and reference electrodes in all experiments.

Cardex AG® ECG #2566 Tab electrodes (*PSS World Medical, Inc., Jacksonville, FL, USA*) with a Ag/AgCl sensing system covered by a conductive hydrogel and low impedance were used as working and reference electrodes at the surface of plants.

Platinum electrodes were used for plant electrostimulation and prepared from Teflon coated platinum wires (*A-M Systems, Inc., Sequim, WA, USA*) with a diameter of 0.076 mm. Platinum electrodes were used for electrostimulation of plants. We allowed the plants to rest for



Schematic 1. Experimental setup. Two Pt-electrodes were used for electrostimulation of a leaf from a function generator and 8 Ag/AgCl electrodes were used for measurements of plant electrical responses. For measurements of dependencies of distance between Pt-electrodes on amplitude of electrical responses in a leaf, 7 Pt electrodes were inserted on the top of a leaf.