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Green plants: electrochemical interfaces☆

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Abstract

The most rapid methods of long distance communication between plant tissues and organs are bioelectrochemical signals. The action potential propagates rapidly throughout the plant along electrified interfaces. A potential pathway for transmission of this electrical signal might be the phloem sieve-tube system since it represents a continuum of plasma membranes. A phloem is an electrical conductor of bioelectrochemical impulses over long distances. At the cellular level in plants, electrical potentials exist across membranes, and thus between cellular compartments, as well as within specific compartments. Electrochemical phenomena are primary, and then deep cytophysiological reactions occur. Possible effects of the electric double layer of the Earth on the electrical activity of plants are discussed. Effects of uncouplers and acid rains on green plants are studied. Uncoupler carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone (FCCP) induces ultra fast action potentials and decreases the resting potential in a soybean. The speed of the propagation of action potentials in a soybean induced by FCCP reaches up to 40 m s^{-1}. The duration of single action potentials after treatment by FCCP was 0.0003 s. Adding FCCP to soil decreases the resting potential to zero level. The automatic measurements of the electrical potential difference can be effectively used in bioelectrochemistry, environmental plant electrophysiology, for the study of molecular mechanisms of transport processes and the influence of external stimuli on plants.

Keywords: Bioelectrochemistry; Electrophysiology; Liquid interfaces; Uncoupler; Action potential; Acid rain

1. Introduction

The 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 electrochemical conduction of electrochemical excitation over specialized structures must be regarded as one of the most universal properties of living organisms. It arose at the early stages of evolution in connection with the need for transmission of a signal about an external influence from one part of a biological system to another. 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.

Any living cell continuously receives information on the environment. Its surface membrane has numerous protein receptors, which interact with practically all the vitally important molecules. Plants have a specific property, which is excitability [1]. This is the property of cells, tissues and organs to change their internal condition and external reactions under the action of various environmental factors, referred to as irritants. The high sensitivity of protoplasm and all cell organelles to any natural and electrochemical effects is the basis for excitability. The excitation waves or action potentials in higher plants could be information carriers in intercellular and intracellular communication in the presence of environment changes. The integral organism of a plant can be maintained and developed in a continuously varying environment only if all cells, tissues and organs function in concordance. Plants are continuously balancing with the external world. The coordination of internal processes and their balance with the environment are connected with the excitability of plant cells.

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The huge amount of experimental material testifies that the main laws of excitability such as the inducement of nonexcitability after excitation, the summation of subthreshold irritations and so on were developed in the vegetative and animal kingdoms in protoplasmatic structures earlier than the morphological differentiation of nervous tissues. These protoplasmatic excitable structures consolidated into the organs of a nervous system and adjusted the interaction of the organism with the environment. Volkov and Haack [2,3] studied the role of electrical signals induced by insects in long-distance communication in plants and confirmed the mechanism by which electrical signals can directly influence both biophysical and biochemical processes in remote tissues.

Mechanical, physical or chemical external irritants act not only at the place of occurrence, but the excitation can be also transferred along the whole plant [1–4]. The speed of transfer depends on many factors, such as the intensity of the irritation, temperature, chemical treatment or mechanical wounding, and is also influenced by previous excitations. The excitation reaction goes in both directions, from the top of a stem to the roots and conversely, but not always at identical rates. The transfer of excitation has a complicated character, accompanied by an internal change in cells and tissues. Bose [5] has established the availability of a reflex arch in such plants as *Mimosa pudica*. By being excited, sensor cells generate impulses, which reach motor cells. The character of their distribution depends upon the physiological condition of plants. The signal from a beam of sunlight is transmitted to the tissues of a stem with an extremely high speed, and the stem begins to curve toward the source of light. The illuminated top of a stem, being excited, causes an impulse, which begins to be distributed among tissues. When the impulse reaches motor cells, the stem bends. Electrochemical phenomena are primary, and then deep cytophysiological reactions occur. Of particular concern is the redistribution of biologically active substances.

Conductive bundles of vegetative organisms not only facilitate the movement of substances, but also actuate the transfer of bioelectrical impulses. This phenomenon underlies the coordination of processes of the vital activity of vegetative organisms. The bioelectrical impulses arise under the influence of various chemical compounds (herbicides, salts, water, plant growth stimulants) and physical factors such as mechanical wounding, electromagnetic or gravitational fields, and temperature effects.

The most rapid methods of long distance communication between plant tissues and organs are bioelectrochemical or electrophysiological signals. The effectiveness of such long-distance communication is clear, since plants can respond rapidly to external stimuli (e.g. changes in temperature or osmotic environment, illumination level, wounding, cutting, mechanical stimulation or water availability) and changes can be detected in distant parts of the plant soon after the injury [1,2]. Wounding stress is accompanied by the rapid long-distance propagation of electrochemical signals, known as action potentials, which switch on the membrane enzymatic systems that realize biochemical reactions, accelerate ethylene synthesis, increase the concentration of proteinase inhibitor, decrease or increase formation of polysomes and protein synthesis. Action potentials have been induced in higher plants by cold or heat shock, wounding, chemical treatment, and by changing of orientation of gravitational or magnetic fields [1–3].

The plant cell is controlled by a molecular stochastic computer (MC), which operates with molecule-words (DNA, RNA, proteins) according to the program recorded in DNA and RNA. In the cell, the main programs are written in the molecules of DNA by a four-letter code. Operations are produced by molecular devices operating with molecule words and recorded on molecules themselves, and they are read off by ribosomes [6]. MC operates with molecular words having definite addresses. Green plants measure many parameters in order to have orientation in the outer medium. The plant cell continuously receives information on the environment. Its surface membrane has numerous protein receptors, which interact with practically all the vitally important molecules. Wounding of plants influences the gene expression [7].

The velocities of the propagation of electrical signals that have values from 0.05 to 4000 cm s^{-1} are sufficiently high to facilitate rapid long-distance communication and account for the rapid response phenomena observed in plants. Both the speed of propagation and the amplitude of action potential depend on the type of external stimulus [8].

The action potential propagates rapidly throughout the plant. A potential pathway for transmission of this electrical signal might be the phloem sieve-tube system since it represents a continuum of plasma membranes. A phloem is an electrical conductor of bioelectrochemical impulses over long distances. From an electrochemical point of view, structures of phloem and axon can be pictured as hollow tubes filled with electrolyte solutions (Fig. 1). The length of an axon or phloem can be several meters and its diameter is about 1–100 μm. At the cellular level in plants, electrical potentials exist across membranes, and thus between cellular compartments, as well as within specific compartments. Ca^{2+}, K^+, H^+, Na^+, and Cl^- ions represent electrolytic species involved in the establishment and modulation of electrical potentials. Electrical potentials have been measured at the tissue and whole plant level [2,3]. Since electrical potential differences are expressed spatially within biological tissue and are modulated over time, many investigators have postulated the involvement of electrical potentials in inter- and intra- cellular communication and thus in the regulation of such physiological plant processes as phloem unloading. Action potentials represent the primary candidate for signaling between cells since they can be induced and transmitted rapidly within plant tissue. Specificity of communication can be achieved through modulation of the amplitude, the duration, the direction of the polarity change,

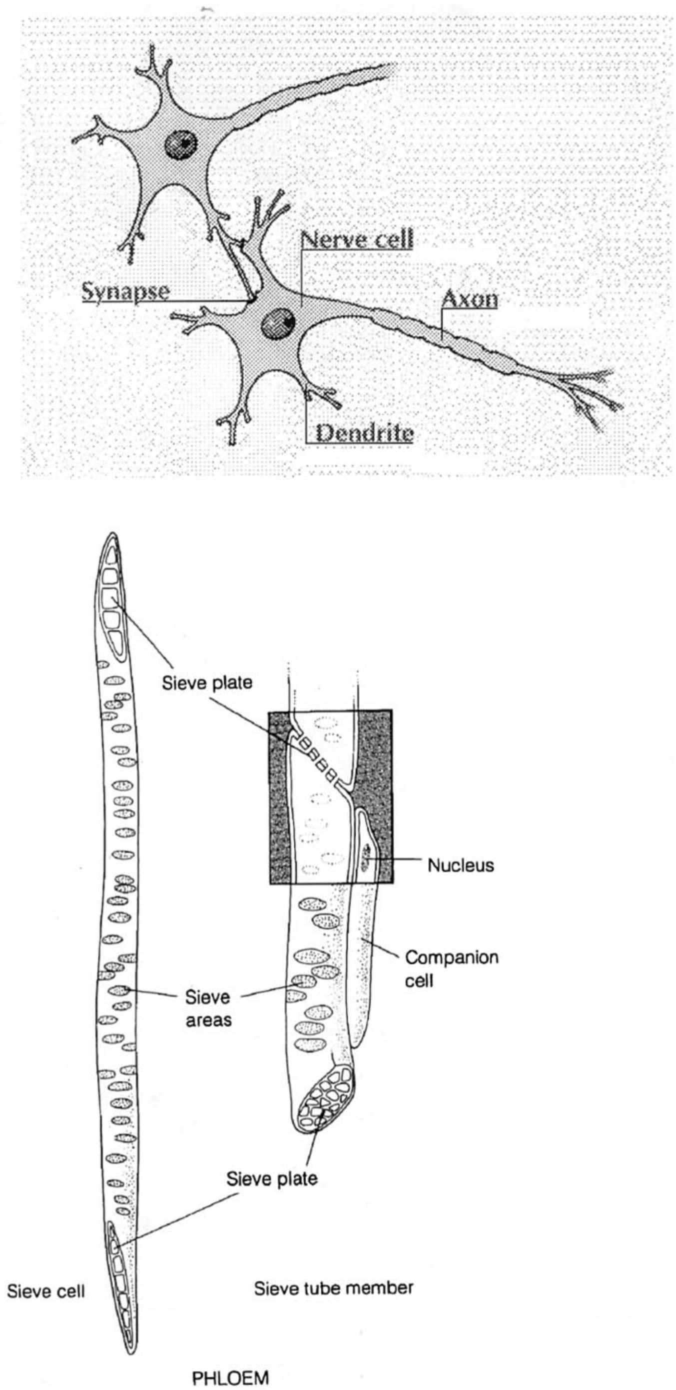

Fig. 1. Structures of a nerve and phloem.

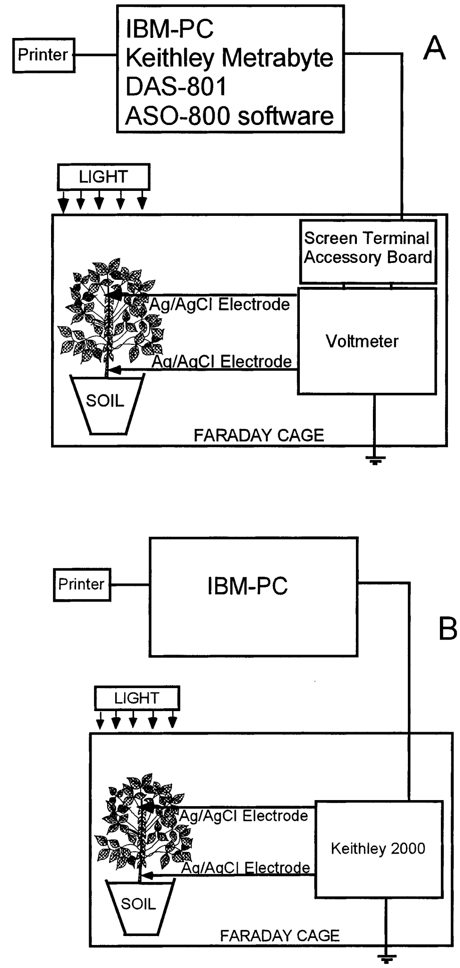

Fig. 2. Apparatus for measuring dc and ac signals in green plants. Ag | AgCl electrodes were connected to a Cole Palmer Microcomputer pH-vision Model 05669-20 voltmeter/pH meter (A) or a Keithley-2000 programmable electrometer/amplifier (B).

and the rate of propagation of the electrical potential signal.

2. Experimental

All electrical measurements were conducted inside a Faraday cage (Fig. 2). Ag | AgCl electrodes were connected to a voltmeter/pH meter (Cole Palmer Microcomputer pH-vision Model 05669-20, Fig. 2A) or a programmable electrometer/amplifier (Keithley-2000, Fig. 2B). An IBM-compatible microcomputer with a multi I/O plug-in data acquisition board DAS-801 (Keithley MetraByte) was interfaced with the voltmeter and used to record the digital data. The measuring signals were recorded as ASCII files using software ASO-801 or EASYEST AG-tool kits for fast startup and automatic data acquisition application from Keithley. For potential difference measurements we used nonpolarizable reversible Ag | AgCl-electrodes with a diameter of 0.25 mm as a reference and working electrodes connected to an electrometer and interfaced with the computer through a multiplexed screw terminal accessory board STA-08 (Keithley). Ag | AgCl electrodes were prepared from a Teflon coated silver wire (A-M Systems, Inc.) according to Ksenzhek and Volkov [1].

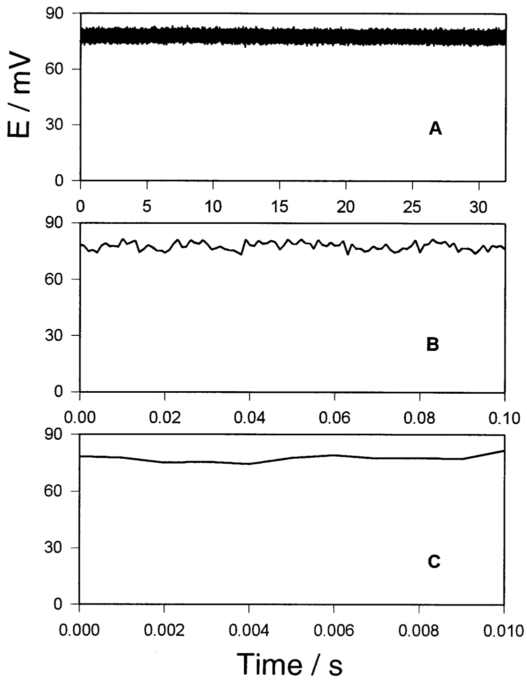

Fig. 3. Potential differences between two Ag | AgCl electrodes in the stem of a soybean before any treatment of the plant. Frequency of scanning was 1000 Hz. Distance between electrodes was 8 cm. Volume of soil was 0.5 l. The soil around the plant was treated with water every day. Room temperature was 24°C. Ag | AgCl electrodes were inserted in the stem of soybean 3 h before measurements.

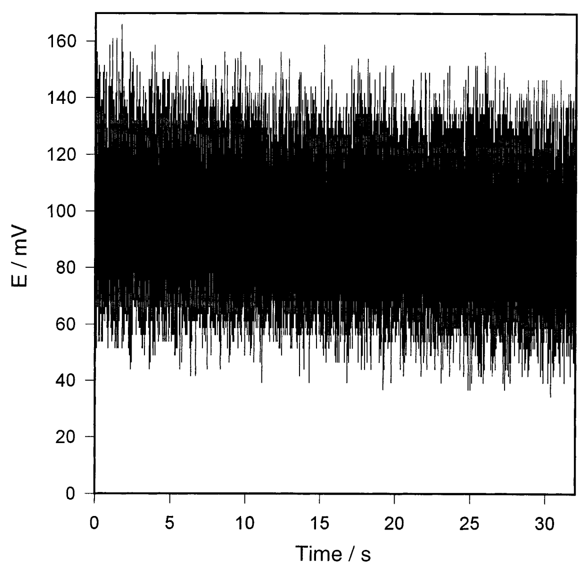

Fig. 4. Potential differences between two Ag | AgCl electrodes in the stem of a soybean 10 s after insertion of electrodes in the stem of a soybean. Distance between electrodes was 8 cm. Volume of soil was 0.5 l. The soil around a plant was treated with water every day. Room temperature was 24°C. Frequency of scanning was 1000 Hz.

The speed of action potential propagation along the phloem was measured as described by Volkov and Haack [2,3].

Three-week-old soybean seedlings (*Glycine max* (L.) Merrill), cultivar Hutchenson were used in all studies; such plants usually had five to six well-developed leaves. Plants were grown in clay pots with sterilized potting soil and were grown in a growth chamber (Environmental Corporation) at 28°C with a 12:12 h light:dark photoperiod. Plants were watered every other day. The laboratory in which the experiments were carried out was a room without windows with the temperature kept at 24°C and humidity of 70%. The light was turned off automatically at 18:00 h, creating total darkness, and turned on again at 06:00 h.

Carbonyl cyanide-*p*-trifluoromethoxyphenyl hydrazone (FCCP) was obtained from Fluka (New York, NY).

3. Results and discussion

3.1. Electrodes insertion

The generation and propagation of action potentials and electrical impulses between the tissues in higher plants can be measured by reversible nonpolarizable electrodes [2,9]. 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. 3). Insertion of electrodes in plants induces action potentials across the stem and slow fluctuations of the resting potential of phloem (Fig. 4). After approximately 1–2 h, the resting potential stabilizes and action potentials induced by wounding disappear.

3.2. Atmospheric electrochemistry: effects of the electric double layer of the earth and acid rains

Chemical reactions involving aerosol particles in the atmosphere derive from the interaction of gaseous species with the liquid water associated with aerosol particles and with dissolved electrolytes. For example, the generation of HONO from nitrogen oxides takes place at the air | water interface in seawater aerosols or in clouds. Clouds convert between 50 and 80% of SO_2 to H_2SO_4. This process contributes to the formation of acid rain. Acid rain is the most serious environmental problem and has an impact on agriculture, forestry and human health. The existence of ions in the atmosphere is the fundamental reason for atmospheric electricity. The voltage between earth's surface and the ionosphere

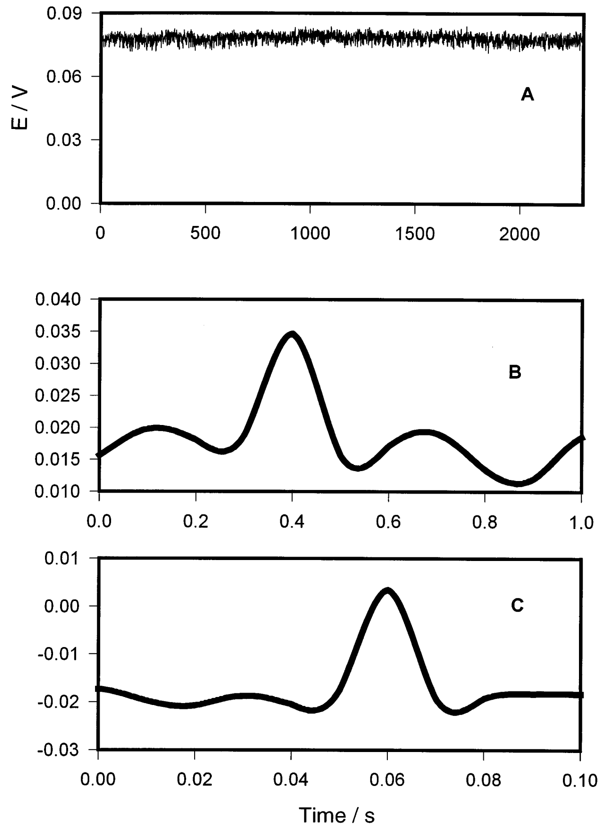

Fig. 5. Potential differences between two Ag | AgCl electrodes in the stem of a soybean: (A) After single spray of a soybean with 0.1 ml of H_2SO_4 at pH 5.0; (B) action potential after single spray of a soybean with 0.1 ml of HNO_3 at pH 3.0; (C) action potential after single spray of a soybean with 0.1 ml of H_2SO_4 at pH 3.0. The soil was preliminary treated by water every day. Ag | AgCl electrodes were inserted in the stem of soybean 3 h before a chemical treatment of plants.

is about 40 kV, the electrical current is about 2000 A and a current density of about 5 pA m^{-2} results. The Earth is an electrode immersed in a weak gaseous electrolyte, the naturally ionized atmosphere. The earth's surface absorbs anions and has a negative charge. The electrostatic field strength at the earth's surface is about 110–220 V m^{-1} and depends on the time of day. Usually it is about 110 V m^{-1}, but at 19:00 h Greenwich time, the electrostatic field strength is about 220–250 V m^{-1} [10]. Oceans, lakes, rivers cover a significant part of the Earth and their surface is also charged negatively against the atmosphere. Electrical polarity in soybean, potato, tomato, and cacti coincides with the electrical field of the electric double layer of Earth-($-$) in roots and ($+$) on the top of the plants. Atmospheric change of the electrostatic field strength at 19:00 h. Greenwich time does not induce action potentials or changes the resting potential of soybean or potato plant.

Acid rain has a pH below 5.6. Sulfuric and nitric acids are two predominant acids in acid rain. Spraying the soybean by an aqueous solution of H_2SO_4 in the pH region from 5.0 to 5.6 does not induce action potentials or variations of the resting potential (Fig. 5A). Spraying the plant (0.1 ml) or deposition of 10 μl drops of aqueous solution of H_2SO_4 or HNO_3 in the pH region from 0 to 4.9 on leaves induced action potentials in soybean with 55 ± 5 cm s^{-1} speed of propagation (Fig. 5B, C). The duration of single action potentials, after treatment by HNO_3 and H_2SO_4, was 0.2 and 0.02 s, respectively. The speed of propagation of the action potential does not depend on the location of the working electrode in the stem of the plant or in the leaves, or on the distance between the working and reference electrodes [1–3].

Action potentials were generated in a soybean if the pH of the soil was acidified even without spraying the plant (Fig. 6). The action potential induced by acid rain reaches a working electrode in the soybean, which changes its potential relative to the reference electrode. Hence, it is possible to measure the peak potential of the working electrode compared to the reference electrode. The action potential propagates along the stem and reaches the reference electrode, which gives a mirror image of the potential peak with an opposite sign from that of the first peak (Fig. 6) because during this process the potential of the reference electrode changes relative to that of the working electrode.

The cells of many biological organs generate an electric potential that may result in the flow of electric current. Electrical impulses may arise spontaneously or they may result from stimulation. Once initiated, they

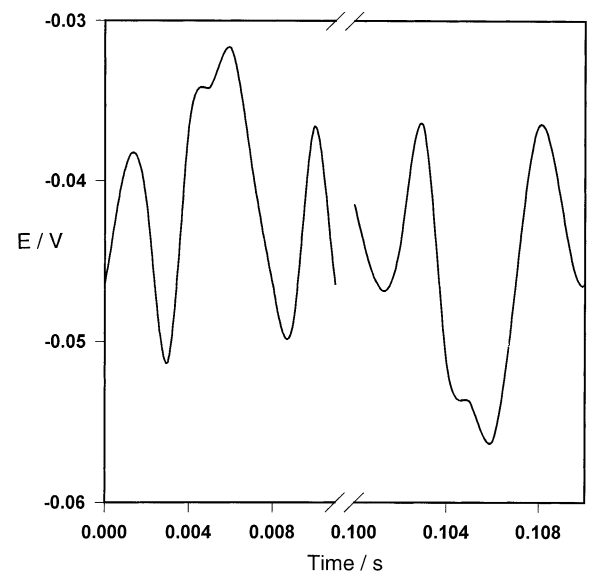

Fig. 6. Potential differences between two Ag | AgCl electrodes in the stem of a soybean measured after adding to soil H_2SO_4. The pH of soil was 3.0. Distance between Ag | AgCl electrodes was 5 cm. The soil was preliminary treated by water every day. Ag | AgCl electrodes were inserted in the stem of soybean 3 h before a chemical treatment of soil.

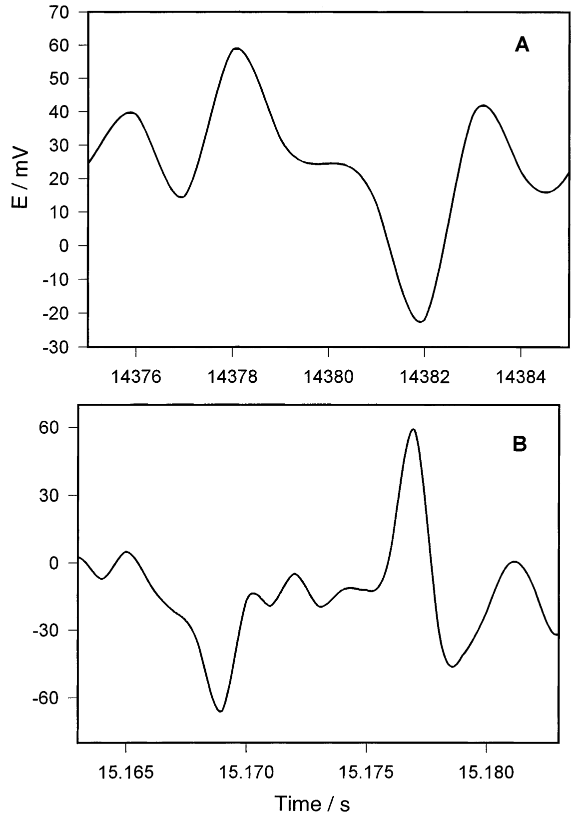

Fig. 7. Potential differences between two Ag | AgCl electrodes in the stem of soybean 4 (A) and 20 (B) h after adding 30 ml of 10^{-6} M FCCP to soil. Distance between electrodes was 7 cm. The soil was preliminary treated by water every day. Volume of soil was 0.5 l. Ag | AgCl electrodes were inserted in the stem of soybean 3 h before a chemical treatment of soil.

can propagate to adjacent excitable cells. The change in transmembrane potential creates a wave of depolarization, or action potential, that affects the adjoining, resting membrane. Thus, when the phloem is stimulated at any point, the action potential is propagated over the entire length of the cell membrane and along the phloem with a constant voltage. The propagation of each impulse is followed by the absolute refractory period during which the fiber cannot transmit a second impulse. The high sensitivity of protoplasm and all cell organelles to any natural and chemical effects is the basis for excitability. The integral organism of a plant can be maintained and developed in a continuously varying environment only if all cells, tissues and organs function in concordance. Plants are continuously balancing with the external world.

3.3. Effects of uncouplers and protonophores

FCCP is known as a protonophore or uncoupler of oxidative phosphorylation in bioelectrochemistry. Addition to the soil of aqueous solution of FCCP induces action potentials in a soybean (Figs. 7–9). After treatment of soil by an aqueous solution of FCCP, the resting potential, measured between two Ag | AgCl microelectrodes in a stem of a soybean, slowly decreases from 80–90 mV (− in a root, + on the top of the soybean) to zero level over 20 h. Figs. 8A and 9A show that positive and negative spikes appear during measurement of the electrical potential difference between two reversible silver chloride electrodes. High-resolution analysis of shorter intervals shows that these spikes are action potentials (Figs. 8B,C and 9B,C). The action potential induced by FCCP reaches a working electrode in the soybean, which changes its potential relative to the reference electrode. Hence, it is possible to measure the peak potential of the working electrode compared to the reference electrode. The action potential propagates along the stem and after a few milliseconds reaches the reference electrode, which gives a mirror image of the potential peak with an opposite sign from that of the first peak (Figs. 7–9) because during this process the potential of the reference electrode changes relative to that of the working electrode. The distance between Ag | AgCl-electrodes divided by time between positive and negative mirror spikes corresponds to the speed of action potential propagation. The duration of single action potentials during the first 20 h after treatment by FCCP varies from 2 to 0.002 s (Fig. 7). The

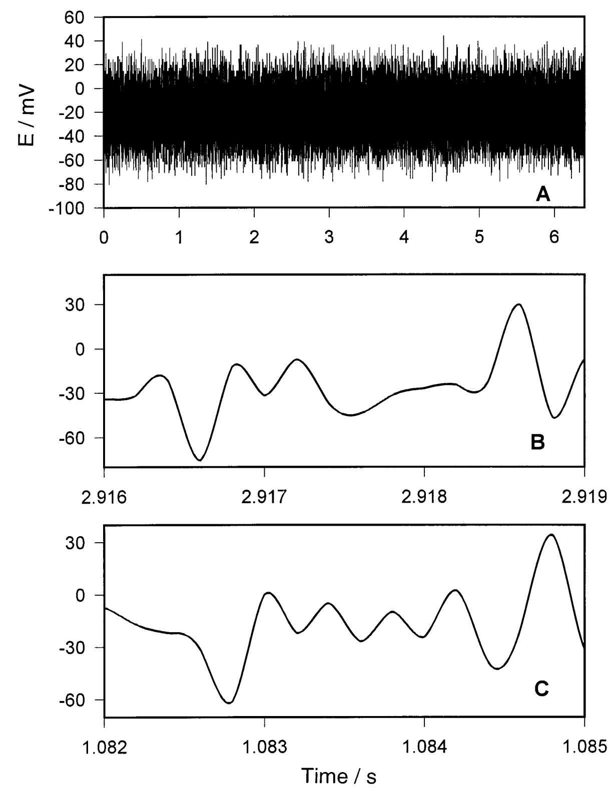

Fig. 8. Potential differences between two Ag | AgCl electrodes in the stem of soybean 100 h after adding 30 ml of 10^{-6} M FCCP to soil. Frequency of scanning was 5000 Hz. Distance between electrodes was 8 cm. The soil was preliminary treated by water every day. Volume of soil was 0.5 l. Ag | AgCl electrodes were inserted in the stem of soybean 3 h before a chemical treatment of soil.

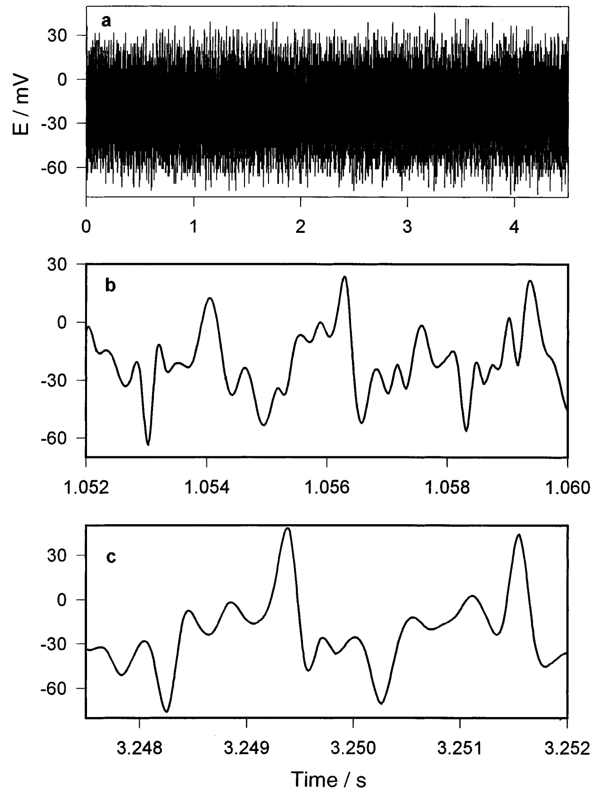

Fig. 9. Potential differences between two Ag | AgCl electrodes in the stem of soybean 100 h after adding 30 ml of 10^{-6} M FCCP to soil. Frequency of scanning was 5000 Hz. Distance between electrodes was 5 cm. The soil was preliminary treated by water every day. Volume of soil was 0.5 l. Ag | AgCl electrodes were inserted in the stem of soybean 3 h before a chemical treatment of soil.

amplitude of action potentials is about 60 mV (Fig. 7B). The maximum speed of action potential propagation during the first 20 h is 10 m s^{-1} (Fig. 7B). Fig. 2B shows action potentials in soybean 20 h after treatment of soil by FCCP. After 100 h, action potentials with amplitude about 60 mV, duration time 0.0003 s, and a propagation speed of 40 m s^{-1} (Figs. 8 and 9) generate in a soybean. The speed of propagation, duration, and amplitude of action potentials do not depend on the location of the working electrode in the stem or the root of the plant or in the leaves, or on the distance between the working and reference electrodes. Action potentials take an active part in the expedient character of response reactions of plants as a reply to external effects. These impulses transfer a signal about the changes of conditions in a conducting bundle of a plant from the root system to the point of growth and conversely. The response reactions of plant tissues and organs can be local or they can be transmitted from cell to cell over long distances via the plasmodesmata. Excitation, due to electrical impulses generated by changes in environmental conditions, functions as a carrier of information in a soybean. Action potentials are signals caused by the depolarization of a plasma membrane. Mechanical, physical or chemical external irritants act not only at the place of occurrence, but the excitation can also be transferred along the whole plant. The speed of excitation transfer depends on many factors, such as the intensity of the irritation, temperature, chemical treatment or mechanical wounding, and is also influenced by previous excitations. The conduction reaction goes in both directions, from the top of a stem to roots and conversely. The transfer of excitation has a complicated character, accompanied by an internal change in cells and tissues. The most rapid methods of long distance communication between plant tissues and organs are bioelectrochemical or electrophysiological signals. The effectiveness of such long-distance communication is clear since plants can respond rapidly to external stimuli (e.g., changes in temperature or osmotic environment, plant pathogens, insects, illumination level, wounding, cutting, mechanical stimulation or water availability), and changes can be detected in distant parts of the plant soon after the injury.

The fastest velocity reported for propagation of action potentials in green plants was 0.2 m s^{-1} in *Dionaea* flytrap [11]. Action potentials induced in a soybean by FCCP are 200 times faster (Figs. 8 and 9). The automatic measurements of the electrical potential difference can be used effectively in environmental plant electrophysiology, for the study of molecular mechanisms of transport processes, and the influence of external stimuli on plants.

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