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BRIEF COMMUNICATION

A low-noise multi-channel device for the monitoring of systemic electrical signal propagation in plants

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Abstract

Long-distance electrical signals generated in locally stimulated plants are linked with systemic physiological responses. The propagation of electrical signal through a plant can be measured by multiple electrodes attached to different sites of a plant body. As this signal has to be measured with the sensitivity of tens of microvolts, it can be easily disturbed by power-line hums or external electromagnetic fields. These disturbances can mimic the action potentials generated by a plant. In this work, we present a brief summary of various experimental approaches to the measurement of surface electrical potential (SEP) on a plant and a description of our multi-channel device for the SEP measurement. The main advantages of our measuring system are galvanic separation of the measuring unit, resulting in the elimination of power-line disturbances, and simple and stable contact of Ag/AgCl-peletted electrodes with the plant surface, facilitated by an ordinary gel used in human electrocardiography. These improvements enabled us to detect unperturbed variation (slow) and action (fast) potentials on a plant, as demonstrated by the four-electrode measurement of the electrical signal propagation in a locally wounded tomato plant.

Additional key words: action potential, local wounding, systemic response, variation potential.

The first evidence of the generation and propagation of electrical signal in a plant body was given in the 19th century by Burdon-Sanderson (1873). He observed changes in the surface electrical potential of trap lobes of a carnivorous plant *Dionaea muscipula* during trap closure following the irritation of lobes sensitive protuberances. Nowadays, it is well known that also other plants generate electrical signals when they are locally stimulated or wounded (for review see Thain and Wildon 1992, Malone 1996, Davies 2006). These signals propagate to distant parts of the plant and are linked to various physiological responses, such as changes in respiration, photosynthesis or phloem translocation (for recent reviews see Fromm and Lautner 2007, Hlaváčková 2009).

Two types of long-distance electrical signals can be distinguished - action potentials (APs) and slow wave (variation) potentials (VPs). Although both signals reflect the changes in plasma membrane potential, they differ in their origin and characteristics. APs in plants are self-propagating electrical signals mediated through voltage-gated channels and transmitted preferentially in phloem (Fromm and Bauer 1994, Rhodes *et al.* 1996, Dziubinska 2003, Davies 2006). APs appear after non-damaging stimuli (*e.g.* electrical stimuli, light/dark transitions, cooling or pollination) and are characterized by a steady amplitude, signal shape and propagation rate (Stanković *et al.* 1998, Dziubinska *et al.* 2001). APs are usually observed as spikes (Davies *et al.* 1991, Malone 1996, Stanković *et al.* 1998, Krol *et al.* 2006) and fulfill classical electrophysiological laws: all-or-none law, strength-duration relation and the existence of refractory periods (Zawadski *et al.* 1991).

On the contrary to APs, VPs appear to be a local consequence of long-distance signals of different nature (hydraulic, chemical or both). These long-distance signals are rapidly transmitted in xylem throughout the whole

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Abbreviations: AP - action potential, ECG - electrocardiography, EEP - extracellular electrical potential, IEP - intracellular electrical potential, SEP - surface electrical potential, VP - variation potential.

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plant and elicit local electrical changes along their pathway (Davies 2004, 2006). The origin of VPs is still a matter of debate (for recent review see Hlaváčková 2009). VPs have been observed in a variety of plant species in response to strong damaging stimuli such as wounding, *e.g.*, heat treatment or crushing (Van Sambeek and Pickard 1976, Malone and Stanković 1991, Stanković and Davies 1998), or in response to localized increase in xylem pressure (Malone and Stanković 1991, Stahlberg and Cosgrove 1997). The amplitude and propagation rate of VPs decrease with increasing distance from the wounded site (Davies *et al.* 1997, Mancuso 1999), thus, on the contrary to APs, VPs do not follow the all-or-none rule. VPs are usually observed as broad waves rather than narrow spikes (Davies *et al.* 1991, Malone 1996, Stanković *et al.* 1998).

The appearance and propagation of electrical signals is detected *via* the changes in the extracellular or intracellular electrical potential (EEP or IEP) in different parts of a plant. IEP is measured by microelectrodes impaled into a cell using a micromanipulator (see *e.g.* Shabala 2006) and reflects the imbalances in ion activities inside one particular cell. On the contrary, EEP reflects these imbalances in the apoplast. EEP can be measured either invasively, by piercing of thin electrode into the plant tissue, or non-invasively, by attaching the electrode to a plant surface. When using the invasive approach, a long stabilization of the electrode-plant contact is required. Nevertheless, once this contact is established, it provides a stable ion connection for

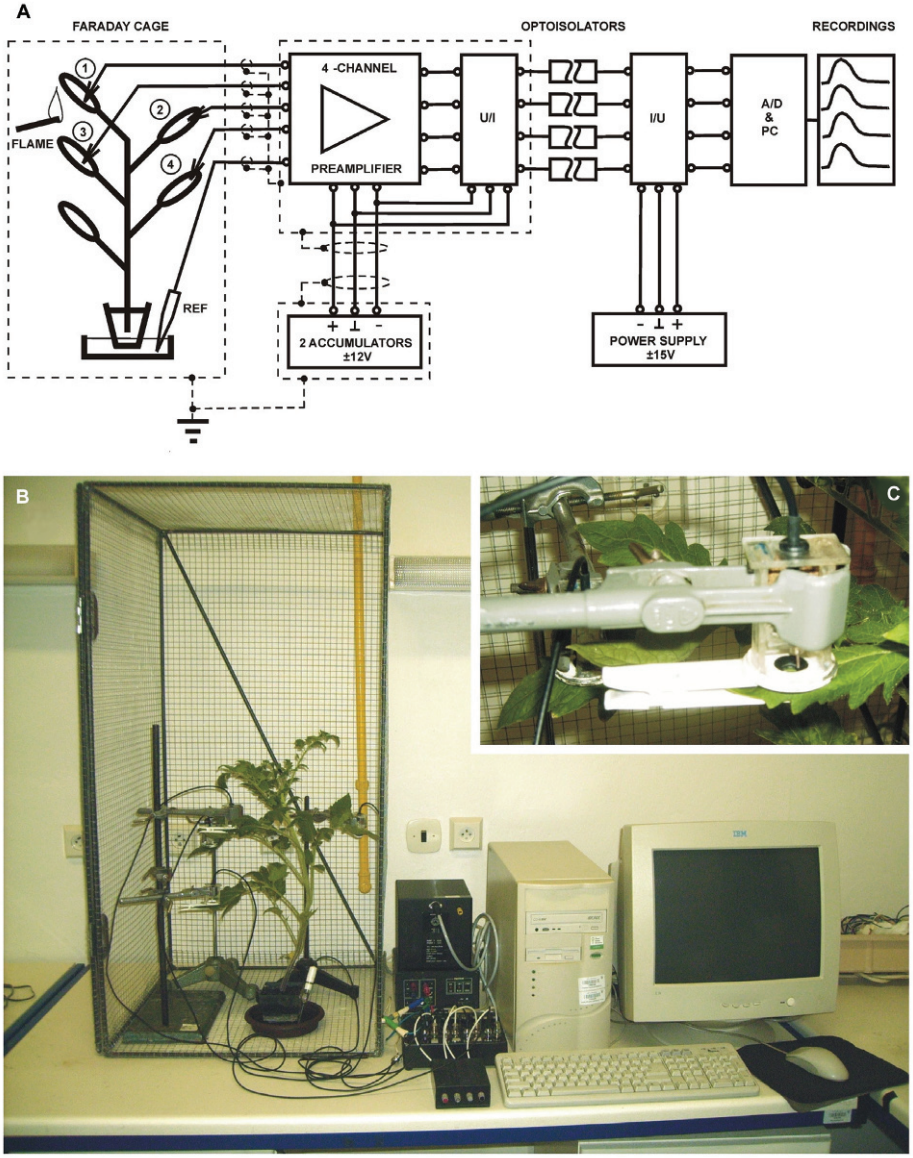

Fig. 1. *A:* Electrical scheme of our four-channel device for the measurement of surface electrical potential changes on a plant placed in a Faraday cage. *Dashed lines* represent grounded shielding circuits. *B:* Our device for the measurement of surface electrical potential changes on a plant. *C:* The surface electrode with a clipped leaf in a holder. For details see the text.

several days (Zawadski *et al.* 1995). However, it should be kept in mind that any piercing of the plant tissue causes damage and therefore, from this point of view, the non-invasive approach is better. The surface electrodes are more regardful of a plant, but their disadvantage is that the electrode-plant contact is stable only for several hours (see below). Although both methods of EEP measurement often give similar results (*e.g.* Mancuso 1999), the non-invasive surface method should be generally preferred. According to our knowledge, so far no survey has been published dealing with the approaches used in the measurement of the surface electrical potential on a plant. Therefore, in the following we present a brief summary of the methods reported in the literature.

Generally, a surface electrode used for the measurement of extracellular electrical potential consists of a non-polarizable wire electrode (Ag/AgCl-pelleted or calomel) inserted into a salt-bridge with a well-defined concentration of chloride. A stable conductive contact between the bridge and the plant surface is ensured by a wick. Each research group studying electrical signals on plants aims at the construction of the electrode, which would fulfill the requirement of the maximum stability of its electrical response and of its minimal effect on the plant surface. While a high concentration of chloride is necessary to obtain high stability, it can significantly affect and damage the plant surface. Therefore, a suitable compromise has to be achieved between these two requirements. Recent constructions of surface electrodes (*e.g.* Shabala 1997, Mancuso 1999, Živanović *et al.* 2001, Stahlberg *et al.* 2001, 2005) have been widely inspired by the electrodes designed by Van Sambeek and Pickard (1976). These authors made the electrode from a small glass pipette. They got the agar-coated cotton thread through its tip, filled the pipette with 0.1 M KCl (gelled with 1 % agar) and impaled the Ag/AgCl-pelleted electrode into the pipette. Nowadays, some researchers fill the electrode with an isotonic physiological buffer containing KCl instead of a pure KCl solution (Favre *et al.* 1991, Shabala 1997), which is more regardful of the plant surface.

The maintenance of a stable conductivity of the interface between the salt bridge and the plant surface is a stumbling block to the proper measurement of the electrical potential of a plant. The partial desiccation of the wick, changing the ion concentration inside the wick, is a serious problem especially for the long-term measurements. Some authors have overcome this problem by moistening the wick from time to time during the measurement by the addition of a drop of KCl solution (*e.g.* Van Sambeek and Pickard 1976, Roblin 1985, Zawadski *et al.* 1991). However, a proper construction of the electrode allows performing even the long-term SEP measurements without the need of external intervention. Malone and Stanković (1991) reported that by using silicone rubber tubing on the end of a salt bridge they obtained a good surface contact for over 12 h without the need of rewetting. The problem of wick desiccation is also avoided by the use of a so-called bath electrode, *i.e.* an Ag/AgCl-pelleted electrode impaled into the agar block with KCl, which is plunged into a saline bath surrounding the plant surface (*e.g.* leaf petioles) (Rhodes *et al.* 1996). A very convenient solution of the problem of the wick dessication has been offered by Mancuso (1999), who simply connected the Ag/AgCl-pelleted electrode to the plant surface with the help of a conductive gel widely used in electrocardiography (ECG).

The electrical potential of a particular part of a plant with the attached surface electrode (measuring electrode) is measured against some reference electrode in a complete circuit. The reference electrode is usually of the same type as the measuring one and is either attached to another part of a plant (detection of relative changes) or impaled in wet soil and grounded (detection of absolute changes). In the latter case the reference electrode should have a larger salt bridge (see *e.g.* Van Sambeek and Pickard 1976), because otherwise the leakage of ions from the salt bridge to soil can significantly change the ion concentration inside the bridge. After the attachment of the surface electrode to the plant, the contact has to be stabilized for some time. Usually, the electrolytic equilibrium between the electrode wick and leaf tissue is established within 1-2 h (*e.g.* Malone and Stanković 1991, Volkov and Brown 1998). This stabilization is accompanied by a drift of the measured potential difference between the measuring and the reference electrode.

The maximum measured amplitudes of SEP in plants are within the range of tens of milivolts and therefore the measuring devices must have high input resistance and their resolution should be at least several tens of microvolts. The input resistance of the devices currently used for these measurements is usually in the range of 10^{12} - 10^{15} Ω. When the propagation of an electrical signal through a plant is to be detected, the signals from multiple electrodes in different parts of a plant body must be detected simultaneously. As multi-channel sensitive voltmeters are not readily available, researchers often construct their own devices for this purpose. The output signal from the voltmeter is digitalized by an analog/digital (A/D) converter that is today represented by a data acquisition card inside a computer. The recordings are stored and handled by computer software.

As bioelectric signals are weak, their detection is sensitive to the changes in the external electromagnetic field. Any plugged electrical device around an investigated plant or swinging mains can induce electrical potential changes on a plant and distort the proper measurement of electrical potentials on a plant. Therefore, a plant and sometimes also the electrical devices connected to a plant are placed inside a Faraday cage that is grounded together with the reference electrode. Naturally, the electrical potential of the ground is considered to be constant.

We constructed a four-channel sensitive apparatus for the detection of SEP changes on plants. This device has

been already used for the two-electrode measurement of electrical signal propagation through tobacco plants after local burning (Hlaváčková *et al.* 2006). The apparatus consists of an amplifier, a voltage/current converter, four optoisolators, a current/voltage converter and an A/D converter inside a computer (Fig. 1*A*,*B*). A key component of the amplifier is a differentiating preamplifier with high input resistance. We chose INA 116 (*Burn-Brown*, Tucson, USA) with a declared input resistance of 10^{15} Ω. Taking into account the line wires to the electrodes and input connectors, the resulting input resistance of our amplifier was about 10^{13} - 10^{14} Ω. The gain of the whole amplifier was adjustable from 100 to 1000. In order to avoid external electrical interferences caused by possible ground loops and by the penetration of disturbing electrical signals from the electrical mains, we decided to use an independent power supply to the amplifier (two 12 V Pb batteries) and to galvanically separate the amplifier and the A/D converter by the optoisolators. This construction led to a significant reduction of the noise (see also below). As our A/D converter had analog inputs, we connected the optoisolators in front of the A/D converter. Before entering the optoisolators, the voltaic signal need to be converted to the changes of current, therefore a voltage/current converter had to be inserted. A reverse converter was inserted behind the optoisolators (Fig. 1*A*). The A/D converter is represented by a commercial 12-bit data acquisition card PCA-7228AL (*Tedia*, Pilsen, Czech Republic) plugged into a PC. Electrical signals were sampled and stored using the *ScopeWin* software (*Tedia*).

SEP changes on a plant were detected with silver wire electrodes (1 mm diameter) peletted with AgCl (*Scanlab Systems*, Prague, Czech Republic). The electrodes were simply connected to the adaxial surface of a leaf by a conductive gel commonly used in ECG (*VUP*, Prievidza, Slovak Republic), as proposed by Mancuso (1999). To prevent the changes in the position the electrode on the leaf surface during the measurement, the measured leaf was placed into a leaf clip (a standard clip to a *PEA* chlorophyll fluorimeter, *Hansatech*, Norfolk, UK) with an electrode holder (Fig. 1*C*). Moreover, the clip was fixed by another holder clamped to a stable stand. The hole in the center of the upper part of the clip (3 mm diameter) defined the contact area between the gel and the leaf surface. The reference electrode was of the same type as the measuring one and was inserted into a thin glass tube tipped by a glass frit. The tube filled with 0.3 M KCl represented a salt bridge of the reference electrode. The electrode tip was immersed into the basin with water, which was placed below the pot with the measured plant (Fig. 1*B*). Before the measurement of SEP changes, the measuring and reference electrodes were immersed into 0.3 M KCl for 1 h.

In order to reduce the surface potential changes induced by external electromagnetic fields, the plant was placed inside a Faraday cage that was grounded together with a conductive screen covering the amplifier (see Figs. 1*A*,*B*). After the attachment of the measuring electrodes to the plant it is necessary to wait for 1 h to attain steady state levels of electrical potentials. During the measurement, a sampling rate of 1 point per 30 ms was used. The level of input noise of the device did not exceed 20 μV (peak-to-peak value). When the detection unit of the device was not galvanically separated, *i.e.* the optoisolators were omitted and the batteries were replaced by a power supply connected with electrical mains, the input noise increased by more than four times.

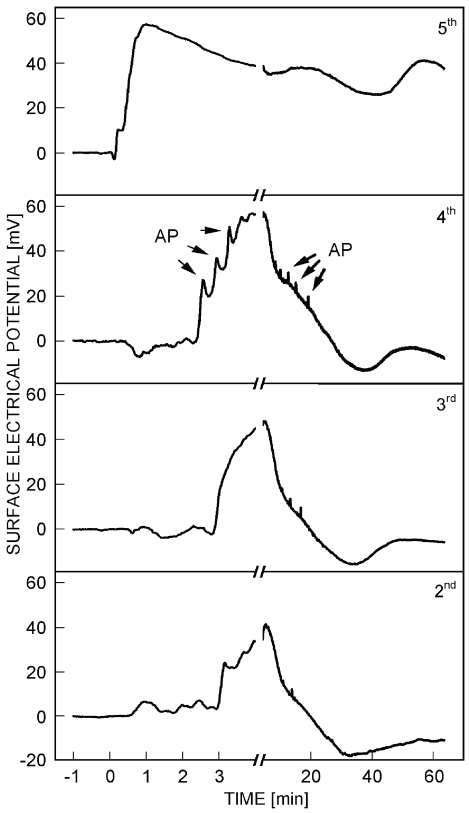

Fig. 2. Changes in the surface electrical potential on selected leaves (indicated) of a tomato plant after local burning of the 5th leaf (counted from the base of the stem). Zero time represents the beginning of the burning. AP denotes action potential. For other details see the text. The plant was cultivated in compost soil in pots for 6 weeks under growth chamber conditions (23 °C, 16-h photoperiod, PAR 130 μmol photons m^{-2} s^{-1}, RH 45 %).

In this case the humming noise dominated, as can be deduced from the appearance of a characteristic frequency (not shown). Such hums originate from the ground loops that are formed when the grounding of the individual electrical components connected in the device is realized differently. Therefore, the humming noise has to be taken into account when measuring the electrical potential using home-made devices. A galvanic separation of the detection unit that we used in our device overcomes these possible problems and ensures that the input noise of the device reflects just the unavoidable electrical noise.

The use of the ECG gel as a conductive medium

between the Ag/AgCl electrode and a plant surface is very advantageous. The gel ensures a stable conductive contact and enables continuous measurement of SEP for more than 12 h without any intervention (not shown). In general, the length of this period depends on the wettability of the plant surface (this differs from tissue to tissue and plant species to species), on ambient conditions (temperature, humidity) and on gel volume (determining the rate of desiccation). On the other hand, when the traditional thread electrode is used for the SEP measurement, it is necessary to overcome the problems with finding a balance between the desiccation and excessive moistening of the thread even during the short-term measurements. A proper function of the thread electrode depends on a combination of many variables. According to our practice, these include the characteristics of the thread (thickness, length below the electrode tip), the compression of the thread in the electrode tip, gelation of KCl in the salt bridge, ambient conditions (humidity, temperature) and wettability of the plant surface. Therefore, in our opinion, the Ag/AgCl electrode with ECG gel represents a better alternative to the more traditional thread electrode.

We used our low-noise multi-channel device for the measurement of SEP changes on a tomato plant after local burning. This treatment is often used to study the propagation of electrical signal through a plant (*e.g.* Hlaváčková *et al.* 2006). A top leaflet of the youngest fully developed leaf (the 5th, counted from the base of the stem) was burned (12 s) with a flame from a burning wooden stick. The measuring electrodes were attached to the adaxial side of the burned leaf and to three other leaves localized below the burned one. Our measurement demonstrated that the electrical signal from the burned leaf propagated to more distant leaves (Fig. 2). The amplitude of the signal decreased with increasing distance from the site of burning, which is a typical feature of VP (Davies *et al.* 1997, Mancuso 1999). APs were observed as several seconds long spikes on the VP wave, mainly on the 4th leaf. These APs were generated systemically, as no APs were detected on the burned leaf (Fig. 2).

In summary, in this paper we have presented an overview of the technical approaches to the measurement of SEP changes on plants. At the same time, we introduced our approach to the SEP measurement, involving the use of the ECG gel and a home-made device with electrically separated detection unit. Our device enables the measurement of slow VP as well as fast AP signals without distortions caused by power-line disturbances and by the instability of the contact between the Ag/AgCl-peletted electrodes and the plant surface.

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