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Electrical signaling in *Aloe vera* induced by localized thermal stress

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Abstract

Action potentials in higher plants are theorized as the information carriers in intercellular and intracellular communication in the presence of environmental stressors. Among the most common stressors is heat shock. Under stressful conditions, the response reactions of plant tissues and organs can be local or transmitted over long distances. In this article, the speeds of propagation of thermally induced action potentials in green plants are discussed, and their speeds were found to be comparable to those occurring in various mammalian species. These rapid action potentials in green plants were recorded in real time using modern data acquisition techniques. According to our measurements, a single application of localized heat stress induces fast action potentials in *Aloe vera* (67 m/s). Electrical signals propagated along all leaves of the *A. vera* plants were studied. Possible pathways for electrical signal propagation in vascular plants are also discussed.

Keywords: Action potential; Plant electrophysiology; Electrical signaling; Heat stress; Cold stress; *Aloe vera*

1. Introduction

Excitability is a fundamental property that plants exhibit at the whole plant, tissue, and cellular levels. This property allows the cells, tissues and organs of plants to operate in concert to adapt its internal conditions and external reactions in response to environmental stimulants referred to as irritants. According to Goldsworthy [1], electrochemical signals that resemble nerve impulses exist in plants at all evolutionary levels. The excitation waves, or action potentials, in higher plants are thought to be the mechanism behind intercellular and intracellular communication [2–15]. Action potentials are signals caused by the depolarization of cellular membranes. Mechanical, physical or chemical irritants affect not only the location of occurrence, but these irritants may affect the entire plant as well [3–12]. In some plants, such as Venus Flytrap and *Mimosa pudica*, action potentials are localized [16,17]. In other plant species, the speed of transfer depends on many factors, including the intensity of the irritation, temperature, chemical treatment or mechanical wounding [4–7,16]. The excitation reaction may travel between the top of the stem and the root in either direction.

The cells of many biological organs generate electric potentials that result in the flow of electric currents. Electrical impulses may arise as a result of stimulation. Once initiated, these impulses can propagate to adjacent excitable cells [17–22]. The change in transmembrane potential creates a wave of depolarization, or an action potential, which affects the adjoining, resting membrane. Thus, while the plasma membrane is stimulated at any point, the action potential is propagated over the entire length of the cell membrane and along the conductive bundles of tissue with constant amplitude. Once initiated, the action potential has a stereotyped form and, essentially, a fixed amplitude — an “all-or-none” response to a stimulus. The propagation of each impulse is followed by an absolute refractory period during which a second impulse cannot be transmitted. The ability to become excited resumes shortly after the membrane depolarizes. Electrical signals can propagate along the plasma membrane on short distances in plasmodesmata, and on long distances in conductive bundles [18,22].

Bioelectrochemical phenomena may be the underlying mechanism behind the adaptation which ensures the survival of the plant organism during unfavorable environmental conditions [23]. An important trigger mechanism in the development of the adaptive syndrome is propagation of action potentials. At the cellular level, electrical potentials exist across membranes, and thus between

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cellular and specific compartments. Electrolytic species such as K^+, Cl^-, Ca^{2+}, and H^+ ions are actively involved in the establishment and modulation of electrical potentials. Electrical activity can be the direct result of abiotic stress. Abiotic stress is physical stress caused by extreme temperatures, light, drought, herbicides, salt, osmotic pressure, cutting, and wounding. The most common abiotic stress in plants is extreme temperature, more specifically, heat stress.

Heat stress, also known as thermal shock, occurs in plants, animals, insects, bacteria, and fungi in response to drastic temperature increase [24–32]. Heat shock is a treatment given to plant cells or to an entire plant and is characterized by elevated temperature around 15 °C above ambient. Thermal shock alters gene expression and leads to increased heat tolerance in a wide range of organisms. The responses of many organisms to elevated temperature have been characterized and described as the heat shock response.

Heat stress in plants stimulates the production of heat shock proteins (hsp), which assist plants in the adaptation to and tolerance of extreme temperatures. Heat shock proteins are important homeostatic and defense mechanisms in plants. Kinetics of hsp synthesis, their electrophoretic profiles, and intracellular localization have been characterized in soybean, tomato and other plants [33,34]. In the case of the tomato, exposure to extreme heat activates protease inhibitor (PIN) genes. This occurs through a unique signal transduction process. A common second messenger in this signal transduction pathway is inositol phosphate. Ca^{2+}-mediated second messenger systems have also been hypothesized as possible systems in the heat shock response of tomato plants and other plants. Lin et al. [34] demonstrated the development of thermal tolerance in soybean seedlings.

Low-temperature stress retards growth of most crop plants, especially in tropical and subtropical region. Cold tolerance is associated with the action of multiple low-temperature regulatory pathways.

Aloe vera L., also known as *A. barbadensis* Miller, is a xerophyte in the Liliaceae family. Its succulent leaves contain substances used for numerous medical and cosmetic applications. The natural habitats of *A. vera* are the subtropical parts of the world. *A. vera* is considered to be intolerant of low temperatures.

Electrophysiologists have measured the generation of action potentials in plants induced by flaming or brief chilling and report different amplitudes, durations, and speeds of propagation of action potentials [2,27,29–32]. These measurements were taken with extremely slow digital and analog registration systems without low-pass filters and with different time constants $t = RC$ of voltmeters, depending on input resistance R and capacitance C. It was shown [23] that the difference between publications [2,27–32] can be caused by electronic effects of aliasing. If the sampling rate is too slow, the rapid changes in the original signal between any two consecutive samples cannot be accurately recorded. As a result, higher frequency components of the original signal will be misrepresented as lower frequencies. In signal processing, this problem is known as aliasing [23]. Undersampling may result in the mispresentation of the measured signal.

The purpose of this study is to evaluate in real time the electrical signaling generated by *A. vera* in response to localized heat and cold stresses using a new ultra fast data acquisition system with high input impedance. The information gained from this study can be used to elucidate the effects of extreme temperature on higher plants and to observe the speed of intracellular and intercellular communication within plants affected by thermal stress.

2. Materials and methods

All electrochemical measurements were collected at constant temperature (22 °C) inside a Faraday cage mounted on a vibration-stabilized table in a laboratory (Fig. 1). High-speed data acquisition of low-pass filtered signals was performed using microcomputers with simultaneous multifunction I/O plug-in data acquisition board NI-PXI-6115 or NI-PCI-6115 (*National Instruments*) interfaced through an NI SCB-68 shielded connector block to 0.1 mm nonpolarizable reversible Ag/AgCl electrodes attached to the plant. The results were reproduced on a workstation with data acquisition board NI 6052E DAQ (National Instruments) with input impedance of 100 GΩ interfaced through an NI SC-2040 Simultaneous Sample and Hold (National Instruments). The multifunction NI 6115 data acquisition board provides high resolution and a wide gain range. NI 6115 features continuous, high-speed data acquisition. NI 6115 boards feature large 64 Msample onboard memory. Any single channel can be sampled at any gain up to 10 MSamples/s. Measuring signals were recorded as ASCII files using *LabView* (National Instruments) software.

Ag/AgCl electrodes were prepared from Teflon coated silver wire (A-M Systems, Inc.). Ag/AgCl electrodes were maintained at constant temperature because of their high temperature sensitivity.

A fundamental rule of sampled data systems is that the input signal must be sampled at a rate greater than twice the highest frequency component in the signal. This is known as the Shannon sampling theorem, and the critical sampling rate is called the Nyquist rate. Stated as a formula, it says that $f_s/2 > f_a$, where f_s is the sampling frequency and f_a is the maximum frequency of the signal being sampled. Violating of the Nyquist criterion is called undersampling and this results in aliasing [35,36]. Theoretically, it

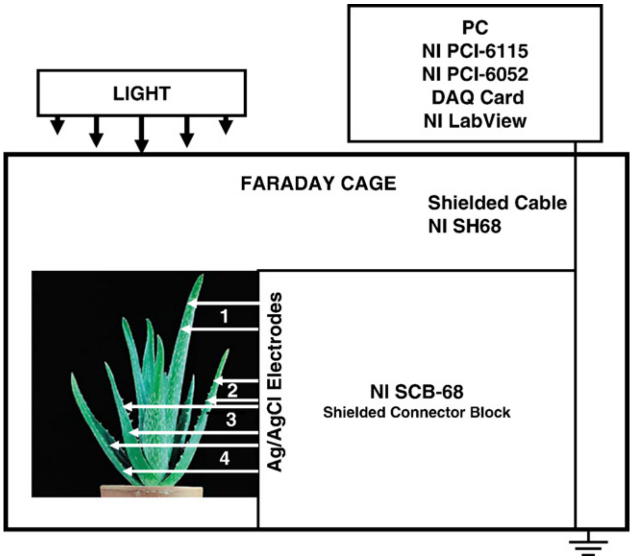

Fig. 1. Experimental setup.

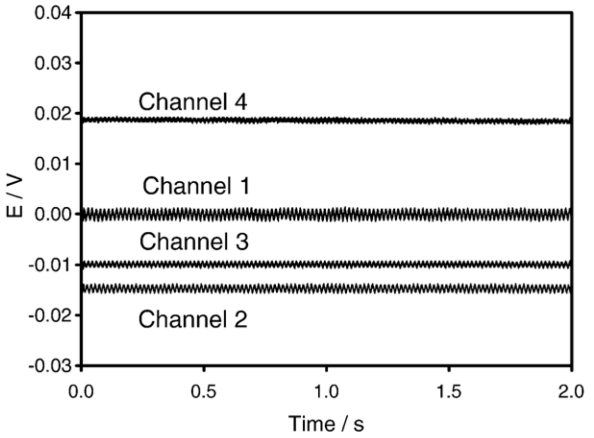

Fig. 2. Potential difference between Ag/AgCl electrodes in the leaves of *Aloe vera* before any treatment of the plant. Measurements were recorded at 500,000 scans/second. Distance between electrodes for each channel was 1 cm.

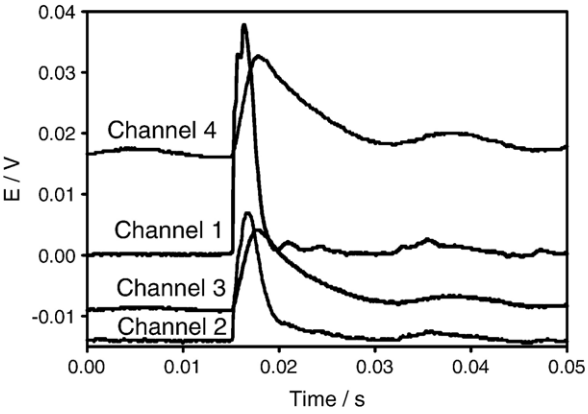

Fig. 4. Action potential (AP) induced in leaves of *Aloe vera* plant by cold shock (ice). Measurements were recorded at 100,000 scans/second and 400,000 scans/sample. Distance between electrodes for each channel was 1 cm.

is impossible to recover information from signals with frequencies at or below the Nyquist frequency. Due to this limitation, all data presented in this paper were collected using a high-speed data acquisition system.

Flame was applied continuously for 1–2 s to a pre-determined leaf using a *BIC* utility lighter.

A. vera L. was grown in clay pots. Fourteen plants were watered daily and exposed to a 12:12 h light/ dark photoperiod (*Environmental Corporation*) at 22 °C. Volume of soil was 0.5 L. *A. vera* plants had 20–25 cm leaves. The average humidity was 45–50%.

3. Results

Plants were exposed to a diverse array of continuously varying stressors, including variations of temperature. Plants generate different types of extracellular electrical events in connection to varying environmental stressors. Following insertion of the electrodes, the plants were allowed to rest until a stable potential difference was obtained between the measuring and reference electrodes (Fig. 2).

Action potentials in higher plants hold promise as the information carriers in intracellular and intercellular communication during environmental changes. Heat stress induces high-speed electrical signals in *A. vera* (Fig. 3) with an average speed of propagation of 67 m/s (S.D. 8 m/s, $n=7$ plants). We approximated the velocity of AP propagation as the distance between two channels (pairs of Ag/AgCl electrodes) divided by time between maximum values of AP registered by these channels.

To induce action potentials, heat shock was applied to a pre-determined leaf of *A. vera* chosen at random. Fig. 3 shows an action potential generated in a flame-treated leaf (channel 1) and in another leaf not treated with flame (channel 2). Action potentials propagated with constant amplitudes and speeds along all leaves of the *A. vera* specimens studied. Duration of action potential at treated by flame leaf is about 1 ms and at all other leaves about 2 ms.

Fig. 4 shows fast excitation waves in *A. vera*, induced by a small section of ice placed on a pre-determined leaf. A cold shock induces fast excitation waves in *A. vera* with an average speed of propagation of 132 m/s (S.D. 12 m/s, $n=7$ plants). These electrical waves propagate along all leaves in *A. vera*, but the amplitude of a signal on a treated leaf negatively correlates with an increased distance from the point of treatment.

Amplitude and speed of propagation of action potentials on all leaves should be constant and independent of the distance from treatment. The reason why the amplitude of electrical signaling near a point of a cold stress is higher and the duration time of signal is lower than these same values after treatment on other leaves remains unclear.

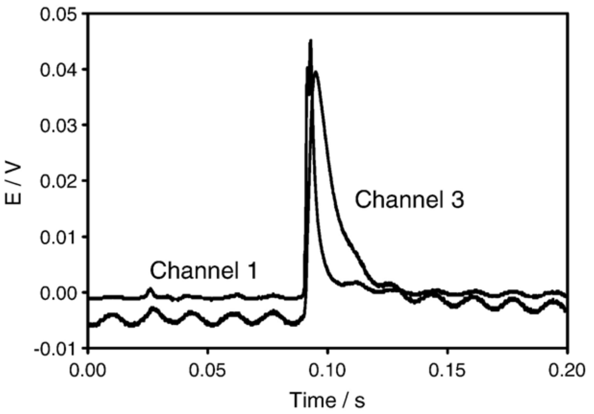

Fig. 3. Action potential (AP) induced in leaves of *Aloe vera* plant by thermal shock (flame). Measurements were recorded at 500,000 scans/second and 2,000,000 scans/sample. Channel 1 is located on the leaf treated by thermal shock and channel 2 is located on a different leaf of the same plant. Distance between Ag/AgCl electrodes for each channel was 1 cm.

4. Discussion

Plants possess most of the same chemistry of the neuromotoric system in animals. For example, they employ neurotransmitters such as acetylcholine, dopamine, noradrenalin, serotonin and histamine [37], cellular messengers like calmodulin, the cellular motors actin and myosin, and voltage-gated ion channels. Plants also utilize a variety of sensors to detect touch, light, gravity and

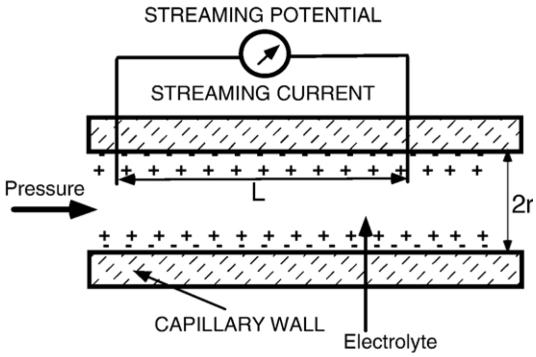

Fig. 5. Streaming potential and current induced by flow of electrolyte solution in a capillary produced by pressure difference.

temperature. The reason why plants have developed pathways for electrical signal transmission most likely lies in the necessity to respond rapidly to environmental stress factors. 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. Pathways for long-distance electrical signal transduction may include conductive bundles and plasmodesmata.

Upon perception, electrical signals can be propagated via plasmodesmata to other cells of the symplast. Electrical coupling via plasmodesmata was demonstrated in a variety of species, indicating that plasmodesmata are relays in the signaling network between cells. Low-resistance connections extend between organs. These low-resistance bridges are located throughout the whole plant. The sieve tube system appears to possess these qualities. The structures of the sieve tube members are unique and appear to be suitable for the transmission of electrical signals due to the relatively large, unoccluded sieve plate pores. The continuity of the plasma membrane appears to play a role in this process as well. Moreover, the low degree of electrical coupling in a lateral direction, caused by plasmodesmata at the interface between companion cells and phloem parenchyma cells, facilitates long-distance signaling.

One of the most important components of cell-to-cell electrical communication is the plasmodesmata [18]. Electrical waves spread in a lateral direction, most likely via the plasmodesmata. Theoretical substantiation of the function of the plasmodesmata can be found in the studies of Spanswick [38], who demonstrated in very precise experiments that the specific resistance of plasmodesmata is approximately 100 times lower than that of the plasmalemma or the tonoplast.

According to Wildon et al. [30] estimation, the theoretical limit of physical signal propagation in plants is 1500 m/s, which is equal to the speed of sound or of a soliton in water. A hydraulic pressure wave can induce the propagation of electrical signals. Electrokinetic phenomena play important roles in the transport of water through the xylem. Even in iso-osmotic conditions, water can flow from the soil through the xylem due to electro-osmosis [39,40]. Heyl [41] and Keller [42] found that electro-osmotic flow of water from soil through xylem is caused by root pressure.

Movement of an electrolyte solution along a capillary generates a streaming electrical potential between the upstream and downstream ends of liquid flow in *A. vera* (Fig. 5). Electrokinetic phenomena reflect the relation between the relative motion of the two phases and the electrical properties of the separating interface. The occurrence of electrokinetic phenomena is an indication that there is an electrical double layer at the boundary between a wall and a liquid and these phases are electrically charged. The electrokinetic movement of the liquid would be impossible if the liquid had no electrical charges, which are affected by an electrical field. The electrical double layer is formed at the internal surface of the sieve tube because of either the selective adsorption of ions of an electrolyte present in the liquid of the capillary or the dissociation of molecules of the substance from which the capillary wall is made. Fig. 5 represents an electrical double layer formed in a capillary, and it is assumed that the potential-determining ions are charged negatively, whereas the counter-ions are positively charged. The layer of counter-ions moves toward the cathode, parallel to the boundary layer of the potential-determining ions. The motion of the liquid in the capillary towards the cathode is due to the forces of friction and molecular cohesion. The layer of liquid bordering the capillary wall remains stationary. The remaining liquid that is near the surface of the wall, but does not border it, moves and obeys the friction law which is applied to ordinary liquids.

Equations for the streaming potential *E*; the streaming electrical current *I* and resistance *R* can be written as follows:

$$E = \frac{\varepsilon_0 \varepsilon \varsigma}{\eta \kappa} P, \tag{1}$$

$$I = \frac{\pi \varepsilon \varepsilon_0 \mathrm{Pr}^2 \xi}{\eta L}, \tag{2}$$

$$R = \frac{l}{\pi r^2 \kappa} \tag{3}$$

where ε_0 is the dielectric permittivity of a vacuum (8.85×10^{-12} C/Vm), ε is the dielectric permittivity of the aqueous phase, *P* is the pressure causing the relative movement of the

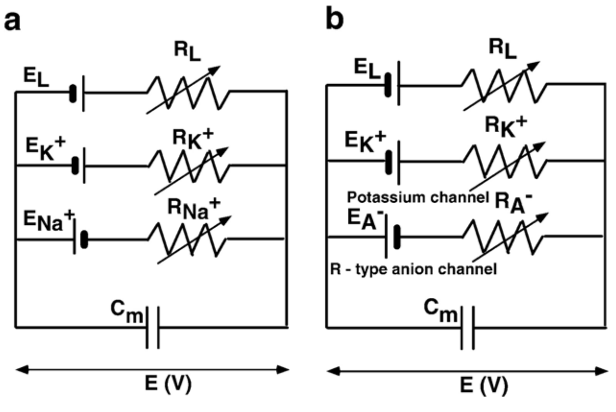

Fig. 6. The Hodgkin–Huxley equivalent circuit for an axon (a) and the modified HH circuit for sieve tubes in phloem (b). The three conductive branches represent ion channels and leak conductance with their different electromotive forces.

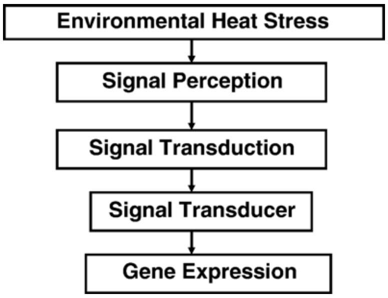

Fig. 7. A generalized sequence of events induced by heat shock in higher plants.

phases along the interface, η is the viscosity of the aqueous phase, κ is the conductivity of a aqueous phase, l is the capillary length, ζ is the electrokinetic or zeta potential equal to the potential difference between the immobilized and mobile phases of the electric double layer which varies from zero to some tens of millivolts, and r is the radius.

In electrokinetic phenomena, the streaming potential is equal to the streaming current multiplied by resistance, agreeing with Ohm's law. Therefore, the xylem may also be involved in propagation of long-distance electrical signals, caused by the change of turgor, surface charge, or ξ potential of a xylem wall.

The conductive bundles are responsible for long-distance propagation of electrical signals. One of the potential pathways for transmission of action potentials is the phloem sieve-tube system, since it represents a continuum of plasma membranes. Phloem is an electrical conductor of bioelectrochemical impulses over long distances. Phloem consists of two types of conducting cells: the characteristic type known as sieve-tube elements and another type known as companion cells. Sieve-tube elements are elongated cells made up of end walls that are perforated by numerous minute pores through which dissolved materials are conducted. Such sieve-tube elements are connected in a vertical series known as sieve tubes.

Hodgkin and Huxley's membrane model [43] accounts for K^+, Na^+ and ion leakage channels in axons (Fig. 6a). The membrane resting potential for each ion species is treated like a battery and a variable resistor models the degree to which the channel is open. Fromm and Spanswick [44] discovered that the electric stimulation of the plant is followed by ion shifts, which are most striking in the phloem cells. The amount of cytoplasmic calcium increased slightly while the content of K^+ and Cl^- was diminished after stimulation. Such evidence leads to the conclusion that the rapidly activating anion channels (Cl^-, NO_3^-) [45] as well as K^+ channels [4–10,46] are involved in the propagation of action potentials (Fig. 6b).

All processes of life have been found to generate electric fields in every organism that has been examined with suitable and sufficiently sensitive measuring techniques. The conduction of electrochemical excitation must be regarded as one of the most universal properties of living organisms. It arose in connection with a need for the transmission of a signal in response to an external influence from one part of a biological system to another. The study of the nature of regulatory relations of the plant organism with the environment is a basic bioelectrochemical problem, one that has a direct bearing on tasks of controlling the growth and development of plants. Fig. 7 illustrates the simplified mechanism of biosignaling in plants induced by a heat stress.

Despite the existence of great amounts of accumulated information concerning electric effects in plants, their physiological and electrochemical mechanisms remain poorly understood. Further investigation could provide information into the outlook of possible uses of these phenomena for improvement of agricultural technologies. These reasons provide significant basis to the importance of further profound investigations of electrical phenomena in plants.

According to modern measurements in real time, the APs in green plants and animals have similar speeds of propagation and duration. The automatic measurements of the extracellular and intracellular electrical potential difference can be effectively used in plant electrophysiology to study the molecular mechanisms of ion transport, the influence of external stimuli on plants, and for investigating the bioelectrochemical aspects of the interaction between insects and plants. The use of new computerized methods provides opportunities for detection of ultra fast APs in green plants in real time.

Acknowledgements

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