# Plants as Environmental Biosensors: Non-invasive Monitoring Techniques

**A.G. Volkov, M.I. Volkova-Gugeshashvili, and Albert J. Osei**

**Abstract** Plants are continuously exposed to a wide variety of perturbations including variation of temperature and/or light, mechanical forces, gravity, air and soil pollution, drought, deficiency or surplus of nutrients, attacks by insects and pathogens, etc. It is essential for all plants to have survival sensory mechanisms against such perturbations. As a consequence, plants generate various types of intracellular and intercellular electrical signals mostly in the form of action potentials or variation potentials in response to these environmental changes. However, over a long period, only certain plants with rapid and highly noticeable responses to environmental stresses have received much attention from plant scientists. Of particular interest to our recent studies on ultra fast action potential in green plants, we discuss in this review the possibility of utilizing green plants as fast biosensors for molecular recognition of the direction of light, monitoring the environment, and detecting the insect attacks as well as the effects of pesticides and defoliants.

**Keywords** Bioelectrochemical signaling · biosensors · action potential · phototropism · acid rain

## 1 Introduction

A biosensor is defined as a device that either detects, records, and transmits information related to a physiological change/process in a biological system, or uses biological materials to monitor the presence of various chemicals in a substance. A variety of

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plant and animal-tissues have been incorporated into various electrochemical transducers to detect and quantify a range of biologically important analytes including drugs, hormones, toxicants, neurotransmitters and amino acids. A detailed discussion on biosensors utilized in chemical or biological analysis is beyond the scope of this review. Based on our investigations on fast bioelectrochemical signaling events in green plants and similar examples reported in the literature by other plant scientists, we discuss here the evidence supporting the foundation for utilizing the entire green plant as a fast biosensor for monitoring the environmental perturbations in the close vicinity of a living plant.

Nerve cells in animals and phloem cells in plants share one fundamental property: they possess excitable membranes through which electrical excitations can propagate in the form of action potentials (Ksenzhek and Volkov 1998; Bose 1925; Volkov et al. 2000; Mwesigwa and Volkov 2001a, b). Plants generate bioelectrochemical signals that resemble nerve impulses, and are present in plants at all evolutionary levels. Prior to the morphological differentiation of nervous tissues, the inducement of nonexcitability after excitation and the summation of subthreshold irritations were developed in the vegetative and animal kingdoms in protoplasmatic structures.

The cells, tissues, and organs of plants transmit electrochemical impulses over short and long distances. It is conceivable that action potentials are the mediators for intercellular and intracellular communication in response to environmental irritants (Mwesigwa et al. 2000; Shvetsova et al. 2001; Volkov 2006a, b, c; Brown and Volkov 2006). Action potential is a momentary change in electrical potential on the surface of a cell that takes place when it is stimulated, especially by the transmission of an impulse (Brown and Volkov 2006).

Initially, plants respond to irritants at the site of stimulation; however, excitation waves can be distributed across the membranes throughout the entire plant. Bioelectrical impulses travel from the root to the stem and vice versa. Chemical treatment, intensity of the irritation, mechanical wounding, previous excitations, temperature, and other irritants influence the speed of propagation (Ksenzhek and Volkov 1998; Volkov 2006a, b, c).

Conductive bundles of vegetative organisms sustain the flow of material and trigger the conduction of bioelectrical impulses. This feature supports the harmonization of biological processes involved in the fundamental activity of vegetative organisms.

The conduction of bioelectrochemical excitation is a rapid method of long distance signal transmission between plant tissues and organs. Plants quickly respond to changes in luminous intensity, osmotic pressure, temperature, cutting, mechanical stimulation, water availability, wounding, and chemical compounds such as herbicides, plant growth stimulants, salts, and water. Once initiated, electrical impulses can propagate to adjacent excitable cells. The change in transmembrane potential creates a wave of depolarization or action potential, which affects the adjoining resting membrane (Brown and Volkov 2006).

Electrical potentials have been measured in our laboratory at the tissue and whole plant level by using the experimental set-up described in Fig. 1. Measurements were taken inside a Faraday cage mounted on a vibration-stabilized table. An *IBM*-compatible microcomputer with multi I/O plug-in data acquisition board NI 6052E DAQ (*National Instruments*) was interfaced through a NI SC-2040 Simultaneous Sample and Hold (*National Instruments*). The multifunction NI 6052E data acquisition board provides high resolution and a wide gain range and supports continuous, high-speed data acquisition. Single channels can be sampled at any gain up to 333 kSamples/s. The digitized data includes negligible time skew (less than 50 ns) between channels. Measuring signals were recorded as ASCII files using *LabView (National Instruments)* software. Nonpolarizable reversible Ag/AgCl electrodes were used to measure the electrical signals. The temperature was held constant since these electrodes are sensitive to the temperature. Ag/AgCl electrodes were prepared from Teflon coated silver wire (*A-M Systems, Inc*.). Plants were irradiated in directions A or B at different wavelengths using narrow band pass interference filters from *GS Edmund Scientific* (Barrington, NJ) with a central wavelength tolerance of ±1 nm.

## 2 Plants as Biosensors for Monitoring the Acid Rain

Acid rain is the most serious environmental problem and has impact on agriculture, forestry, and human health (Shvetsova et al. 2002; Volkov et al. 2002). Chemical reactions involving aerosol particles in the atmosphere are derived from the interaction of

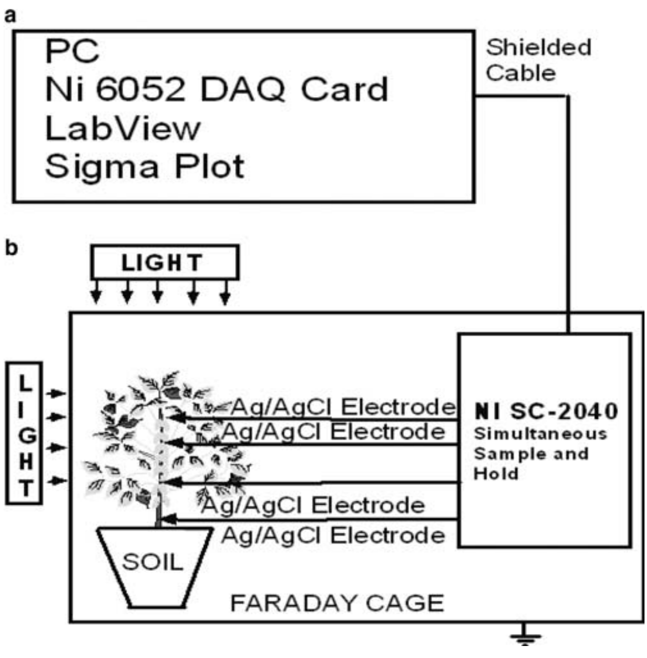


**Fig. 1** Experimental set-up for measuring electrical signals in green plants

gaseous species with the liquid water. These reactions are associated with aerosol particles and dissolved electrolytes. For example, the generation of HONO from nitrogen oxides takes place at the air/water interface of 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 exerts a variety of influences on the environment by greatly increasing the solubility of different compounds, thus directly or indirectly affecting many forms of life.

Acid rain has a pH below 5.6. Sulfuric acid and nitric acid are the two predominant acids in acid rain. Approximately, 70% of the acid content in acid rain is sulfuric acid, with nitric acid contributing to the rest 30%. Spraying the soybean plant with an aqueous solution of $H_2SO_4$ in the pH region from 5.0 to 5.6 does not induce action potentials. However, action potentials were generated in soybean either by spraying the leaves of the plant (1 mL) or deposition of 10 ∝L drops of aqueous solution of

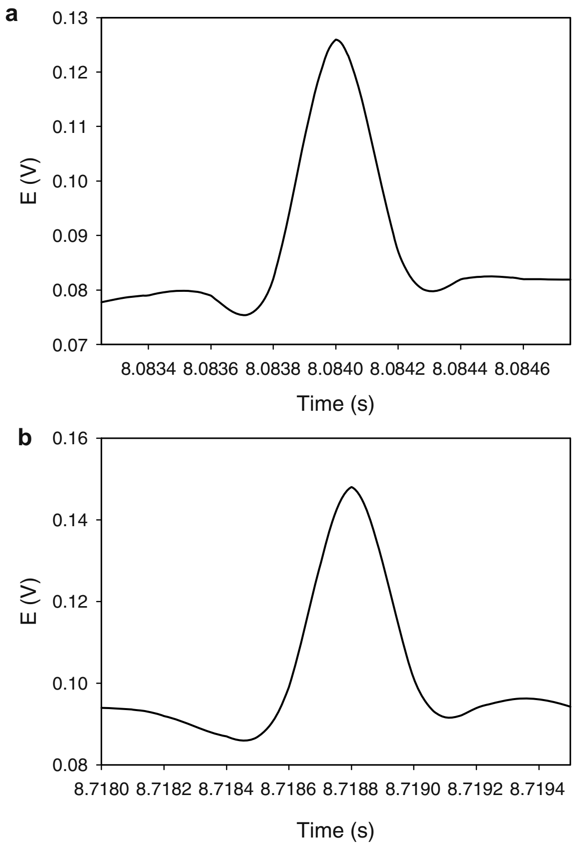


**Fig. 2** Potential difference between two Ag/AgCl electrodes in the stem of the soybean measured 7 (**a**) and 100 (**b**) h after adding 25 mL of 1 mM $Al(NO_3)_3$ to the soil. The soil pH after sterilization was 7. Distance between Ag/AgCl electrodes was 5 cm. Volume of soil was 0.5 L. The soil around the plant was treated with water every day. Room temperature was 22°C. Humidity was 45–50%

$H_2SO_4$ or $HNO_3$ in the pH region from 0 to 4.9 on leaves. The duration of single action potentials after spraying the plant with $HNO_3$ and $H_2SO_4$ was 0.2 and 0.02 s, respectively (Shvetsova et al. 2002; Volkov et al. 2002).

The evolution of plants occurred in the presence of many mineral nutrients even in high to potentially toxic concentrations. Of those nutrients, aluminum in particular is the most abundant metal (8%) in the Earth's crust. Aluminum concentrations in mineral soil solutions are usually well below 50 ∝M at pH values higher than 5.5, but rise sharply at lower pH. Subsequently, plant roots have been continuously exposed to potential toxic concentrations in the soil environment. On the other hand, aluminum is not considered an essential element, but many plants usually contain from 0.1 to 500 ppm and the addition of small amounts of aluminum to a nutrient solution may promote plant growth.

An important advantage of plants at mildly acidic, neutral, or alkaline pH values is that most phytotoxic forms of Al are relatively insoluble in soil. However, at pH 5 and below, Al may accumulate to toxic concentrations that prevent root growth and plant functions. Thus aluminum compounds become more soluble in acidified soils. In accordance with soil science, soil acts as a buffering system when the pH of soil slowly increases to a neutral value after soil acidification due to reaction of protons with metal oxides such as Al which is normally insoluble at a neutral pH. Therefore, we continued our study on the effects of Al salts on electrical signaling in soybean. Figure 2 show that Al ions induce fast action potentials in soybean.

## 3 Electrical Signals Induced by Pesticides

Pesticides 2, 3, 4, 5, 6-pentachlorophenol (PCP), 2, 4-Dinitrophenol (DNP), carbonylcyanide m-chlorophenylhydrazone (CCCP), and carbonylcyanide-4-trifluoromethoxyphenylhydrazone (FCCP) act as insecticides and fungicides. PCP is the primary source of dioxins found in the environment. This pollutant is a defoliant and herbicide. PCP is utilized in termite control, wood preservation, seed treatment, and snail control. The pesticide DNP is used to manufacture dye and wood preservative. DNP is often found in pesticide runoff water. The electrochemical effects of CCCP, PCP, DNP, and FCCP have been evaluated on soybean plants (Mwesigwa et al. 2000; Shvetsova et al. 2001; Volkov et al. 2000, 2001; Labady et al. 2002; Mwesigwa and Volkov 2001a, b).

CCCP decreased the variation potentials of soybean from 80–90 mV to 0 mV after 20 h. CCCP induced fast action potentials in soybean with amplitude of 60 mV (Labady et al. 2002). The maximum speed of propagation was 25 m/s. Exudation is a manifestation of the positive root pressure in the xylem. After treatment with CCCP, the exudation from cut stems of the soybean remains the same. Therefore, the addition of CCCP did not cause a change in the pressure, although it may influence the zeta potential due to depolarization (Labady et al. 2002).

The addition of aqueous solution of PCP also causes the variation potential in soybeans to stabilize at 0 mV after 48 h. Rapid action potentials are induced. These action potentials last for 2 ms, and have amplitudes of 60 mV. The speed of propagation is 12 m/s; after 48 h, the speed increased to 30 m/s.

DNP induces fast action potentials and decreases the variation potential to zero in soybeans (Mwesigwa et al. 2000). The addition of aqueous DNP to the soil induces fast action potentials in soybeans. After treatment with an aqueous solution of DNP, the variation potential, measured between two Ag/AgCl electrodes in a stem of soybean, slowly decreases from 80–90 mV (negative in a root, positive on the top of the soybean) to 0 during a 48-h time frame. The duration of single action potentials, 24 h after treatment by DNP, varies from 3 to 0.02 s. The amplitude of action potentials is about 60 mV. The maximum speed of action potential propagation is 1 m/s. After 2 days, the variation potential stabilized at 0. Fast action potentials were generated in a soybean, with amplitude of about 60 mV, 0.02 s duration time, and a speed of 2 m/s. Fromm and Spanswick studied the inhibiting effects of DNP on the excitability of willow by recording the resting potential in the phloem cells (Fromm and Spanswick 1993). In willow, $10^{-4}$ M DNP rapidly depolarized the membrane potential by about 50 mV.

The FCCP also induced action potentials in soybean (Shvetsova et al. 2001). The maximum speed of these action potentials within 20 h after the treatment was 10 m/s. After 100 h, the action potentials were still being produced. The amplitude of 60 mV remained constant. The duration was 0.3 ms, and the speed of propagation was 40 m/s (Shvetsova et al. 2001).

Constant release of hazardous metal pollutants into the environment has become a global problem. Contamination of soil, ground and surface waters with such pollutants can negatively affect all levels of an ecosystem, and thus, the clean-up of contaminated soils and waters is one of the most important challenges the environmental scientists face today.

## 4 Insect-Induced Electrochemical Signals in Potato Plants

Volkov and Haack were the first to afford a unique opportunity to investigate the role of electrical signals induced by insects in long-distance communication in plants (Haack and Volkov 1995a, b).

Action and resting potentials were measured in potato plants (*Solanum tuberosum* L.) in the presence of leaf-feeding larvae of the Colorado potato beetle (*Leptinotarsa decemlineata* (Say); Coleoptera: Chrysomelidae). When the larvae were allowed to consume upper leaves of the potato plants, after 6–10 h, action potentials with amplitudes of 40 ± 10 mV were recorded every 2 ± 0.5 h during a 2-day test period. The resting potential decreased from 30 mV to a steady state level of 0 ± 5 mV. The action potential induced by the Colorado potato beetle in potato plants propagates slowly and hence, the speed of propagation can be measured with two Ag/AgCl electrodes. The action potential propagates from plant leaves with Colorado potato beetles down the stem, and to the potato tuber (Volkov and Haack 1995). The speed of propagation of the action potential does not depend on the location of a working electrode in the stem of the plant or tuber, or the distance between the working and reference electrodes (Volkov and Haack 1995).

## 5 Molecular Recognition of the Direction of Light by Green Plants

Phototropism is one of the best-known plant tropic responses. A positive phototropic response is characterized by a bending or turning toward the source of light. When plants bend or turn away from the source of light, the phototropic response is considered negative. A phototropic response is a sequence of the four following processes: reception of the directional light signal, signal transduction, transformation of the signal to a physiological response, and the production of directional growth response. Phototropin is a blue light (360–500 nm) flavoprotein photoreceptor responsible for phototropism and chloroplast orientation.

Inside the Faraday cage the soybean plant was irradiated in the direction A (Fig. 1) with white light for 2 days with a 12:12 h light: dark photoperiod prior to the conduction of experiments. Action potentials are not generated when the lights are turned off and on. Changing the direction of irradiation from direction A to direction B generates action potentials in soybean approximately after 1–2 min. These action potentials depend on the wavelength of irradiation light. Irradiation at wavelengths 400–500 nm induces fast action potentials in soybean with duration time of about 0.3 ms; conversely, the irradiation of soybean in the direction B at wavelengths between 500 and 630 nm fails to generate action potentials. Irradiation between 500 and 700 nm does not induce phototropism. Irradiation of soybean by blue light induces positive phototropism (Volkov et al. 2004).

## 6 Conclusion and Future Perspective

Green plants interfaced with a computer through data acquisition systems can be used as fast biosensors for monitoring the environment, detecting effects of pollutants, pesticides, defoliants, predicting and monitoring climate changes, and in agriculture, directing and fast controlling of conditions influencing the harvest.

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