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Electrical signal propagation within and between tomato plants

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ABSTRACT

According to literature, electrostimulation of plants can induce plant movement, activation of ion channels, ion transport, gene expression, enzymatic system activation, electrical signaling, plant-cell damage, enhanced wound healing, and can also influence plant growth. Many plants can communicate above ground and underground between adjacent plants. Electrostimulation by square pulses induces passive electrotonic potentials propagating within and between tomato plants. The amplitude and sign of electrotonic potentials, in both the electrostimulated and neighboring tomato plants depends on the amplitude, rise and fall of the applied voltage. Electrostimulation by the pulse train, sinusoidal and triangular saw-shape voltage profile shows the existence of electrical differentiators and refractory periods in cell-to-cell electrical coupling in tomato plants. Electrical networks within one tomato plant can communicate underground with electrical circuits in another tomato plant. Here, we present the mathematical model of electrotonic potentials transmission between tomato plants which is supported by the experimental data. The information gained from this mathematical model and analytical study can be used not only to elucidate the effects of electrostimulation on higher plants, but also to observe and predict the intercellular and intracellular communication in the form of electrical signals within the electrical networks within and between tomato plants.

1. Introduction

1.1. Signaling between plants

Plants can communicate with each other using different pathways above ground such as volatile organic compounds (VOC) emission and sensing [1–3]; electromagnetic interactions [4], and acoustic or sound vibrations [5, 6]. Some plants can take up volatiles through the stomata and by adsorption on the leaf's surface [7]. There are many publications about possible underground communication pathways between plants, bushes and trees. These communication pathways include: root grafting, the plants' rhizosphere (root ball), and mycorrhizal networks in the soil [8–15]. There are different pathways for communication within and between plants such as cell-to-cell, root-to-root, shoot-to-shoot, and between roots and shoots [17, 18, 20–31]. Soil is a good electrical conductor between neighboring plants [32]. The soils electrical resistivity depends on moisture, ion exchange capacity, porosity, pore size distribution, solute concentration, temperature, and chemical content. The resistivity ρ (Ωm) is defined as follows:

$$\rho = R \times (S/L)$$

with R being the electrical resistance (Ω), L the length of the cylinder (m) and S its cross-sectional area (m^2). The resistivity usually ranges between 1 Ωm for saline soil and 100 kΩm for dry soil overlaying crystalline rocks [32].

The experimental and analytical study of electrotonic signal conduction during the day between *Aloe vera* plants using underground pathways in soil was published recently [20]. *Aloe vera* L. is a xerophyte in the *Liliaceae* family with crassulacean acid metabolism (CAM). Stomata in *Aloe vera* open at night and close during the day.

1.2. Electrostimulation of tomato plants

The electrostimulation of plants can induce plant movement, activation of ion channels, ion transport, gene expression, enzymatic systems activation, action and electrotonic potentials, plant-cell damage, enhanced wound healing, and influence plant growth [22, 23, 27, 33–41]. Herde et al. [33, 34] found that electrical current application (10 V, 30 s) activates *pin2* gene expression in tomato plants and increases endogenous levels of abscisic acid. Stanković and Davies [35] demonstrated that the electrolysis of tomato plants by 9 V for 3–4 s

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Abbreviations: *C*, Capacitance; *R*, Resistance; *TEACl*, Tetraethylammonium chloride; *V*, Voltage; V_{in}, Input voltage; *VOC*, Volatile organic compounds; λ, The length of electrotonic potential.

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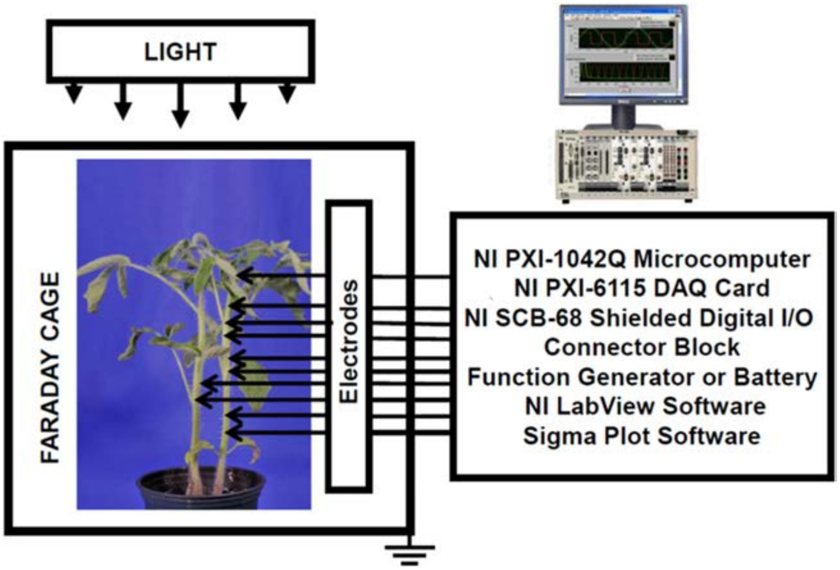


Scheme 1. Diagram of experimental setup. Two Pt-electrodes were used for electrostimulation of a plant from a function generator or 1.5 V D-battery and 8 Ag/AgCl electrodes were used for measurements of plant electrical responses.

occasionally induces electrical signals with amplitude of 40 mV and a speed of 3.5–4.5 mm/s, it also elicits systemic *pin2* gene expression. Transmission of these electrical signals in response to electrostimulation was found in only 20% of tomato plants [35]. Stanković and Davies [35] found after 9 V electrostimulation that “5-fold or greater increase in *pin2* mRNA levels occurs within 1 hour”. Authors did not analyze the threshold level of stimulation voltage and the possible dependence of electrical responses on amplitude and polarity of applied voltage.

The goal of this work is to find if fast underground electrical signal conduction exists between neighboring tomato plants. Tomato plants (*Lycopersicon esculentum* Mill. cv Cosmonaut Volkov) are dicots and their stomata are open during the day and closed at night.

2. Materials and methods

2.1. Plants

Fifty tomato (*Lycopersicon esculentum* Mill. cv Cosmonaut Volkov) plants were grown in plastic pots with sterilized potting soil in a plant growth chamber (*Environmental Corporation*). Plants were exposed to a 12:12 h light/ dark photoperiod at 22° C. The average air humidity was 40% and the irradiance was 250–300 μmol photons m^{-2} s^{-1} PAR at plant level. All measurements were performed on 21-to 28-day-old plants. The soil around the plants was treated with water every day. All experiments were performed on healthy adult specimens. To avoid root-to-root connections or mycorrhizal networks between plants, we moved tomato plants from their pots to a new large pot and separate soil between plants by a non-penetrable barrier before experiments.

2.2. Electrodes for extracellular measurements

All measurements were conducted in the laboratory at 21 °C inside a Faraday cage mounted on a vibration-stabilized table. Teflon coated silver wires (*A-M Systems, Inc., Sequim, WA, USA*) with a diameter of 0.2 mm were used for preparation of non-polarizable electrodes. Reversible Ag/AgCl electrodes were prepared in the dark by electrodeposition of AgCl on 5 mm long silver wire tips without Teflon coating in a 0.1 M KCl aqueous solution. The anode was a high-purity silver wire and the cathode was a platinum plate. Electrical current in the electrolytic cell was limited to 1 mA/cm^2 of the anode's surface. Stabilization of electrodes was accomplished by placing two Ag/AgCl electrodes in a 0.1 M KCl solution for 24 h and connecting a short circuit between them. The response time of Ag/AgCl electrodes was less than 0.1 μs. Identical Ag/AgCl electrodes were used as working and reference electrodes for measurements of potential differences in the plants.

Platinum electrodes were used for tomato plant electrostimulation and prepared from Teflon coated platinum wires (99.99% purity; *A-M Systems, Inc., Sequim, WA, USA*) with a diameter of 0.127 mm. Platinum electrodes are stable over a wide range of potentials, in acidic and alkaline solutions, and in the presence of redox components [42]. We allowed the plants to rest for 2 h after electrode insertion. Electrodes were placed along the vascular bundles of a stem and also in soil in some control experiments.

2.3. Chemicals

Daconil containing 0.087% of chlorothalonil (GardenTech, Colorado Springs, USA) known also as 2,4,5,6-tetrachloroisophthalonitrile was used as an antifungal treatment of soil in control experiments. It may also be used to kill mildew, bacteria, algae, and insects.

2.4. Data acquisition

Experimental setup is shown in Scheme 1. High speed data acquisition was performed using microcomputers with simultaneous multifunction I/O plug-in data acquisition board NI-PXI-6115 (*National Instruments, Austin, TX, USA*) interfaced through a NI SCB-68 shielded connector block to Ag/AgCl electrodes. The system integrates standard low-pass anti-aliasing filters at one half of the sampling frequency.

2.5. Electrostimulation

We used two methods of plant electrostimulation: the function generator or the 1.5 V D-batteries. The function generator FG300 (*Yokagawa, Japan*) was interfaced to the NI-PXI-1042Q microcomputer (*National Instruments, Austin, TX, USA*) and used for the electrostimulation of plants (Scheme 1). The function generator gives many options for the electrostimulation, such as shape, duration, and frequency of stimulation.

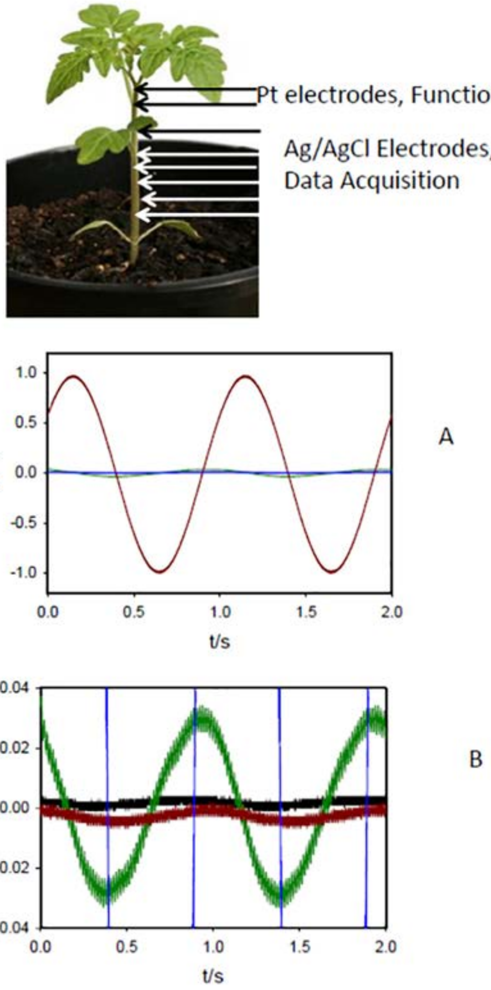


Fig. 1. Potential difference V between Ag/AgCl electrodes inserted to the tomato stem along vascular bundles was induced by ±1V sinusoidal waive from function generator, which was connected to Pt-electrodes above Ag/AgCl electrodes in the same stem. Distance between Pt electrodes was 0.8 cm. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s. Panel B was extracted from Panel A to show high resolution responses.

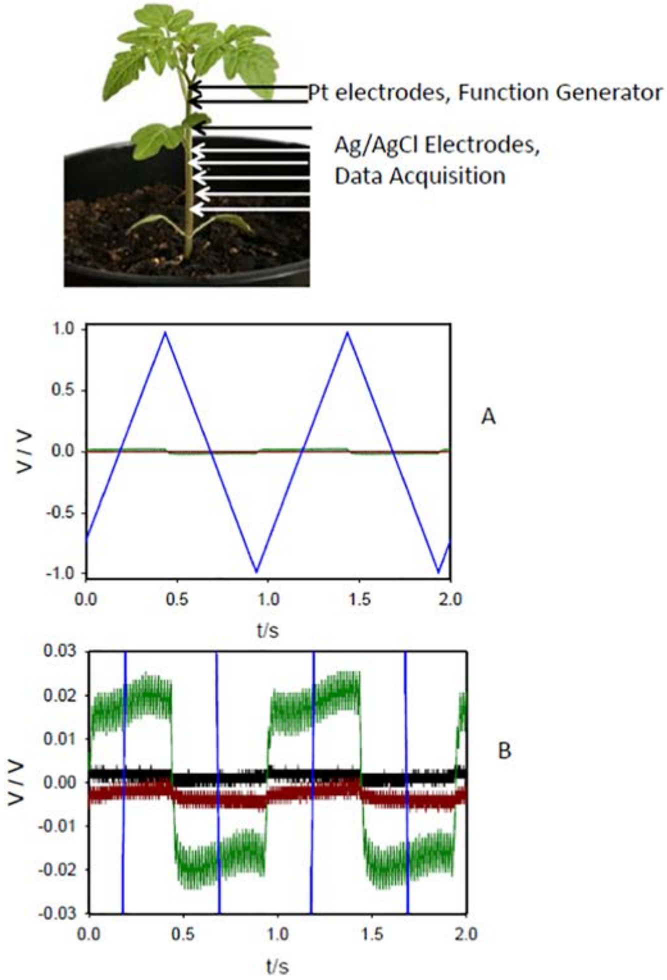


Fig. 2. Potential difference V between Ag/AgCl electrodes inserted to the tomato stem along vascular bundles was induced by ±1V triangular wave from function generator, which was connected to Pt-electrodes above Ag/AgCl electrodes in the same stem. Fig. B was extracted from Fig. A to show high resolution electrical responses. Distance between Pt electrodes was 0.8 cm. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s.

2.6. Images

A photo camera Nikon D3x with AF-S Micro Nikkor 105 mm 1:2.8 G ED VR lens was used for the photography of plants.

2.7. Statistics

All experimental results were reproduced at least 25 times using different tomato plants. Software SigmaPlot 12 (*Systat Software, Inc., San Jose, CA, USA*) was used for statistical analysis of experimental data.

3. Results

3.1. Electrostimulation of electrotonic potential propagation in a stem of tomato plant

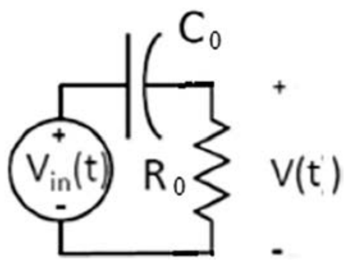
Following insertion of the electrodes, the plants were allowed to rest until a stable potential difference was obtained between the electrodes. During the day, electrical potential differences between the Ag/AgCl electrodes in the plants were stable in the absence of stresses or external stimuli. The mechanism of generation of these potential differences in leaves, roots and a stem of plants is unknown.

Electrostimulation of a tomato plant by a sinusoidal (Fig. 1), triangular (Fig. 2), or square pulse trains (Fig. 3A) from a function generator induced electrical signals along the stem that was registered by Ag/AgCl electrodes. The reaction of the plant strongly depends on the shape of electrical stimulus. Fig. 1 shows the differences in response to sinusoidal stimuli. The panels show both the stimulation and response of tomato plant stem.

If the stimulus changes very fast (Fig. 3A), the plant's response is very significant and nonlinear: the square impulses initiate electrical responses with shapes completely different from the stimulating voltage, which look like spikes or “action” potentials. Any stimulation that is not instantaneous, such as a sinusoidal or triangular function, does not induce electrical spikes as responses in the case of square pulses, but the responses are different from stimuli (Figs. 1, 2).

Sinusoidal waves transform to cosine waves with a phase shift of 90^{o} (Fig. 1) and triangular waves transforms to steps (Fig. 2). This phenomenon shows that electrical networks in plant tissue have electrical differentiators. A real differentiator that describes cell-to-cell electrical coupling can be presented as an equivalent electrical circuit in Scheme 2, which output is directly proportional to the rate of change of the difference between the input $V_{in}(t)$ and the output $V(t)$ voltages:

$$V(t) = C_0 R_0 \frac{d}{dt}(V_{in}(t) - V(t)) \qquad (1)$$



Scheme 2. Equivalent electrical circuit of a differentiator.

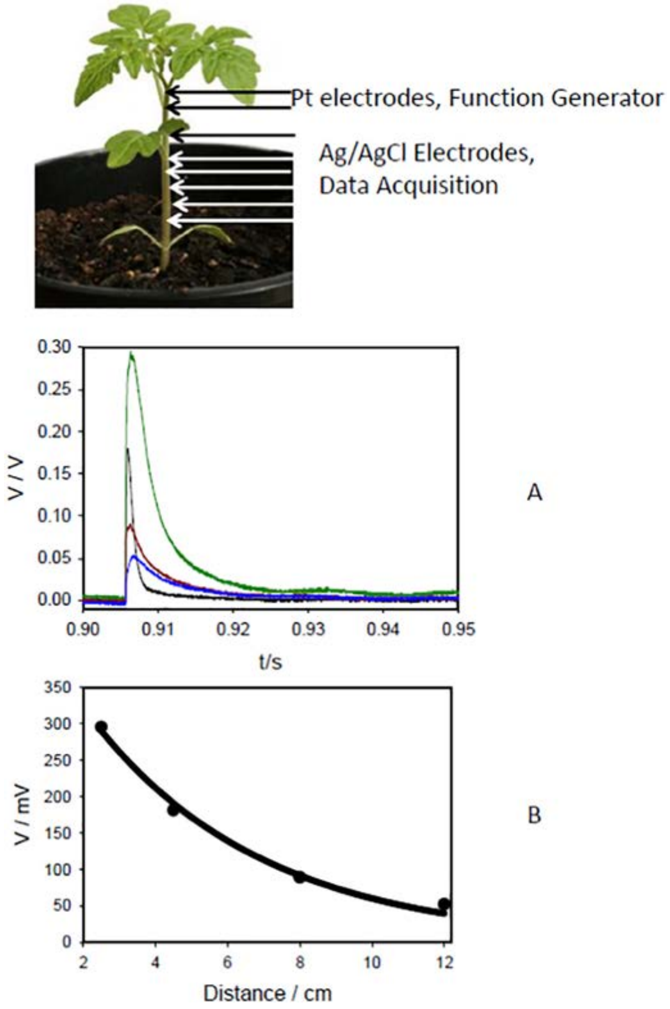


Fig. 3. Dependencies of electrotonic potentials on time (A) and distance between polarizing Pt electrode and working Ag/AgCl electrodes (B). Potential difference *V* between Ag/AgCl electrodes inserted to the tomato stem along vascular bundles was induced by 1 V square pulse train from a function generator with 1 Hz frequency as in Fig. 2, which was connected to Pt-electrodes above Ag/AgCl electrodes in the same stem. Distance between Pt electrodes was 0.8 cm. Distance between upper polarizing Pt-electrode and working Ag/AgCl electrodes is shown in panel B. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s.

where V_{in} is the input voltage, V is the output voltage, R_0 is the resistance and C_0 is the capacitance of the equivalent circuit.

If

$$\frac{dV(t)}{dt} << \frac{dV_{in}(t)}{dt} \tag{2}$$

then.

$$V(t) = C_0 R_0 \frac{d}{dt} V_{in}(t) \tag{3}$$

According to eq. (3), $V_{in} = A\ \sin(\omega t)$ yields

$$V(t) = \omega R_0 C_0 A\ \ \cos(\omega t) \tag{4}$$

i.e. the 90^o phase shift is expected and shown in Fig. 1. The existence of electrical differentiators [20, 27] and cell-to-cell electrical coupling was demonstrated in different plants [20, 27, 42–45].

Eqs. (3) and (4) can be confirmed via analytical frequency domain analysis. The transfer function derived from Eq. (1) is presented as

$$G(s) = \frac{V(s)}{V_{in}(S)} = \frac{\tau_1 s}{\tau_1 s + 1}$$

where s is a Laplace variable, and $\tau_0 = R_0 C_0$. For $\tau_0 = 10^{-2} s$ the amplitude and phase in Bode plots are presented in Fig. 4.

Based on Bode plots, it is clear that Eq. (1) corresponds to the real differentiator, while Eqs. (3) and (4) are valid for the sinusoidal input $V_{in}(t) = A\sin(\omega t)$ at the approximate frequency range $0 < \omega < 75\ \ 1/s$.

Amplitude and the sign of this response depend on the polarity of electrostimulating electrodes (Figs. 1, 2, 3A, 5) and the amplitude of applied voltage. So, the response does not obey the all-or-none rule and it is not an action potential but rather corresponds to the propagating electrotonic potential.

Amplitude of the electrical response decreases with distance from the electrostimulating electrodes. In the process of electrical signal transmission along the stem, its amplitude exponentially decreases (Fig. 3B) and it can be described by equation

$$V = a\ \ \exp(-bx) \tag{5}$$

For the results shown in Fig. 5B, parameters are $a = 0.49$ V and $b = 0.21$ cm^{-1}. The constant of length in this electrotonic potential transmission is $\lambda = 1/b = 4.8$ cm. λ is the distance from the point at which voltage is injected to the point at which the electrotonic depolarization has fallen to e^{-1}, i.e. 0.37, of its original value (V_{in}).

3.2. Electrostimulation of electrical signals transmission between two tomato plants

If two different tomato plants are placed in the same pot, there is a possibility of electrical communication between both plants through wet soil as an electrical conductor (Fig. 5). Electrostimulation of one tomato plant by a sinusoidal, triangular, or square pulse trains from a function generator induces electrical signals propagating in the stem of an adjacent tomato plant (Fig. 5). Amplitude of electrical response increases with decreasing distance between Pt-electrodes or plants. The similar dependence of electrical responses on distance between stimulating electrodes was also shown for single plants in our previous publications [23, 27, 40].

If $t = 0$ and $\cos(\omega t) = 1$, $V(t)$ will be equal *to* ωRCA according to eq. (4) and we can expect that V should be proportional to frequency ω (Fig. 6A). If frequency of the applied voltage is below 70 Hz, the amplitude of $V(t)$ is proportional to the frequency of applied sinusoidal or triangular voltage to the neighbor tomato plant (Fig. 6) as it is expected for the electrical differentiator. Amplitude of $V(t)$ decreases at high frequencies (Fig. 5) and differentiation of applied electrical signals disappears (Fig. 7). These effects can be caused by a refractory period during generation of electrotonic potentials in tomato plants. The refractory period is the time during which another stimulus given to tomato plant will not lead to differentiation in cell-to-cell electrical signal transduction. The refractory period in Figs. 4A and 5C is equal to

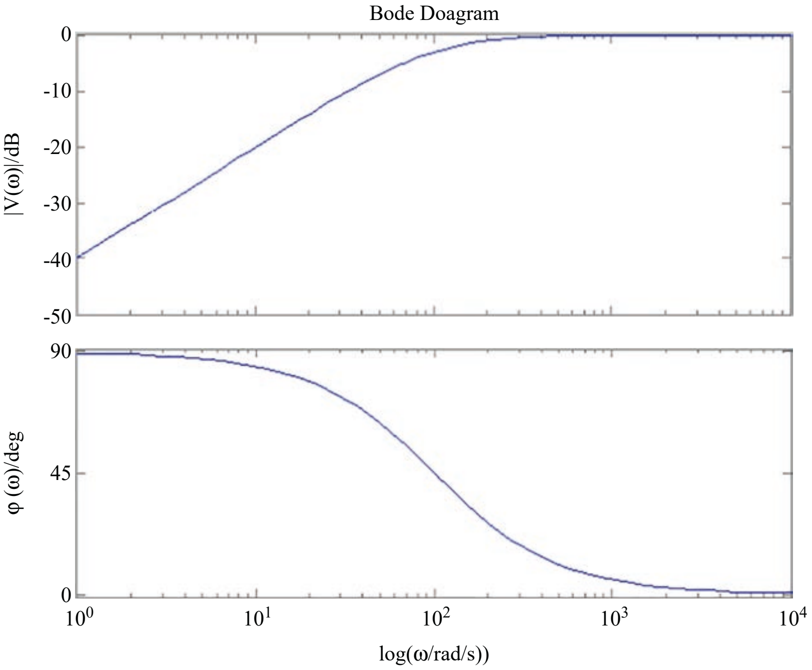


Fig. 4. Bode plots obtained via simulations.

13 ms. If frequency of electrostimulation is less than 76 Hz, the eq. (4) will describe electrical differentiator properties in tomato plant correctly (Fig. 6 A,B). If amplitude of electrostimulation is higher than 77 Hz, phase shift during differentiation of sinusoidal wave will disappear. Potential difference, V, between electrodes inserted in the stem of tomato plant induced by high frequency triangular, sinusoidal, or square pulse train from a function generator will have the same shapes as the applied voltage V_{in} (Fig. 7).

Results shown in Figs. 6 and 7 can be confirmed/reproduced via analytical frequency and time domain analysis (Fig. 8) of the propagation of the electrotonic stimulation potential V_{in} through the system of two tomato plants along stems connected through soil, which equivalent electrical circuit is presented below in Scheme 3.

In this schematic, C_{0eq} and R_{0eq} are the equivalent differential capacitance and the resistance respectively, C_{1eq} is the equivalent capacitance due to electrostatic forces along the length of the tomato stem; R_{1eq}, R_{2eq} are equivalent membrane resistance and a resistance along a tomato stem due to the resistance to a current respectively, R_{3eq}, R_{4eq} are the resistances that characterize the voltages V_1, V_2 distribution along the soil length. The parameters of the equivalent circuit (Scheme 3) can be calculated as in [43]:

$$R_{1eq} = \frac{\overline{R}}{L}\ [\Omega \cdot m],\ \ R_{2eq} = \frac{\tilde{R}}{S}\ [\Omega/m],\ \ C_{1eq} = \overline{C}L\ [F/m],$$

where $\overline{R}\,[\Omega \cdot m^2]$, and the specific capacitance $\overline{C}\,[F/m^2]$ correspond to the measured resistance and the capacitance of one unit area of the membrane respectively; the measured area of a cross-section, and the length of the cross-section contour of the studied tomato stem are S $[m^2]$ and $L\,[m]$ respectively; $\tilde{R}\,[\Omega \cdot m]$ is the measured specific resistance of the tomato stem tissue.

The dynamics of the propagation of the electrotonic stimulation potential V_{in} through the system of two tomato plants along stems connected through soil can be described by a transfer function that is straightforwardly derived for the electrical circuit in Scheme 3:

$$W(s) = \frac{V(s)}{V_{in}(S)} = K_{1eq}\frac{s}{(\tau_{0eq}s + 1)(\tau_{1eq}s + 1)}$$

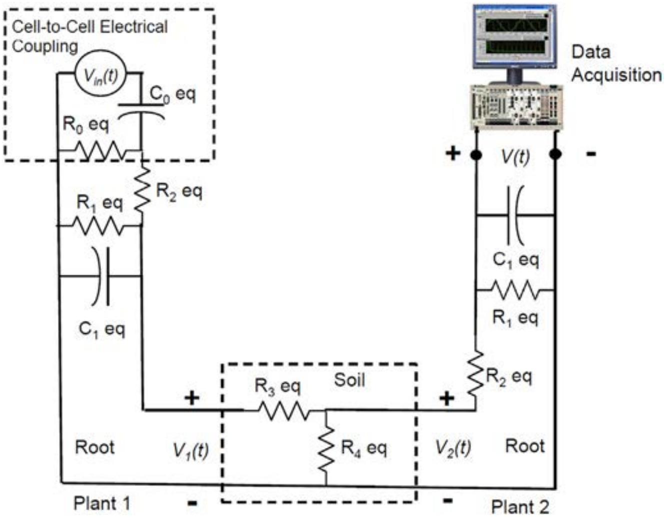
where

$$K_{1eq} = \frac{R_{1eq}R_{4eq}R_{0eq}C_0}{(R_{1eq} + R_{2eq})(R_{3eq} + R_{4eq})},\quad \tau_{oeq} = R_{0eq}C_{0eq},\quad \tau_{1eq} = C_{1eq}\frac{R_{1eq}R_{2eq}}{R_{1eq} + R_{2eq}}$$

and

$$C_{0eq} = 150 \cdot 10^{-9}F,\quad R_{0eq} = 86 \cdot 10^3\Omega,\quad C_{1eq} = 1 \cdot 10^{-9}F,$$
$$R_{1eq} = 150 \cdot 10^3\Omega,\quad R_{2eq} = 86 \cdot 10^3\Omega$$
$$R_{4eq} = 100 \cdot 10^3\Omega,\quad R_{3eq} = 790 \cdot 10^3\Omega$$

The amplitude $20\log_{10}|W(j\omega)|$ in Bode plot is produced by MATLAB and is presented below.



Scheme 3. The equivalent electrical circuits in vascular bundles of two neighboring tomato plants in the same pot with moist soil.

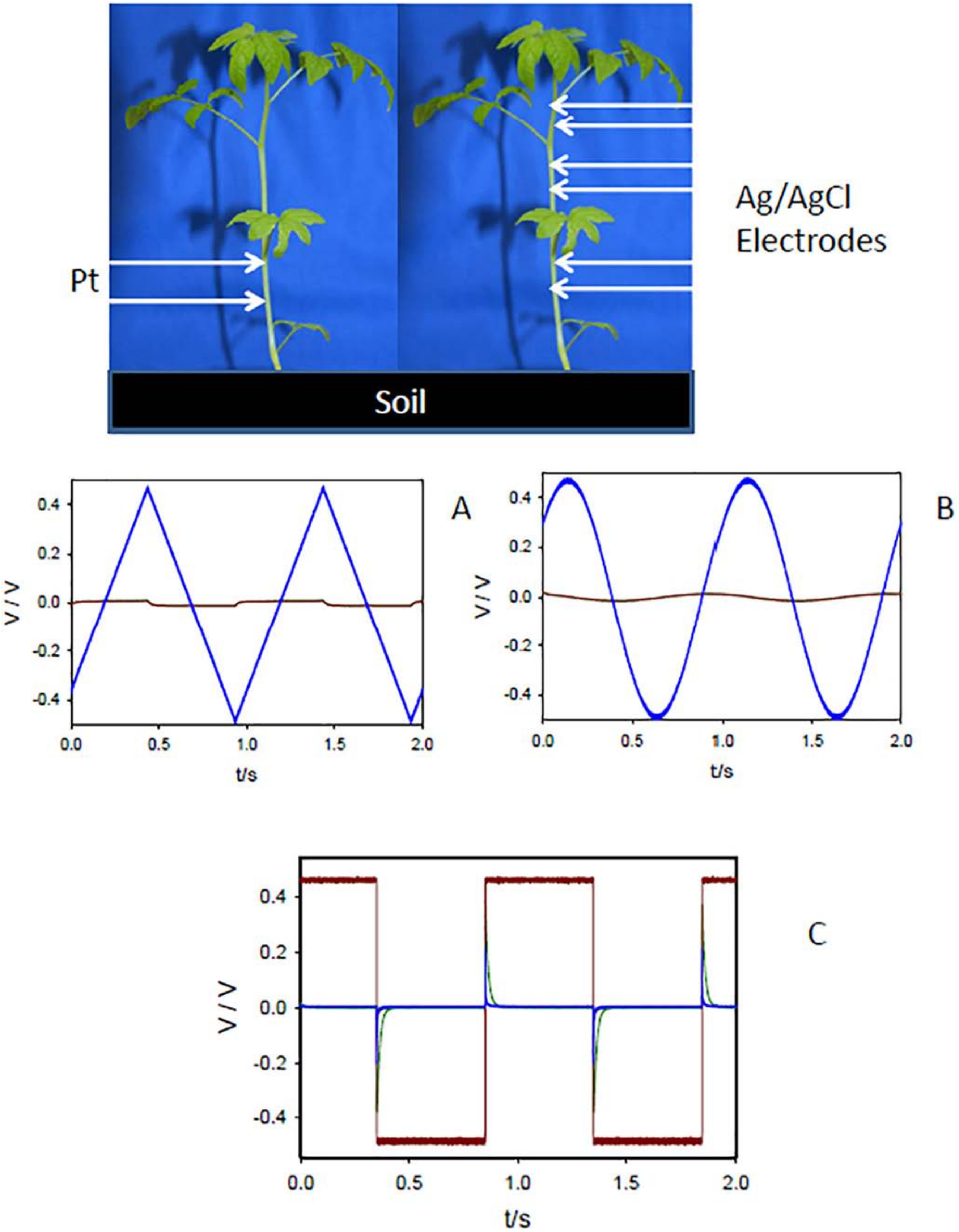


Fig. 5. Potential difference *V* between electrodes inserted in the stem of tomato plant induced by ±0.5 V triangular (A), sinusoidal (B) or square pulse (C) train from a function generator, which was connected to Pt-electrodes in a stem of another tomato plant in the same pot. Distance between Pt-electrodes was 0.3 cm. Distance between lower Pt and Ag/AgCl electrodes and soil was 4 cm. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s.

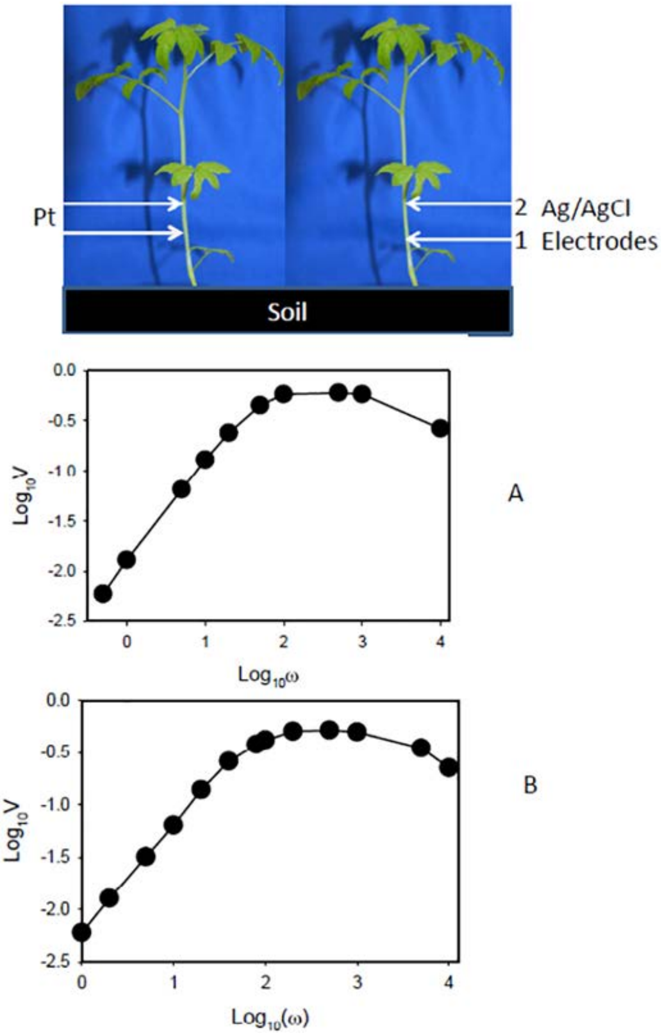


Fig. 6. Amplitude – frequency characteristic of electrical responses *V* between Ag/AgCl electrodes 1 and 2 inserted to the tomato stem along vascular bundles induced by sinusoidal (A) or triangular (B) wave with voltage varies between 0 and 2 V from a function generator, which was connected to Pt-electrodes. Distance between Pt electrodes was 0.2 cm. Distance between lower Pt and Ag/AgCl electrodes and soil was 4 cm.

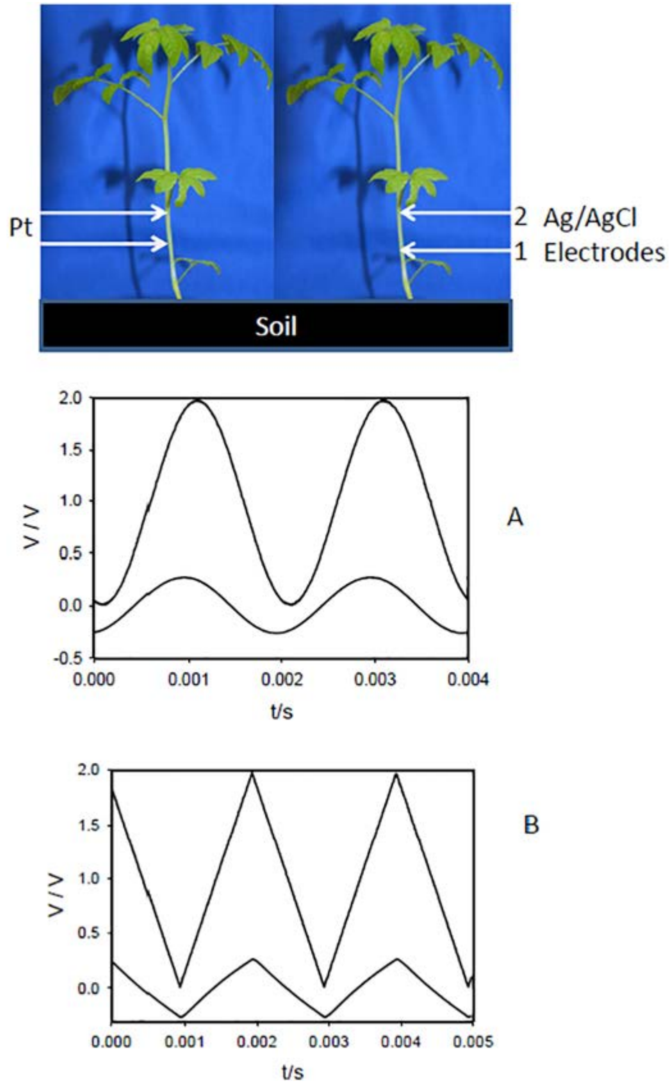


Fig. 7. Potential difference *V* between Ag/AgCl electrodes inserted in the stem of tomato plant induced by 2 V sinusoidal (A) or triangular (B) wave from a function generator with frequency of 500 Hz, which was connected to Pt-electrodes in a stem of another tomato plant in the same pot. Distance between Pt electrodes was 0.2 cm. Distance between lower Pt and Ag/AgCl electrodes and soil was 4 cm. Measurements were performed at 500,000 scans/s with low pass filter at 250,000 scans/s.

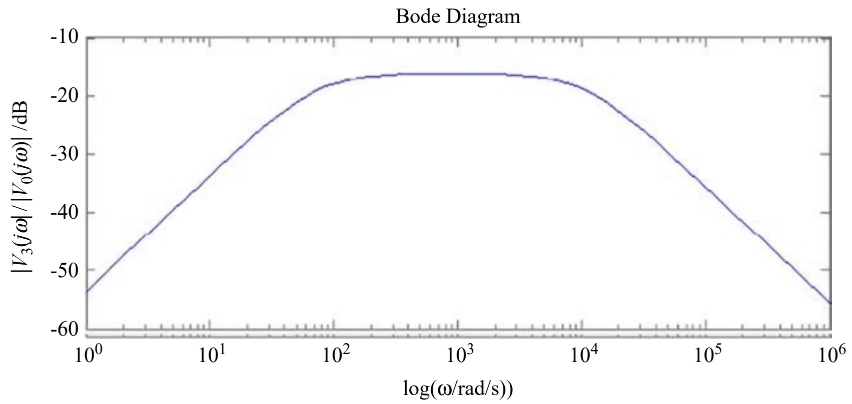


Fig. 8. Bode plot. Amplitude-frequency characteristic 20 log $\frac{|V_3(j\omega)|}{|V_0(j\omega)|}$ obtained via simulations.

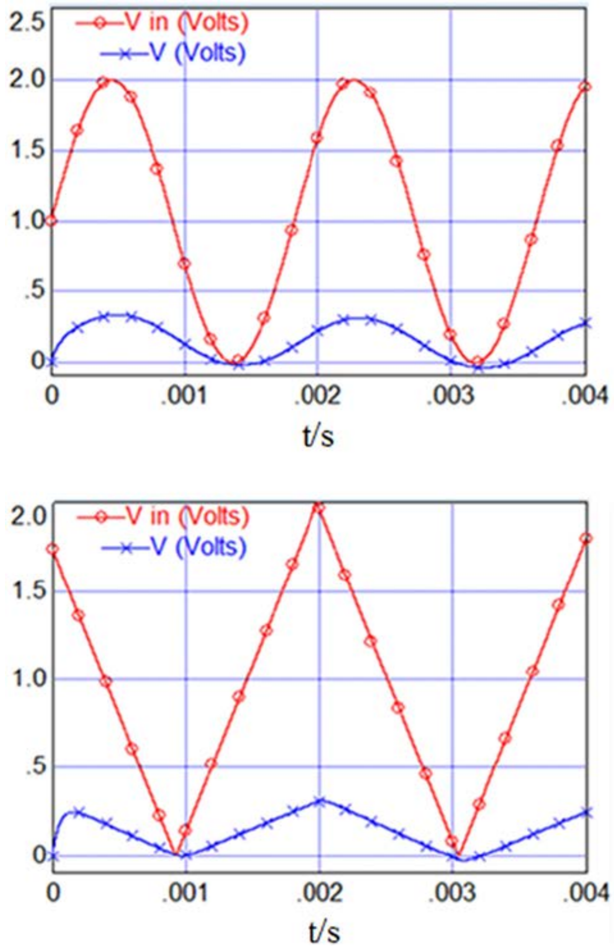


Fig. 9. The results of the analytical/simulation study fit the experimental results closely.

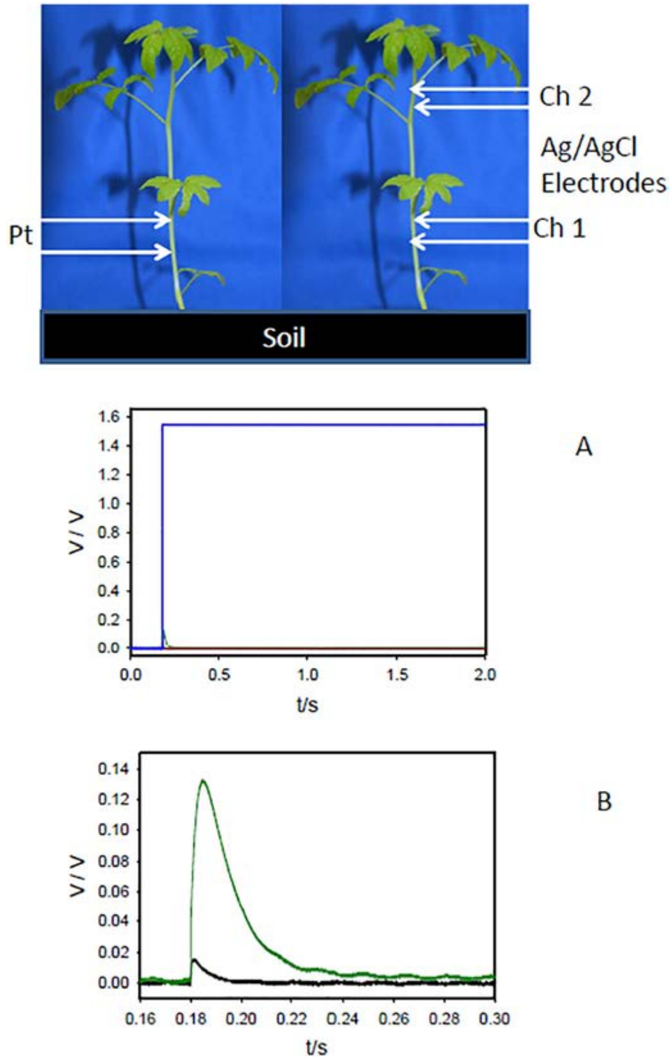


Fig. 10. Dependence of amplitude of electrical responses *V* between electrodes inserted into the tomato stem along vascular bundles. Panel B is extracted from Panel A to show high resolution electrical responses. Distance between Pt electrodes in a stem of another tomato plant in the same pot was 0.4 cm during electrostimulation by 1.5 V square pulse from a battery. Distance between lower Pt and Ag/AgCl electrodes and soil was 4 cm. Distance between of Channel 1 and Channel 2 was 9 cm. Distance between stems of plants was 11 cm. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s.

One can conclude that the amplitude in Bode Plot obtained experimentally and presented at Fig. 6 fits closely the one obtained using the frequency analysis of the equivalent electrical circuit.

The experiment, which results are presented in Fig. 7, was repeated/reproduced via simulations using the transfer function$W(s)$. Potential difference, *V*, between electrodes inserted in the stem of tomato plant induced by high frequency triangular or sinusoidal input from a function generator will have the same shapes as the applied voltage V_{in}.

Electrostimulation of a plants underground electrical communication can be done by a battery (Fig. 10) instead of a function generator (Fig. 9) to avoid possibility of a ground loop or direct electrical communication between a function generator and data acquisition system. Results obtained by both methods are similar and our function generator can be used for such experiments.

Daconil, containing 0.087% of chlorothalonil, was used as antifungal treatment of soil in control experiments. It may also be used to kill mildew, bacteria, algae, and insects. Soil was treated by 5 ml of Daconil 12 h before electrostimulation and response measurements. Daconil did not influence the electrical responses in tomato plants during electrostimulation by the pulse train, sinusoidal or a triangular saw-shape voltage profiles.

If two tomato plants are grown in different pots without electrical connection between them, there is no electrical coupling between electrical networks of both neighboring plants (Fig. 11).

Direct electrostimulation of a tomato plant by electrodes located in soil near a plant is shown in Fig. 12. Electrostimulation of platinum electrodes in soil by a sinusoidal, triangular, or square pulse trains from a function generator induces electrical signals propagating in a stem of an adjacent tomato plant (Fig. 5). Amplitude of electrical response increases with decreasing distance between Pt-electrodes in soil. Since fungi can contribute to underground communication between plants, we used Daconil for a soil treatment against fungi for a week and received the same results as shown in Fig. 12.

4. Discussion

Plants communicate with other plants using different pathways: (1) volatile organic compounds' (VOC) emission and sensing; (2) mycorrhizal networks in the soil; (3) the plants' rhizosphere (root ball); (4) electrostatic or electromagnetic interactions; (5) roots of the same species can sometimes naturally graft. We found the additional signaling pathway of electrotonic potentials propagation between roots of neighboring plants through soil.

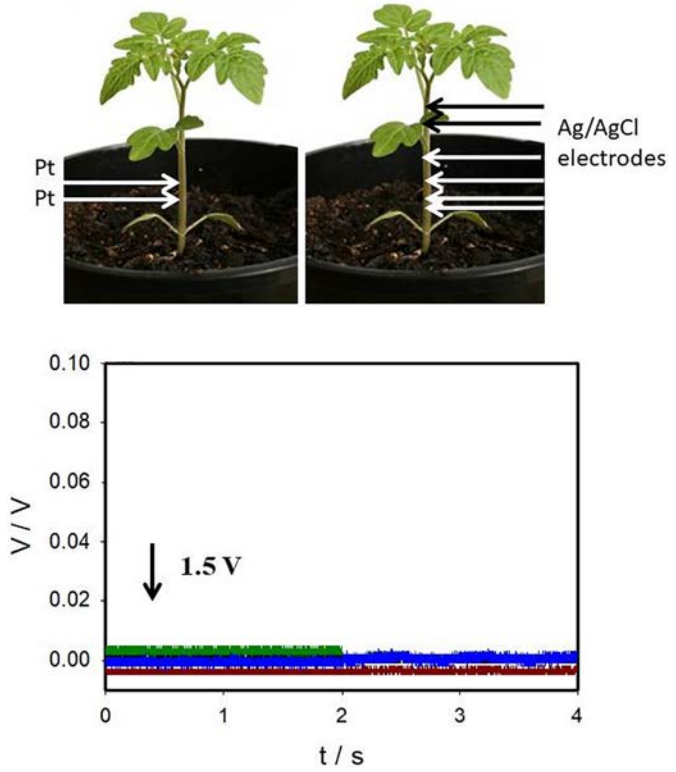


Fig. 11. Dependence of amplitude of electrical responses *V* between electrodes inserted into the tomato stem along vascular bundles. Distance between Pt electrodes in a stem of another tomato plant in the different pot was 0.4 cm during electrostimulation by 1.5 V square pulse from a battery. Distance between lower Pt and Ag/AgCl electrodes and soil was 4 cm. Distance between stems of plants was 11 cm. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s.

Electrical signaling on long and short distances exists in plants. Electrical networks in plants consist of different electrical circuits [19]. Bioelectrical impulses travel from the root to the stem and vice versa [16, 20, 23, 28, 29]. Chemical treatment, intensity of the irritation, mechanical wounding, previous excitations, temperature, and other irritants influence the speed of electrical signal propagation.

The action potential can propagate over the entire length of the cell membrane and along the conductive bundles of tissue with constant amplitude, duration, and speed. Electrotonic potentials in plants exponentially decrease with distance.

Electrotonic potentials are also well known in animal tissue such as neurons, heart, and muscles tissue [44, 45]. The amplitude of an electrotonic potential exponentially decreases with distance both in plants and animal tissues. These potentials play a rather important role. For example, the spread of a receptor potential is accomplished by means of electrotonic potentials [45]. Some small neurons have only electrotonic potentials; some neurons utilize electrotonic potentials to trigger the action potential. Electrotonic potentials can influence the duration of action potentials [44]. In animal tissue, studying both action potentials and electrotonic potentials is very advanced and can be done at the level of a single excitable cell. This is almost impossible to do with plant tissue. The rare exceptions are *Chara* and *Nitella* [46]. In other cases we deal with the ensemble of cells, but we are unsure of the position of the electrodes.

The pulse train, sinusoidal and a triangular saw-shape voltage profiles can be used for electrostimulation of plants and fast underground electrotonic signal transmission between plants. Electrical voltage from a function generator $V_{in}(t)$ applied to Pt-electrodes inserted to a stem is converted to electrotonic potentials due to electrical cell-to-cell coupling between cells in plant tissue by an electrical differentiator. The amplitude and sign of electrotonic potentials in both electrostimulated and neighboring tomato plants depends on the amplitude, rise and fall of the applied voltage. Electrostimulation by a sinusoidal wave from a function generator induces electrical response in plants with a phase shift if frequency of applied voltage does not exceed 76 Hz. At higher frequencies a phase shift decreases and finally disappears. Electrostimulation by a saw-shape voltage wave from a function generator induces electrical response in plants in forms of horizontal positive and negative steps if frequency of applied voltage does not exceed 76 Hz. At higher frequencies responses reproduce a saw-shape voltage wave. Decreasing amplitude of responses with frequencies of sinusoidal or triangular waves higher than 100 Hz shows the existence of a refractory period at the cell-to-cell electrical coupling. The most common pathway for the cell-to-cell electrical communication in higher plants is plasmodesmata [47–50]. Spanswick estimated that plasmodesmata have an electrical "resistance about 60 times higher than would be the case if they were completely open channels" [49]. The amplitude of electrotonic potentials are sensitive to various membrane active drugs. Injection of tetraethylammonium chloride (TEACl) near or between Pt-electrodes in plants decreases the amplitude without decreasing the duration of electrotonic potential [40]. TEACl is known as a blocking agent of K^+ ion channels and aquaporins. We can assume that ion channels are involved in cell-to-cell electrical coupling in plants and the generation of electrotonic responses. The refractory period in Fig. 6 is a well-known property of voltage gated ion channels.

If voltage gated ion channels are closed and not involved in signal transmission along a plasma membrane in vascular bundles, the propagation of passive electrotonic potentials can be described by a cable theory [10, 27, 51, 52] along a circuit consisting of plasma membrane capacitors C_1, resistors R_1, resistance along a plasma membrane R_2, soil capacitance C_3 and resistance R_3 (Scheme 3). Electrical circuits in the roots and at the root/soil interface are very complicated and many authors propose different active and passive equivalent electrical schemes. Due to additional RC-circuits in a root and soil, the duration of electrotonic potentials can increase. The analytical/simulation study of the equivalent circuit in Scheme 3 fits well the experimental time domain and frequency domain results.

In this article, we investigated passive electrotonic signal transmission between tomato plants. In the future, it will also be very interesting to investigate the possibility of active electrical signal communication between plants if the length of action potentials is larger than distance between roots of two plants. Propagation of action potentials through dead plant tissue on short distances is well known in literature [53–55].

Knowledge of electrical networks in plants is required in order to successfully influence plant metabolism and function [5–10]. The reason why plants have developed pathways for electrical signal transmission probably lies in the plants necessity to respond rapidly to environmental stress factors. Different environmental stimuli evoke specific responses in living cells, which have the capacity to transmit a signal to the responding region. In contrast to chemical signals, such as hormones, electrical signals are able to rapidly transmit information over long distances with high speed. Electrical potentials have been measured at the tissue and whole plant levels. The generation of electrical gradients is a fundamental aspect of signal transduction. In plants, these potentials can be induced through mechanical or electrical stimulation, changing the direction of light, chemical treatment, and plant-insect interaction. Biologically closed electrical circuits play a very important role in the physics of living systems and they operate over large distances in biological tissues. The activation of such a circuit can lead to various physiological and biophysical responses.

Disclosure of potential conflicts of interest

The authors declare no competing financial interest.

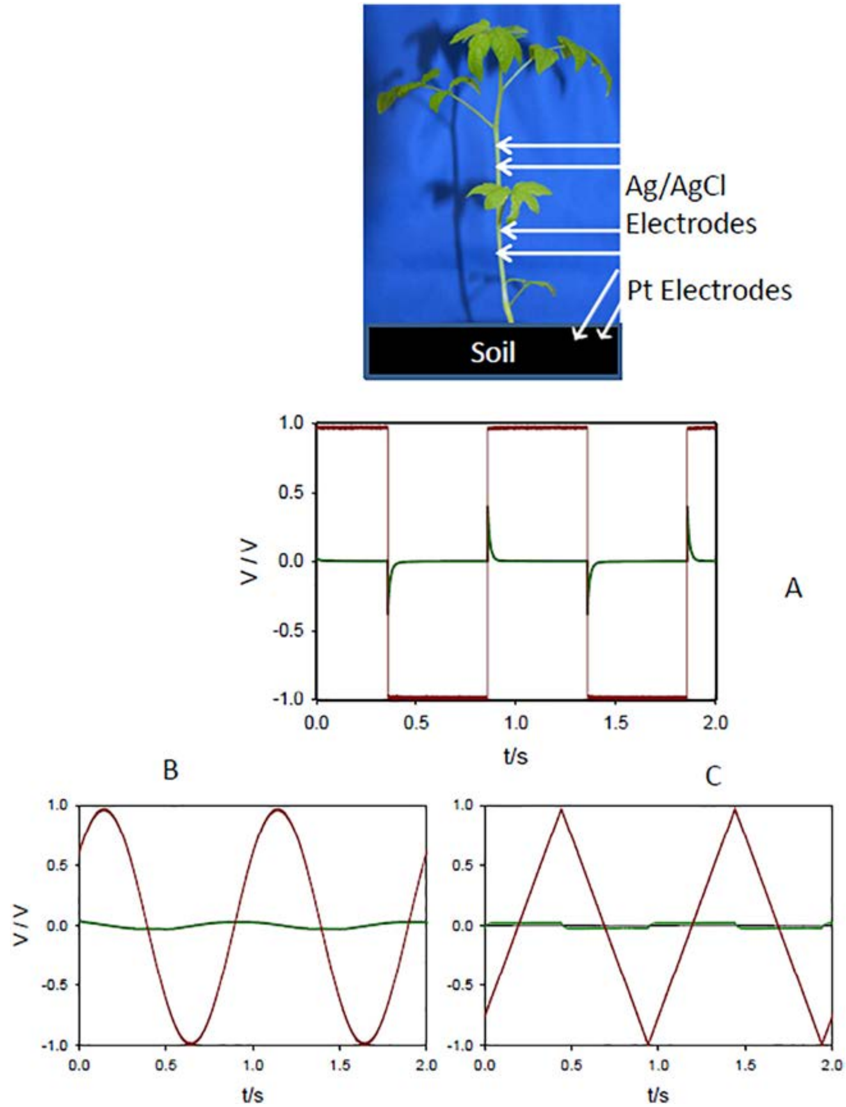


Fig. 12. Potential difference *V* between Ag/AgCl electrodes inserted to the tomato stem along vascular bundles was induced by ±1V square pulse train (A), sinusoidal (B) or triangular (C) waves from a function generator, which was connected to Pt-electrodes in the soil. Measurements were performed at 250,000 scans/s with low pass filter at 125,000 scans/s. Distance between the lower Ag/AgCl electrode and soil was 4 cm.

Conflict of interest

The authors declare no conflict of interest in this paper.

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References

[1] M.E. Maffei, J. Gertsch, G. Appendino, Plant volatiles: production, function and pharmacology, Nat. Prod. Rep. 28 (2011) 1359–1380.
[2] I.T. Baldwin, J.C. Schultz, Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants, Science 221 (1983) 277–279.
[3] R. Karban, Plant Sensing and Communication, University of Chicago Press, Chicago, 2015.
[4] Z. Tronteli, G. Thiel, V. Jazbinsek, Magnetic measurements in plant electrophysiology, in: A.G. Volkov (Ed.), Plant Electrophysiology, Springer, Berlin 2006, pp. 187–218.
[5] R.C. Mishra, R. Ghosh, H. Bae, Plant acoustics: in the search of a sound mechanism for sound signaling in plants, J. Exp. Bot. 67 (2016) 4483–4494.
[6] M. Gagliano, Green symphonies: a call for studies on acoustic communication in plant, Behav. Ecol. (2016) 1–7, https://doi.org/10.1093/beheco/ars206.
[7] K. Sugimoto, K. Matsui, J. Takabayashi, Uptake and conversion of volatile compounds in plant-plant communication, in: J.D. Blande, R. Glinwood (Eds.), Deciphering Chemical Language of Plants Communication, Springer International Publishing Switzerland, In 2016, pp. 305–316.
[8] Z. Babikova, D. Johnson, T.J.A. Bruce, J.A. Pickett, L. Gilbert, How rapid is aphid-induced signal transfer between plants via common mycelial networks? Commun. Integr. Biol. 6 (2013), e25904. .
[9] H.P. Bais, S.W. Park, T.L. Weir, R.M. Gallaway, J.M. Vivanco, How plants communicate using the underground information superhighway, Trends Plant Sci. 9 (2004) 26–32.
[10] D. Johnson, L. Gilbert, Interplant signaling through hyphal networks, New Phytol. 205 (2015) 1448–1453.
[11] S.W. Simard, D.A. Perry, M.D. Jones, D.D. Myrold, D.M. Durall, R. Molina, Net transfer of carbon between ectomycorrhizal tree species in the field, Nature 388 (1997) 579–582.
[12] S.E. Smith, D.J. Read, Mycorrhizal Symbiosis, 3rd ed. Academic Press, London, 2008.
[13] T. Helgason, T.J. Daniell, R. Husband, A.H. Fitter, J.P.W. Young, Ploughing up the wood-wide web? Nature 394 (1998) 431.
[14] D. Robinson, A. Fitter, The magnitude and control of carbon transfer between plants linked by common mycorrhizal network, J. Exp. Bot. 50 (1999) 9–13.
[15] Plant electrophysiology, in: A.G. Volkov (Ed.), Methods and Cell Electrophysiology, Springer, Berlin, 2012.
[16] Plant electrophysiology, in: A.G. Volkov (Ed.), Signaling and Responses, Springer, Berlin, 2012.
[17] A. G. Volkov (2012) Plant biosensor and method. US Patent Number 8,205,502.
[18] A. G. Volkov (2014) Plant biosensor and method. US Patent Number 8.893.551.

[19] A.G. Volkov, Biosensors, memristors and actuators in electrical networks of plants, Intern. J. Parallel Emerg. Distrib. Syst. 32 (2017) 44–55.

[20] A.G. Volkov, Yu.B. Shtessel, Electrotonic signal transduction between *Aloe vera* plants using underground pathways in soil: experimental and analytical study, AIMS Biophys. 4 (2017) 576–595.

[21] Y.Y. Song, R.S. Zeng, J.F. Xu, J. Li, X. Shen, W.G. Yihdego, Interplant communication of tomato plants through underground common mycorrhizal networks, PLoS One 5 (2010), e13324. https://doi.org/10.137/journal.pone.0013324.

[22] A.G. Volkov, J.C. Foster, T.A. Ashby, R.K. Walker, J.A. Johnson, V.S. Markin, *Mimosa pudica*: electrical and mechanical stimulation of plant movements, Plant Cell Environ. 33 (2010) 163–173.

[23] A.G. Volkov, L. O'Neal, M.I. Volkova, V.S. Markin, Electrostimulation of *Aloe vera* L., *Mimosa pudica* L. and *Arabidopsis thaliana*: Propagation and collision of electrotonic potentials, J. Electrochem. Soc. 160 (2013) G3102–G3111.

[24] D.C. Wildon, J.F. Thain, P.E.H. Minchin, I.R. Gubb, A.J. Reilly, Skipper, H.M. Doherty, P.J. O'Donnell, D.J. Bowles, Electric signaling and systemic proteinase inhibitor induction in the wounded plan, Nature 360 (1992) 62–65.

[25] R.L. Overall, B.E.S. Gunning, Intercellular communication in Azolla roots: II, Electr. Coupling Protoplasma 111 (1982) 151–160.

[26] B.J.W. Chen, H.J. During, N.R.P. Anten, Detect thy neighbor: identity recognition at the root level in plants, Plant Sci. 195 (2012) 157–167.

[27] A.G. Volkov, Yu.B. Shtessel, Propagation of electrotonic potentials in plants: experimental study and mathematical modeling, AIMS Biophys. 3 (2016) 358–378.

[28] A.G. Volkov, R.A. Haack, Insect induces bioelectrochemical signals in potato plants, Bioelectrochemistry 35 (1995) 55–60.

[29] O.S. Ksenzhek, A.G. Volkov, Plant Energetics, Academic Press, San Diego, 1998.

[30] E. Jovanov, A.G. Volkov, in: A.G. Volkov (Ed.), Plant Electrostimulation And Data Acquisition. Plant Electrophysiology- Methods And Cell Electrophysiology, Springer, Berlin 2012, pp. 45–67.

[31] S.A. Zebelo, K. Matsui, R. Ozawa, M.E. Maffei, Plasma membrane potential depolarization and cytosolic calcium flux are early events involved in tomato (*Solanum lycopersicon*) plant-to-plant communication, Plant Sci. 196 (2012) 93–100.

[32] I. Samouëlian, I. Cousin, A. Tabbagh, A. Bruand, G. Richard, Electrical resistivity survey in soil science: a review, Soil Tillage Res. 83 (2005) 173–193.

[33] O. Herde, H. Peña-Cortés, J. Fisahn, Proteinase inhibitor II gene expression induced by electrical stimulation and control of photosynthetic activity in tomato plants, Plant Cell Physiol. 36 (1995) 737–742.

[34] O. Herde, R. Atzorn, J. Fisahn, C. Wasternack, L. Willmitzer, H. Peña-Cortés, Localized wounding by heat initiates the accumulation of proteinase inhibitor II in abscisic acid-deficient plants by triggering jasmonic acid biosynthesis, J. Plant Physiol. 112 (1996) 853–860.

[35] B. Stankovic, E. Davies, Intercellular communication in plants: electrical stimulation of proteinase inhibitor gene expression in tomato, Planta 202 (1997) 402–406.

[36] L.E. Murr, Plant Growth Response in a Simulated Electric Field Environment Nature, vol. 200, 1963 490–491.

[37] Y. Mizuguchi, Y. Watanabe, H. Matsuzaki, Y. Ikezawa, T. Takamura, Growth acceleration of bean sprouts by the application of electrochemical voltage in a culturing bath, Denki Kagaku 62 (1994) 1083–1085.

[38] J. Black, F. Forsyth, D. Fensom, R. Ross, Electrical stimulation and its effects on growth and ion accumulation in tomato plants, Can. J. Bot. 49 (1971) (1971) 1809–1815.

[39] A.G. Volkov, C.L. Vilfranc, V.A. Murphy, C. Mitchell, M.I. Volkova, L. O'Neal, V.S. Markin, Electrotonic and action potentials in the Venus flytrap, J. Plant Physiol. 170 (2013) 838–846.

[40] A.G. Volkov, E.K. Nyasani, C. Tuckett, J.M. Scott, M.Z. Jackson, E.A. Greeman, A.S. Greenidge, D.O. Cohen, M.I. Volkova, Yu.B. Shtessel, Electrotonic potentials in *Aloe vera* L.: Effect of intercellular and external electrodes arrangement, Bioelectrochemistry 113 (2017) 60–68.

[41] J. Fromm, R. Spanswick, Characteristics of action potentials in willow (*Salix viminalis* L.), J. Exp. Bot. 44 (1993) 1119–1125.

[42] A.G. Volkov, D.W. Deamer, D.I. Tanelian, V.S. Markin, Liquid Interfaces in Chemistry and Biology, J. Wiley, N. Y., 1998

[43] W. Rall, Time constants and electrotonic length of membrane cylinders and neurons, Biophys. J. 58 (1969) 1483–1508.

[44] K.J. Sampson, C.S. Henriquez, Electrotonic influences on action potential duration dispersion in small hearts: a simulation study, Am. J. Physiol. Heart Circ. Physiol. 289 (2005) H350–H360.

[45] G.M. Shepherd, Neurobiology, Oxford University Press, New York, 1994.

[46] M.J. Beilby, Action potentials in charophytes, Int. Rev. Cytol. 257 (2007) 43–82.

[47] R.R. Lew, Regulation of electrical coupling between Arabidopsis root hairs, Planta 193 (1994) 67–73.

[48] R.R. Lew, Root hair electrophysiology, in: A.M.C. Emons, T. Ketelaar (Eds.), Root Hairs. Plant Cell Monographs, vol. 12, Springer-Verlag, Berkin 2008, pp. 123–144.

[49] R.M. Spanswick, Electrical coupling between cells of higher plants: a direct demonstration of intercellular communication, Planta 102 (1972) 215–227.

[50] J.M. Frachisse-Stoilskovic, J.L. Julien, The coupling between extra-and intracellular electric potentials in *Bidens pilosa* L, Plant Cell Environ. 16 (1993) 633–641.

[51] A.L. Hodgkin, W.A.H. Rushton, The electrical constants of a crustacean nerve fibre, Proc. R. Soc. B 133 (1946) 444–479.

[52] J.J. Jack, D. Noble, R.W. Tsien, Electric Current Flow In Excitable Cells, Clarendon, Oxford, 1975.

[53] A.L. Houwink, The conduction of excitation in *Mimosa pudica*, Recueil des Travaux Botaniques Neerlandais 32 (1935) 51–91.

[54] J.C. Bose, Researches on Irritability of Plants, Longmans, Green, and Co, London, 1913.

[55] G. Haberlandt, Physiological Plant Anatomy, 4th ed. Macmillan, London, 1884.