

Research Paper

Solitary waves in soybean induced by localized thermal stress

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Abbreviations: AP, action potential; R, resistance C, capacitance; f_s , the sampling frequency; f_{max} , the maximum frequency of the sampled signal

Key words: Action potential, plant electrophysiology, electrical signaling, localized heat stress, excitability

Action potentials in higher plants are believed to be the information carriers in intercellular and intracellular communication in the presence of an environmental stressor. Plant electrophysiologists have recorded long distance electrical signaling in higher plants during the last two hundred years. Reproducing the duration, speed of propagation, and the shape of the action potential is challenging. Early measurements revealed that the speed of action potential propagation in plants is extremely slow - from 0.1 mm/s to 20 cm/s, although many faster plant responses to stress have been recorded as well. We hypothesized that this discrepancy is most likely due to the artifacts of aliasing from slow registration systems. In this study, we employ real time measurements using modern data acquisition techniques to detect ultra fast action potentials in green plants induced by localized heat stress. Thermal shock or heat stress is the most common environmental stress. Based on more sophisticated measuring techniques, we show that plants transmit solitary waves and that the speed of action potential propagation in green plants is similar to the speed of action potentials in mammals, varying from a few meters per second up to 105 m/s. Possible pathways for electrical signal propagation in vascular plants are discussed.

Introduction

Excitability is a specific property that allows cells, tissues and organs to alter their internal condition and external reactions under the impact of various environmental factors, referred to as irritants. According to Goldsworthy,¹ electrochemical signals resembling nerve impulses exist in plants at all evolutionary levels. Action potentials (APs) in higher plants can be generated in response to mechanical, physical or chemical external irritants.²⁻⁹

The existence of electrical signaling in plants has been known for more than two centuries.¹⁰⁻¹⁴ As the field progressed, AP velocities were measured using a variety of methods. These first measurements revealed that the velocities of APs range from 0.1

mm/s to 20 cm/s.^{12,15-18} These slow AP velocities are similar to the speeds of diffusion of 0.6–4.0 mm/s as seen in the phloem¹⁹ and 25 cm/s in the xylem.²⁰ The previous measurements are significantly slower than those recorded in animal neurons. The existence of carnivorous or “motorized” plants and their fast movements cast certain doubts on the accuracy of these measurements. Sachs²¹ discussed the paradox between slow speeds of AP propagation registered by Burdon-Sanderson¹² in the Venus flytrap and ultra fast plant mechanical responses during the trap closing, because such slow action potentials¹² can be a result of the Venus flytrap closure, but not its cause.

We hypothesized that the discrepancies that exist between the traditional AP velocities measured in plants and the more recent recorded velocities are due to aliasing. The Shannon sampling theorem, a fundamental rule of sampled data systems, states that the input signal must be sampled at a rate greater than twice the highest frequency component in the signal. The critical sampling rate is called the Nyquist rate. Mathematically, $f_s/2 > f_{max}$, where f_s is the sampling frequency and f_{max} is the maximum frequency of the signal being sampled. Violating the Shannon sampling theorem is considered undersampling and results in aliasing. Aliasing does not infer that the “Sampled Value” is erroneous, but rather it means that the inferred time dependence of a series of samples is distorted. Due to this limitation, all data presented in this paper were collected on high speed data acquisition systems.

The electrophysiological signaling in higher plants strongly depends on environmental conditions.²²⁻²⁴ Plants are exposed to a diverse array of continuously varying perturbations, including variations of temperature.²⁵⁻²⁶ Plants generate different types of extracellular electrical events in connection to environmental stress.²⁷ Thermal shock is induced in plants, animals, insects, bacteria, and fungi by drastic changes in temperature. Thermal shock alters gene expression and leads to increased heat tolerance in a wide range of organisms. The response of many organisms to elevated temperature has been characterized and described as the heat shock response. In plants, heat stress stimulates the production of heat shock proteins (HSP).²⁸ HSPs assist plants in the adaptation to and tolerance of extreme temperatures.²⁹ None of the mechanisms by which higher plants perceive abiotic stresses has been elucidated. Progress in this crucial area will substantially advance our knowledge of stress initiated signal transduction events. The main goal of this study was to investigate in real time the electrical signaling in higher plants induced by a thermal wounding.

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Results and Discussion

We employed a novel PXI system to measure electrical signaling in plants induced by localized thermal stress. This experimental approach is illustrated in Figure 1. 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.

When electrochemical signals are measured, it is extremely important to take into consideration of the *sampling rate*, which determines how often the measurement device samples an incoming analog signal. According to the sampling theorem, the original analog signal must be adequately sampled in order to be properly represented by the digital signal, which is acquired. If the sampling rate is too slow, the rapid changes in the original signal in 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 (aliasing). In signal processing, this problem is known as *aliasing*. According to the Nyquist Criterion, the sampling frequency must be at least twice the bandwidth of the signal to avoid aliasing. As illustrated in Figure 2A and B, a sinusoidal signal with 500 Hz frequency can be uniquely reconstructed from the digitized signal when sampled at a rate of 300,000 samples/s or 100,000 samples/s, a sampling rate well above the Nyquist frequency limit of 1,000 samples/s. However, when the sampling frequency is at the Nyquist frequency limit, the distortion begins as shown in Figure 2D. According to Figure 2D, it is obviously impossible to reconstruct the original signal when it is under sampled at any frequency below the Nyquist Criterion limit, such as a sampling rate of 10 samples/s. During the last 125 years, plant electrophysiologists have measured action potential in plants for with extremely slow registration systems and without anti-aliasing low pass filters. Due to electronic effects of aliasing and different time constant $\tau = RC$ of analog voltmeters, different authors published different speeds of the action potential propagation (Table 1), different amplitudes and duration time of action potentials even for the same plants such as tomato plant. It is hypothesized that these discrepancies in the results among different groups of researchers were caused by aliasing or large time constants of high input impedance analog or digital voltmeters.

Localized heat wounding induces high speed electrical signals in vascular plants, specifically in soybean (Fig. 3). Our results show that a single application of heat induces fast action potentials in soybean plants (105.5 m/s, s.d. = 5.6 m/s, $n = 9$). These high speed APs are comparable to the recorded AP velocity in the neuron. Figure 3 shows that the duration of a solitary wave in soybean is about 1 ms. A localized thermal stress applied for 1 second generates only one action potential which is transmitted from the injured leaf through stem to roots.

Plants possess many chemical aspects similar to the neuromotoric system in animals.⁴⁰ For example,

Figure 2. Reconstructed 500 Hz sinusoidal signal from the digitized signal sampled at (a) 300,000 samples/second, (b) 100,000 samples/second, (c) the Nyquist rate of 1,000 samples/second; (d) aliased 500 Hz signal due to undersampling at 10 samples/second.

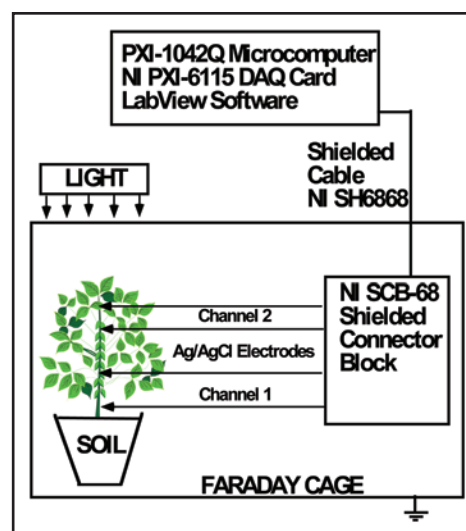


Figure 1. Experimental setup for measuring electrical signals in soybean.

plants employ neurotransmitters such as acetylcholine, dopamine, noradrenalin, serotonin and histamine,⁴⁰ cellular messengers like calmodulin,⁴⁰ the cellular motors actin and myosin, and voltage-gated ion channels.⁹ Plants also utilize a variety of sensors to detect touch, light, gravity and 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. Living cells have the capacity to transmit signals to the other regions of the organism. In contrast to chemical signals such as hormones, electrical signals are able to rapidly transmit information over long distances.

Upon perception, electrical signals in plants can be propagated via the plasmodesmata to other cells of the symplast. Electrical coupling via the plasmodesmata was demonstrated in a variety of species, indicating that plasmodesmata are relays in the signaling network

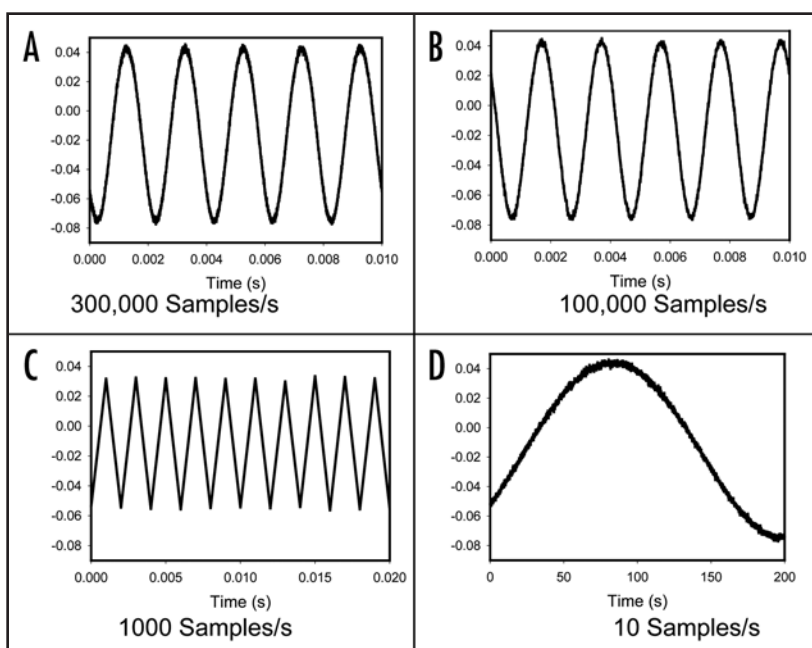


Table 1 **Electrical signals in higher plants induced by thermal stimuli**

	Plant	Stimulus	Potential Amplitude, mV	Potential Duration, s	Potential Speed, cm/s	Potential Length, cm	Low - Pass Anti-Aliasing Filter	Physiological Effect	References
1	Tomato (<i>Lycopersicon esculentum</i> Mill.)	Localized heat	AP: 49	60-300	2.5-4.0	150-1200	No	Induction of <i>pin</i> gene expression	18
2	Tomato (<i>Lycopersicon esculentum</i> Mill.)	Localized heat	AP: 79	10	0.2	2	No	Induction of <i>pin</i> gene expression	30
3	Tomato (<i>Lycopersicon esculentum</i> cv. Heinz 1350)	Localized heat	AP: No VP: 40	No ~60	No 0.4	No ~24	No	Induction of <i>pin</i> gene expression	31
4	Tomato (<i>Lycopersicon esculentum</i> cv. Heinz 1350)	Localized heat	AP: No VP: 74	No >1800	No ?	No ?	No	Induction of <i>pin</i> gene expression	24
5	Wheat (<i>Triticum durum</i> Desf., cv. Iva)	Localized heat	AP: No VP: ?	No ?	No ?	No ?	No	Induction of stomata activity	16
6	Tobacco (<i>Nicotiana tabacum</i> cv. Samsun)	Localized heat	EPP	>3600	1.5-2.0	5400-7200	No	Stomata closure, reductions in the rate of transpiration and CO ₂ assimilation	32
7	Sunflower (<i>Helianthus annuus</i>)	Localized heat	AP: 18.5-24.4	14.7-18.4	1.3-2.7	24-39.7	No	Action potential is the carrier of information	33
8	Bean (<i>Phaseolus multiflorus</i> Willd.)	Localized heat	AP: 22.3-27.0	16.3-26.1	0.5	8.2-13.05	No	Action potential is the carrier of information	33
9	Buckwheat (<i>Fagopyrum sagittaeum</i> Gilib.)	Localized heat	AP: 17.1-19.5	16.9-24.6	0.9-1.2	22.1-20.3	No	Action potential is the carrier of information	33
10	Pumpkin (<i>Cucurbita pepo</i> L.)	Localized heat	AP: 8.5-17.9	12.2-16.4	0.7-1.0	11.5-12.2	No	Action potential is the carrier of information	33
11	Pumpkin (<i>Cucurbita pepo</i> L.)	Gradual cooling	AP: 18 (13°C) 117 (26°C)	30-60	?	?	No	Increase in cold resistance	34
	Pumpkin (<i>Cucurbita pepo</i> L.)	Gradual cooling	~120 mV	?	?	?	No	Increase in cold resistance	35
12	<i>Mimosa pudica</i>	Cooling with ice water	AP: 150	8	2-3	16-24	No	Leaf movements	36
13	<i>Mimosa pudica</i>	Localized heat	AP: 80-100	120-300	0.4-0.8	48-240	No	Leaf movements	37
14	<i>Hibiscus rosasinensis</i> L. (Malvaceae)	Localized heat	AP: No VP: 100	>150	1.3	>195	No	Respiration change; reduced metabolite concentrations in the ovary	38
15	<i>Hibiscus rosasinensis</i> L. (Malvaceae)	Cooling with ice water	AP: 100	2-3	2	4-6	No	Respiration change; reduced metabolite concentrations in the ovary	38
16	<i>Aloe vera</i>	Localized heat	AP: 40	0.01	6,700	67	Yes		39
17	<i>Aloe vera</i>	Ice	AP: 20-35	0.003	13,200	40	Yes		39
18	Soybean (<i>Glycine max</i> (L.) Merrill)	Localized heat	AP: 130	0.001	10,500	10.5	Yes		Present work

AP, action potential; VP, variation potential; EEP, extracellular electrical potential.

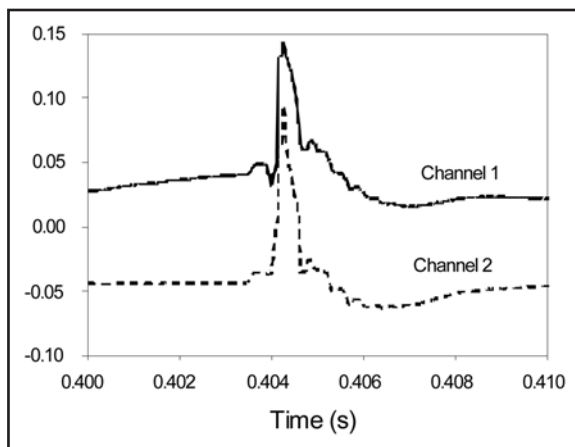


Figure 3. Action potentials induced in a soybean plant by thermal shock (flame). Sampling rate is 100,000 scans per second and distance between channels is 2 cm.

between plant cells.⁴¹ Low resistance connections extend between plant organs, and 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.

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. This reason provides a significant basis to the importance of further profound investigations of electrical phenomena in plants. Green plants generate a wide spectrum of electric signals that arise in response to various external perturbations. These signals propagate for long distances along plant tissues and can cause remote effects in a plant.

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. Electrical signaling in living organism arose to address the need for the transmission of signals in response to an external influence from one part of a biological system to another. The study of the nature of regulatory relations of a plant organism with the environment is the investigation into a bioelectrochemical phenomenon providing insight into the growth and development of 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 plants and other biological organisms. The use of new computerized methods

provides opportunities for detection of ultra fast APs in green plants in real time.

Materials and Methods

A novel real-time experimental setup using an ultra-fast PXI data acquisition system was developed (see Fig. 1). All measurements were conducted in the laboratory at constant room temperature inside a Faraday cage mounted on a vibration-stabilized table. In order to estimate possible high frequency content of the responses evoked, a high performance *National Instruments* data acquisition system was used. 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 a NI SCB-68 shielded connector block to 0.1 mm thick nonpolarizable reversible Ag/AgCl electrodes. The results were reproduced on a workstation with data acquisition board NI 6052E DAQ with input impedance of 100 G Ω interfaced through a NI SC-2040 Simultaneous Sample and Hold. The system integrates standard low-pass anti-aliasing filters at one half of the sampling frequency.

Ag/AgCl electrodes were prepared from Teflon coated silver wire (*A-M Systems, Inc.*). Localized thermal stress was created by flame applied continuously for 1 seconds to a predetermined leaf using a utility lighter (*BIC*). 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.

The soybean seedlings (*Glycine max* (L.) Merrill) were used approximately 3 weeks after germination. Each plant had an average of 5 developed leaves. Plants were grown in clay pots with 0.5 L of sterilized potting soil in a plant growth chamber (*Environmental Corporation*) at 22°C. Plants were watered daily and exposed to a 12/12 hr light/dark photoperiod at 22°C. Humidity remained constant at 45–50%.

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