Plant electrophysiology: pentachlorophenol induces fast action potentials in soybean

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Abstract

In the present work, we show that pentachlorophenol (PCP) induces ultra fast action potentials and decreases the variation potential in a soybean. The speed of the propagation of action potentials in a soybean induced by PCP reaches up to 30 m/s, similar to excitation propagation in animal nerves. Measured velocities for transmission of action potentials in green plants reported by different authors were registered in the range of a few mm/s. Action potentials induced in soybeans by PCP are many times faster. Seventy hours after adding PCP to soil variation (resting) potential decreases to zero level. This is the first attempt of high-speed automatic measurements of fast action potentials in green plants. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

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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 [1]. The electrochemical conduction of excitation over specialized structures must be regarded as one of the most universal properties of living organisms [2]. 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 [3]. Study of the nature of regulatory relations of the plant organism with the environment is a basic electrophysiological problem, one that has a direct bearing on the task of controlling the growth and development of plants [4,5].

Every living cell continuously receives information about its surroundings. Its surface membrane has numerous protein receptors, which interact with practically all vitally important molecules [6]. Plants have a specific property, which is excitability. This property is used by cells, tissues, and organs to change their internal condition and external reactions under the action of various environmental factors, referred to as irritants [7]. Volkov and Haack [8] 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.

Action potentials in higher plants are the information carriers in intercellular and intracellular communication in the presence of environmental changes. Plants are exposed to a diversity of continuously varying perturbations, including air and soil pollution. The presence of pentachlorophenol (PCP) in the soil is the most serious environmental problem and has a hazardous impact on agriculture and human health. PCP is known as a protonophore or uncoupler of oxidative...
phosphorylation in bioenergetics [9]. It is a pollutant and the largest chemical source of dioxins to the environment. PCP is also used as a herbicide, insecticide, and fungicide. PCP is a member of the substituted halogenated aromatic family of pesticides, many of which exhibit insecticide and fungicide activity. In addition, PCP and its products are toxic to plants, making them useful as defoliants and general herbicides. Primary uses of PCP include protection of timber from fungal rot and wood-boring insects. The mechanism of action of PCP is believed to be the inhibition of the formation of ATP by uncoupling oxidative phosphorylation. Presently, we have been able to show that PCP induces fast action potentials and decreases the variation potential to zero level in soybeans.

2. Materials and methods

All electrical measurements were conducted inside a Faraday cage (Fig. 1). Ag/AgCl electrodes were connected to a voltmeter/pH meter Cole Palmer Microcomputer pH-vision Model 05669-20. 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 start-up and automatic data acquisition application from Keithley. The generation and propagation of action potentials and electrical impulses between the tissues in higher plants can be measured by reversible nonpolarizable electrodes [10]. For potential difference measurements we used nonpolarizable reversible Ag/AgCl-electrodes with a diameter of 0.14 mm as the reference and the 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 the method described by Ksenzhek and Volkov [4]. Since both Ag/AgCl electrodes are identical, we decided to call one a reference and the other a working electrode as shown in Fig. 1. The reference electrode (-) was inserted in the stem or root of a soybean. The working electrode (+) was inserted in to the stem or leaf of a soybean.

Three-week-old soybean seedlings (Glycine max (L.) Merrill), cultivar Hutchenson were used in all studies; such plants usually had five and six well-developed leaves. Plants were grown in a growth chamber (Environmental Corporation) at 28°C with a 12:12 h light:dark photoperiod in clay pots with sterilized potting soil. The plants were given water every other day and kept at 24°C. PCP was obtained from Fluka (New York, NY). All experimental data were repeated at least 10 times. Action potential generation induced by PCP was measured at least 1000 times.

3. Results and discussion

Following insertion of the electrodes, the plants were allowed to rest until a stable potential difference was obtained between the working and reference electrodes. Insertion of electrodes in plants induces action potentials across the stem and slow fluctuations of the variation potential. After approximately 1 and 2 h, the variation potential stabilizes, and action potentials induced by wounding disappear [8]. Fig. 2 shows variation potential in soybean before any treatment of the plant.

When the soil is sufficiently wet, further addition of water to soil or spraying of water on the leaves does not result in generation of action potentials or essential changing of the variation potential. If the soil had not been watered for a
Fig. 2. Potential difference between two Ag/AgCl electrodes in the stem of soybean before any treatment of the plant. Distance between electrodes was 8 cm. The soil was preliminary treated by water every day. Volume of soil was 0.5 l. The soil around a plant was treated with water every day. Room temperature was 24°C. Figures b and c show short time intervals of Figure a.

few days, water stress on the plant may have been observed.

Addition to the soil of the aqueous solution of PCP induces fast action potentials in soybeans (Fig. 3a–c). Fig. 3 shows 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 of the same data presented on Fig. 3a and Fig. 4a show that these spikes are action potentials (Figs. 3 and 4). The action potential induced by PCP reaches a working electrode in the soybean, which changes its potential in relation to the reference electrode (Fig. 5). 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 ms it reaches the reference electrode, which gives a mirror image of the potential peak with an opposite sign from that of the first peak (Fig. 5). During this process the potential of the reference electrode changes with regard to the working electrode. The duration of single action potentials, after treatment by PCP, is 2 ms. The amplitude of action potentials is about 60 mV. After treatment of soil by an aqueous solution of PCP, the variation potential measured between two Ag/AgCl microelectrodes in the stem of a soybean, slowly decreased from 75 to 85 mV (− in a root, + on the top of soybean) to zero during 48 h. Fig. 3 shows action potentials in soybeans after the first 24 h after of soil treatment by PCP. During the second day, action potentials reached an amplitude about 60 mV, duration time 2 ms, and propagation speed of 10 m/s generate in a soybean. After 48 h variation potential stabilizes at zero level and fast action potentials with amplitude at about 60 mV, duration time of 2 ms, and propagation speed of 30 m/s generate in soybean

Fig. 3. Potential difference between two Ag/AgCl electrodes in the stem of soybean 24 h after adding 50 ml of $5 \times 10^{-4}$ M PCP to soil. Distance between electrodes was 8 cm. The plants were given water every other day and kept at 24°C. Volume of soil was 0.5 l. Figures b and c show short time intervals of Figure a.
Potential differences between Ag/AgCl electrodes in the stem and Ag/AgCl electrode in a root of soybean measured 70 h after adding 50 ml of $5 \times 10^{-4}$ M PCP to soil. Distance between Ag/AgCl electrodes was 9 cm. The plants were given water every other day and kept at 24°C. Volume of soil was 0.5 l. Figures b, c and d shows short time intervals of Figure a.

(Fig. 4b–d). Duration time of action potentials and speed of excitation propagation does not depend on the distance between working and reference electrodes. The speed of propagation of action potentials was measured as the distance between Ag/AgCl electrodes divided by time between positive and negative peaks of action potentials (Fig. 5).

The speed of propagation, duration, and amplitude of action potentials 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. 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 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 soybean. The speed of propagation of electrical signals is sufficiently high to facilitate rapid long-distance communication and accounts for the rapid response phenomena observed in plants. This has implications for how plants communicate to elicit the production of stress proteins or phytoalexins.

(Fig. 5). Determination of the speed of action potentials in soybean by measuring potential difference between two Ag/AgCl electrodes in the stem of soybean 24 h after adding 50 ml of $5 \times 10^{-4}$ M PCP to the soil. Distance between electrodes was 8 cm. The plants were given water every other day and kept at 24°C. Volume of soil was 0.5 l. Figures a and b were taken from Fig. 3a.
Action potentials are signals caused by the depolarization of the plasma membrane. 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. 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 excitation reaction goes in both directions, from the top of a stem to the roots and conversely. The condition 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. Effectiveness of such long-distance communication is clear since plants can rapidly respond 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. Volkov and Haack [8] 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. Mizuguchi et al. [11] found out that the application of an ac voltage to the cultured solution accelerates a soybean growth rate by 30%. The authors considered such an accelerating effect to be related to the stimulation of the ion pumps for growth metabolism.

Measured velocities for propagation of action potentials in green plants, which have been reported by different authors, include (see for review [4]), 20 cm/s in Dionaea flytrap, 4.4 cm/s in Mimosa pudica leaf stalk, 5 mm/s in Drosera, 1.8 cm/s following cold stimulation in Cucurbita pepo, 0.5–0.8 mm/s in etiolated pea plants that were wounded, up to 10 cm/s via sieve tubes in Cucurbita pepo, and 1–7 mm/s in response to wounding. Action potentials induced in soybean by PCP are much faster, of the order of 30 m/s (Figs. 3 and 4). Similar data were obtained in experiments with other uncouplers — 2,4-dinitrophenol and FCCP [12–14].

Action potentials are surprisingly common in higher plants and lower eucaryotes. Most of the characterization of action potentials has used intracellular measurements of single cells and focused on the identification of the ion fluxes underlying the action potentials. 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 can propagate to adjacent excitable cells. The change in transmembrane potential creates a wave of depolarization, or action potential, that affects the adjoining, variation membrane. Thus, when the phloem is stimulated at any point, the action potential is propagated along the phloem with fixed amplitude. Once initiated, the action potential has a standard form and an essentially fixed amplitude — an ‘all or none’ response to a stimulus. 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 continuously adjust to the external world. The co-ordination of internal processes and their balance with the environment are connected with the excitability of plant cells. The automatic measurements of the electrical potential difference can be effectively used in environmental plant electrophysiology, for the studying of molecular mechanisms of ion transport and the influence of external stimuli on plants.

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