Relationship between wheat root properties and its electrical signature using the spectral induced polarization method

Kuzma Tsukanov | Nimrod Schwartz

Dep. of Soil and Water Sciences, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew Univ. of Jerusalem, Rehovot, Israel

Correspondence
Nimrod Schwartz, Dep. of Soil and Water Sciences, Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew Univ. of Jerusalem, Rehovot, Israel.
Email: nimrod.schwartz@mail.huji.ac.il

Abstract
Measuring root properties, the “hidden half” of the plant, is challenging due to their heterogeneous and dynamic nature. A promising method for noninvasive mapping of roots and their activity, spectral induced polarization (SIP), has been introduced. However, measurements of root properties together with their SIP responses are missing, limiting the interpretation of a root’s SIP signature. In this study, we coupled SIP measurements of roots in hydroponic solution with measurements of root biomass, surface area, and diameter. Furthermore, we monitored the SIP response of roots poisoned by cyanide, which results in depolarization of the root’s cell membrane potential. We found a linear correlation between root biomass and surface area, and the low-frequency electrical polarization. In addition, we demonstrate the relationship between root cell membrane potential and root polarization. Based on the results, we suggest that in comparison with the stem-based approach used by other researchers, the polarization in the contact-free method used in this study is related to the external surface area of the root and external architectural structures such as root diameter and root hair. Overall, a direct link between root properties and their electrical signature was established.

1 | INTRODUCTION

Roots are the “hidden half” of plants (Eshel & Beeckman, 2013), and despite ever-growing efforts to study their structure, dynamics, and functions, our understanding of roots is still incomplete, especially in comparison with the above-ground part of the plant (Ryan, Delhaize, Watt, & Richardson, 2016). The lag in root research is mainly due to root heterogeneity, their dynamic nature, opacity of the growing media, and the absence of a technology that will overcome these challenges and that will allow in situ investigations of roots and their interactions with the surrounding environment.

In situ root-system architectural parameters such as root weight, length, density, radius, branching, etc., are traditionally assessed by trenching, auger sampling, and root excavation (Atkinson, 2000). These methods are limited because they are destructive, highly laborious, and cannot be used to follow root dynamics. To allow direct monitoring of root systems, modern techniques, such as the minirhizotron (Rewald & Ephrath, 2013), rhizotubes (Cai et al., 2016), X-ray computed tomography (CT; Mooney, Pridmore, Helliwell, & Bennett, 2012), and neutron radiography (Zarebanadkouki, Kim, & Carminati, 2013) are used. These imaging techniques (and others) have accelerated the progress in root research and exposed the enormous potential of breeding for root traits.

Abbreviations: CCCP, carbonyl cyanide 3-chlorophenylhydrazone; EDL, electrical double layer; PNP, Poisson–Nernst–Planck; SIP, spectral induced polarization.

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For example, Nagel et al. (2012) used a rhizotron to study root-system architecture and the response of barley (Hordeum vulgare L.) and maize (Zea mays L.) roots to soil compaction. Koebenick et al. (2015) combined X-ray CT with a model for root water uptake to study three-dimensional water transport in a soil–root system, and Leitner, Felderer, Vontobel, and Schnepf (2014) developed an image analysis approach to reconstruct root structural parameters from neutron radiography. However, these methods still suffer from severe limitations, such as small sample size (on the scale of millimeters to centimeters for most of the advanced noninvasive imaging techniques), limited accessibility of the instruments, nonrepresentative geometry (e.g., two-dimensional rhizotron), and more (Oburger & Schmidt, 2016).

Because geoelectrical methods can map the subsurface in a noninvasive fashion, on various spatial and temporal scales, and at relatively low cost (Binley et al., 2015; Hubbard & Rubin, 2005), they hold great promise as a tool for root research (Vanderborght et al., 2013). To use geoelectrical methods for root studies, the relationships between the electrical signature of roots and root properties of interest need to be established. Chloupeck (1972) was one of the first to study the relationship between root electrical capacitance and root parameters such as biomass. Since then, a linear relationship between root mass and root electrical capacitance has been reported by several authors (Kendall, Peferson, & Hill, 1982; Mcbride, Candido, & Ferguson, 2008; Ozier-Lafontaine and Bajazet., 2005; Preston, McBride, Bryan, & Candido, 2004; Postic & Doussan, 2016; Tsukahara, Yamane, Yamaki, & Honjo, 2009; van Beem, Smith, & Zobel, 1998). These results are usually interpreted using the electrical model proposed by Dalton (1995). In that model, the root behaves like a cylindrical capacitor, and the root-system capacitance can be obtained by summing (in parallel) the capacitance of each root segment. In a different model, the root is thought to behave like a continuous dielectric material embedded in a medium of higher capacitance (Dietrich, Bengough, Jones, & White, 2012). The theoretical consequence of that model is that the capacitance measured between the root and the growing medium is related to the root cross-sectional area (Dietrich et al., 2012; Dietrich, Bengough, Jones, & White, 2013).

In the above studies, the electrical capacitance was measured by insertion of one electrode into the plant stem and other electrode(s) into the soil or nutrient solution (stem-based method). A key disadvantage of this method is that it is invasive with respect to the plant. Furthermore, all measurements were performed at relatively high frequencies (in most cases at 1 kHz), which are also less suitable for field measurements and might miss important processes related to electrochemical polarization that can be detected only at lower frequencies (Kemna et al., 2012). In other words, although the capacitance method demonstrates the potential of electrical methods to monitor roots and their activity, it does not take full advantage of modern geoelectrical methods. In particular, spectral induced polarization (SIP) holds great promise for in situ root monitoring, as it measures the electrical impedance in a relatively broad frequency range and can be applied on various spatial scales, ranging from a small laboratory setup to field measurements (Binley et al., 2015).

### 1.1 Spectral induced polarization

In the SIP method, a low-frequency (typically 0.01 Hz to 10 kHz) sinusoidal current is applied through two electrodes, and the electrical potential is measured with two other electrodes. The ratio between the applied current and the potential is the complex electrical conductance of the system and the complex electrical capacitance (σ*) is calculated. The complex electrical conductivity is composed of the in-phase (σ*) and the quadrature (σ”) conductivities according to

\[
σ^* = σ' + iσ'' = |σ^*| \exp(iφ)
\]  

where \(i\) is the imaginary unit, and \(φ\) is the phase shift. In general, the in-phase conductivity is related to energy-dissipation processes, which at very low frequency are identical to the direct-current electrical conductivity. The quadrature conductivity is the result of polarization processes around charged surfaces such as soil particles (Bücker, Flores Orozco, Undorf, & Kemna, 2019; Revil et al., 2017), bacteria cells (Mellage et al., 2018; Zhang, Revil, Fujita, Munakata-Marr, & Redden, 2014), and root cell membranes (Weigand & Kemna, 2019).

When a charged surface is embedded in an electrolyte, the electric field produced by the surface attracts counterions and repels coions, forming the electrical double layer (EDL; Leroy, Revil, Kemna, Cosenza, & Ghorbani, 2008). The EDL is divided into the Stern and diffuse layer, where the former consists of a thin layer of counterions, and the later contains both counter- and coions. The difference between the concentration of the counter- and coions in the diffuse layer decreases as a function of distance from the charged surface until, at the bulk solution, the difference is zero. Application of low-frequency external electrical field results in the transport of

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**Core ideas**

- Noninvasive electrical measurements are sensitive to plant roots’ physical properties.
- The cell membrane potential is the source of the root electrical polarization.
- Root surface area controls the polarization.
- Root diameter controls the relaxation time.
ions in the EDL and its polarization. The two driving forces for the displacement of ions in the EDL and its polarization are the electromigration and diffusion fluxes, which are typically modeled using the Poisson–Nernst–Planck (PNP) equations (Newman & Thomas-Alyea, 2004):

$$\frac{\partial C_n}{\partial t} = \nabla \left( D_n \nabla C_n + z_n e \frac{D_n}{k_B T} C_n \nabla U \right)$$

$$\nabla (-\varepsilon \varepsilon_0 \nabla U) = \sum_n \varepsilon z_n C_n$$

where $C$ is the ion concentration, $D$ is the diffusion coefficient, $z$ is the valence, $e$ is the elementary charge, $k_B$ is the Boltzmann constant, $T$ is the temperature, $U$ is the electric potential, $\varepsilon$ is the relative permittivity, $\varepsilon_0$ is the permittivity of free space, and the subscript $n$ stand for a specific ion in the system (e.g., Na or Cl). Assuming that only the Stern layer is polarized, spherical geometry, and a nonconductive particle, the PNP equations are solved, resulting in a functional relationship between the complex electrical conductivity and surface and electrolyte properties (Bücker et al., 2019):

$$\sigma^* = \frac{2\beta |\Sigma_S|}{r} \frac{i \omega \tau}{1 + i \omega \tau} + i \omega \varepsilon \varepsilon_0$$

$$\tau = \frac{r^2}{2 \alpha D}$$

where $\beta$ is the ion mobility near the surface, $\Sigma_S$ is the surface charge density at the stern layer, $r$ is the radius, $\omega$ is the angular frequency, $\tau$ is the relaxation time, and $\alpha$ is a coefficient related to the coupling between the charges in the Stern layer and in the electrolyte (Bücker et al., 2019, Equation 32). Note that it is typically safe to assume that Stern layer polarization is significantly higher than diffuse layer polarization (Bücker et al., 2019; Leroy et al., 2008; Revil, Koch, & Holliger, 2012).

### 1.2 Spectral induced polarization of roots

Recently, Weigand and Kemna (2017) used SIP imaging to map roots in a rhizotron box. They were able to monitor the spatial extension of the root system and reported a stress-related decrease in electrical polarization (the plant was immersed in nutrient-deprived water). They further reported a change in the scale of the polarization length due to the stress. The focus of that work, however, was on mapping the spatial distribution of the SIP properties, and not on identifying the mechanisms controlling the roots’ SIP responses. In a follow-up study, Weigand and Kemna (2019) showed that the magnitude of polarization is linked to zones with a high density of roots and link it to the total surface area of the root cells EDL. In addition, they showed that the SIP signature of roots is sensitive to day/night cycles, with an increase in polarization during the night. They explained this observation using a conceptual model for polarization of the root cell membrane, arguing that ion uptake during the day lowers the magnitude of the cell membrane potential, and hence also the root polarization. In another study, preliminary results for the polarization of a single root element show a very high polarization (~400 mrad) at 10 kHz (Ehosioke et al., 2018). While the two aforementioned studies presented important advances in imaging roots using SIP, the lack of supportive root measurements, and the absence of a rigorous model for root polarization limited the interpretation of the results. Hence, the objective of this study was to enhance our understanding of the relationship between root properties and processes and their electrical signatures. Specifically, we aimed to determine the major source of the root polarization, and to link root physical properties (mass, surface area, and diameter) with their SIP signatures.

### 2 MATERIALS AND METHODS

We conducted two main experiments and developed a mechanistic model for root polarization. In the first experiment, we combined SIP measurements of roots with measurements of their physical properties (mass, surface area, and diameter), with the aim of linking root physical and electrical properties. The objective of the second experiment was to link root cell membrane potential and polarization. We therefore monitored SIP spectra of roots while manipulating the root cell membrane potential by adding cyanide to the nutrient solution, which is known to reduce root cell membrane potential by the inhibition of membrane proton pumps (Anderson, Robertson, & Wright, 1977; Felle & Bentrup, 1977; Higinbotham, Graves, & Davis, 1970; Li, McGrath, & Zhao, 2008; Mercier & Poole, 1980). In the two experiments, we used a hydroponic solution to eliminate the influence of growing medium on the measured SIP signal.

### 2.1 Spectral induced polarization signature and root physical properties

We grew wheat (*Triticum aestivum* L.) in a half-strength Hoagland (Hoagland & Arnon, 1950) hydroponic solution. The solution was aerated by pumping air into a 70-L stock bucket that was subsequently circulated to a series of 20-L buckets in which plants were grown for a period of 60 d. Before the electrical measurement, we transferred nine plants with their floating rock wool platform to a 15-cm-diam.,
FIGURE 1  Scheme of the experimental column. (a) Dimensions of the cylindrical column and arrangement of the electrodes. (b) Horizontal electrode arrangement. The angle between the current (C+ and C−) and potential (P+ and P−) electrodes is π/4 rad. The electrodes were placed 10 cm below the water table (the upper part of the column). To avoid electrode polarization, the potential electrodes P+ and P− are retracted in their holes.

100-cm-long Plexiglas column equipped with electrode ports for electrical measurements (electrical measurements were made on all nine plants together). We used 6-mm-diam., 5-cm-long brass electrodes. The potential electrodes were retracted from their respective holes to ensure minimum electrode polarization (see Schwartz, Shalem, & Furman, 2014; Zimmermann, Kemna, Berwix, Glaas, & Vereecken, 2008). The column and electrode configuration are shown schematically in Figure 1. The geometric factor (allowing to convert measured impedance to conductivity) was obtained by measuring the impedance and the electrical conductivity of the nutrient solution. We tested the sample holder by measuring the SIP signature of the nutrient solution (without roots) and obtained a very low phase (<0.4 mrad at 1 kHz). Electrical measurements of different root parts were performed by pulling the plant up in five increments of 5 cm each and measuring the SIP signature of each part. We used an impedance spectrometer (PSIP, Ontash & Omercon) in a frequency range of 0.1 Hz to 1 kHz.

2.2 | Root cell membrane potential and the spectral induced polarization signal

We grew wheat as for the previous experiment, but for a period of 45 d. Before the electrical measurements, we transferred two plants to two columns (30 cm long, 3 cm diam., equipped with four equally spaced brass electrodes) filled with half-strength Hoagland nutrient solution and placed the columns in a climate-controlled chamber with constant light (800 μmol m−1 s−1), temperature (26 °C), and relative humidity (60%) (FitoClima 1200, Aralab). At t = 0, we replaced the nutrient solution of one of the columns with a half-strength nutrient solution that, in addition to nutrients, also contains 10 μM carbonyl cyanide 3-chlorophenylhydrazone (CCCP). Note that the electrical conductivity and temperature of the two solutions were identical. After the replacement of the solution, we continuously measured the SIP spectral of the two columns for 2 h. Measurements were performed in a frequency range of 0.1 Hz to 1 kHz.

2.3 | Data processing

We analyzed the electrical spectra using the double Cole–Cole model (see Chen, Kemna, and Hubbard, 2008, and references within):

\[
\rho^* = \rho_0 \left( 1 - \sum_k m_k \left[ \frac{1}{1 + (i\omega\tau_k)^c} \right] \right)
\]

where \(\rho^*\) is the complex electrical resistivity, \(\rho_0\) is zero-frequency resistivity, \(m\) is the chargeability that relates to the magnitude of polarization, \(i\) is the complex unit, \(\omega = 2\pi f\) is the angular frequency, \(\tau\) is the relaxation time that relates to the length scale of polarization, \(c\) is the Cole–Cole exponent that relates to the uniformity of the polarization length scale, and the subscript \(k = 1\) and 2 represents the low- and high-frequency range of the electrical spectrum, respectively. The model was fitted to the data using the Markov chain Monte Carlo (MCMC) method (Chen et al., 2008). One advantage of the MCMC algorithm is that the uncertainty in model parameters can be considered. As in Chen et al. (2008), we use the 95% highest probability domains of unknown parameters as a measure of uncertainty and report the median as the parameter estimator. The goodness of fit was estimated using normalized RMSE (NRMSE). The maximum NRMSE was 2% for the first experiment, and 5% for the second experiment (nutrient solution with CCCP). Below, we only discuss parameters related to the low-frequency range (\(k = 1\)) of the electrical spectra.
FIGURE 2  Electrical spectra as a function of frequency (f) for different zones of the root system. (a) Insensitivity of the in-phase conductivity ($\sigma'$) to the presence of the root (note the scale of the y axes in Panel a), and (b) consistent decrease in quadrature conductivity ($\sigma''$) from Zone 1 to Zone 5. (c) The phase shift ($\phi$). The shadow regions represent the theoretical limit of the accuracy of the measured phase shift of the system (0.2 mrad). The legend demonstrates the correspondence between the measured signal and the root zone. The baseline is the electrical spectra of the sample holder with the nutrient solution (i.e., before root was added).

3 | RESULTS

3.1 | Relationships between root properties and the spectral induced polarization signature

Figure 2 shows the electrical spectra of different zones in the root system. The in-phase conductivity ($\sigma'$) is almost flat, and the differences between the $\sigma'$ values of the different root zones are small (maximum difference of $\sim$1.5%, Figure 2a). Since the measured electrical conductivity of the nutrient solution is 1172 $\mu$S cm$^{-1}$ (i.e., similar to the $\sigma'$), it is safe to assume that the influence of the roots on the in-phase conductivity is negligible, in agreement with the results of Weigand and Kemna (2019).

The volume fraction of the root (volume of root occupying the zone divided by the volume of the zone) is 21% in Zone 1, and consistently decreases to 1% in Zone 5. The insensitivity of the in-phase conductivity to the presence of the highly resistive roots (Cao, Repo, Silvennoinen, Lehto, & Pelkonen, 2011), despite the high volumetric root/solution ratio, is explained in terms of the equivalent circuit model. Assume that the resistance of the nutrient solution is $R_w$, and the resistance of the root is $R_r$. Since the current is not forced to pass through the roots, a parallel connection between the resistors can be considered, and the total resistance of the system is given by $R_T = R_wR_r/(R_w + R_r)$. As $R_r >> R_w$ (e.g., Ehosioke et al., 2018 showed that $R_r \approx 1$ M$\Omega$), the resistor with the low resistivity (the nutrient solution) controls the total resistance of the system. Since the current does not pass through the cell membrane, the major volume of the root can be considered to be highly resistive (Bera, Bera, Kar, & Mondal, 2016). The main outcome of this is that as long as the connection between roots and the growing media can be considered in parallel (i.e., current is not forced to cross through the root), and $R_r \gg R_w$, the contribution of roots to the in-phase conductivity is negligible.
conductivity is expected to be small. Another consequence is that care must be taken in designing rhizotron experiments for electrical studies of roots (Rao et al., 2018; Weigand & Kemna, 2017, 2019), as the two-dimensional-like geometry of the rhizotron might force the current to pass through the roots. This could result in misinterpretation of the data as such a configuration is similar (in terms of equivalent circuit models) to a connection of resistors in series where the resistor with the highest resistance governs the total resistance of the system ($R_T = R_w + R_r$).

The spectra of the quadrature conductivities ($\sigma''$, Figure 2b) are typical for low-frequency electrochemical polarization processes (Leroy et al., 2008). In Zone 1, $\sigma''$ is highest, and it decreases consistently with increasing distance from the plant stem. In Zone 5, $\sigma''$ approaches zero, and the spectrum of this zone is similar to that obtained for the nutrient solution alone (i.e., without roots). The decrease in $\sigma''$ from Zone 1 to Zone 5 is consistent with the decrease in the amount of roots between those zones. This is evident from Figures 3a and 3b, showing a consistent decrease in root mass and root surface area, respectively, from Zone 1 (near the stem) to Zone 5. We found a linear correlation between both the root mass and root surface area and the chargeability (i.e., the magnitude of polarization) ($R^2 = .96$ and .76, respectively; Figure 4). We attribute the better correlation between chargeability and root mass to the simpler and more accurate measurement of root mass compared with root surface area (which includes scanning and an image-analysis algorithm). Note that since the average diameter of the roots between the different zones is relatively constant (except for Zone 5, see Figure 3c), the ratio between the root surface area and root mass is also constant. Therefore, we expect the same trends between root surface area and root mass, and the polarization.

The relaxation time and the Cole–Cole exponent were not sensitive to the measured root parameters. The relaxation times for Zones 1 and 2 were similar (10.8 and 10.5 ms, respectively), and higher for Zones 3 and 4 (13.7 and 13.5 ms, respectively). Since the differences in average diameter between the different root zones are relatively small (Figure 3c), it is unlikely that the increase in the relaxation time between the zones is related to the measured geometry, and at this stage, the 3-ms differences in relaxation time between the zones remain unexplained. The Cole–Cole exponent was $\sim .5$ in Zones 1, 2, and 3 and increased to $.58$ in Zone 4. Since the electrical spectrum in Zone 5 was flat and in the same range as that of the nutrient solution, the Cole–Cole exponent and relaxation time for this zone are meaningless.

### 3.2 Membrane potential as the source of polarization

To test the role of the root membrane potential in determining root polarization, we reduced the root cell membrane potential by adding CCCP. In Figure 5, we show the temporal

![Figure 3](image-url)

**FIGURE 3** Vertical distribution of the root properties. (a) Root mass and (b) total surface area decrease toward the root tip. (c) Changes in root diameter between the different zones are relatively small. Recall that Zone 1 is near the root collar (see Figure 1d)
F I G U R E 4  Chargeability ($m$) as a function of (a) root mass and (b) total surface area. The linear correlations with the coefficient of determination ($R^2$) are shown in the figure. The shadow regions represent the uncertainty in the model parameters.

F I G U R E 5  Electrical spectra at representative times following the addition of CCCP. The in-phase ($\sigma'$) and quadrature ($\sigma''$) conductivity for the full frequency ($f$) range is shown in Panel a and Panel b, respectively. The inset (Panel c) shows an expanded view of the region in the shaded square. The gray rectangular represents the theoretical limit of the measurement system (0.2 mrad). The percentage decrease in quadrature conductivity from the beginning of the experiment is shown in Panel d ($\sigma''_0$ is the quadrature conductivity at the beginning of the experiment).
change in the SIP signature of roots following the addition of CCCP. In general, with time, the in-phase conductivity slightly increases (Figure 5) while the quadrature conductivity decreases (Figures 5b and 5c). The maximum difference in the quadrature conductivity in the times after the addition of cyanide was observed at 30 Hz (Figure 5d), and due to the relatively low signal, we focus our analysis on that frequency.

At 30 Hz, the in-phase conductivity of the control and CCCP treatments consistently increased with time (7 and 2% after 2 h, respectively; Figure 6a). A plausible explanation for the increase in $\sigma'$ in the two treatments is the activity of the root and the release of root exudates to the nutrient solution, which results in an increase in the solution's electrical conductivity. Indeed, the electrical conductivity of the nutrient solution increased from 1,206 to 1,293 $\mu$S cm$^{-1}$ for the control sample, and from 1,207 to 1,236 $\mu$S cm$^{-1}$ for the CCCP treatment. The smaller increase in $\sigma'$ in the poisoned treatments further supports this explanation, as CCCP decreases root activity.

A systematic decrease in the quadrature conductivity was observed for the CCCP treatment, while at the same time the quadrature conductivity of the control treatment was relatively constant (an example at 30 Hz is given in Figure 6b). The decrease in polarization that followed the addition of CCCP is further illustrated in Figure 7a, where the chargeability is shown as a function of time (with the advantage of considering the full electrical spectrum). In the first 30 min, the chargeability decreased by ~65% and then remained more or less constant until the end of the experiment. Interestingly, Higinbotham et al. (1970) observed an increase (less negative) of ~60% in the membrane potential of pea ($Pisum sativum$ L.) root cells 20 min after they were exposed to cyanide, and a relatively constant potential thereafter. We therefore attribute the decrease in polarization that followed the addition of CCCP to depolarization of the root membrane potential. The classical EDL polarization model reinforces the hypothesis of dependency between root membrane potential and the measured polarization. This is as the polarization is related to the surface charge density of the polarized surface ($\Sigma_5$ in Equation 4), and as the surface charge and the surface potential are linked, for example by the Grahame equation.

In addition to chargeability, which reflects the magnitude of the polarization, the relaxation time and the Cole–Cole exponent provide important information on the scale of the polarization length and its broadness, respectively. In the first 1.5 h, the relaxation time consistently increased from ~2 to ~11 ms and then remained relatively constant (Figure 7b). A possible (but at this stage somewhat speculative) explanation for the increase in relaxation time is the faster depolarization of the root hairs compared with the root cells on the main root axis. This is because the root hair has a larger surface area in contact with the nutrient solution, and hence the transport of CCCP to these cells is faster than to cells on the main root axis. The depolarization of the smaller root hairs (diameter of 5–17 $\mu$m; Gahoonia, Care, & Nielsen, 1997; Grierson, Nielsen, Ketelaar, & Schiefelbein, 2014) increases the relative contribution of larger roots to the total polarization, resulting in an increase in relaxation time.

4 | DISCUSSION

Our results demonstrate a linear relationship between architectural structures of wheat root and electrical chargeability (Figure 4). The magnitude of polarization and the range of relaxation time reported here and in Weigand and Kemna (2019) are similar. For example, in our study, the normalized chargeability ($m_n = m\sigma_0$, where $\sigma_0$ is the lowest frequency in-phase conductivity) in Zone 1 is ~5 $\mu$S cm$^{-1}$ and the relaxation time is ~10 ms. In Weigand and Kemna (2019) the mean normalized chargeability of the root system is ~4 $\mu$S cm$^{-1}$ (see their Figure 6), with relaxation time values that range between 5 and 20 ms. Similarities between the electrical properties of the root systems are found despite the different root systems (wheat in our study and oilseeds in the other study), and the different data processing approaches (double Cole–Cole model in this study and Debye decomposition in Weigand and Kemna, 2019).

Interestingly, in the work of Ehosioke et al. (2018), the magnitude of polarization and the relaxation frequency were much higher. In that work, the phase shift at the peak frequency is 400 mrad (in terms of the quadrature conductivity, this is ~1,600 $\mu$S cm$^{-1}$; i.e., three orders of magnitude higher than in this work), and the peak frequency is at ~10 kHz (this is associated with a relaxation time of 0.016 ms). Oziern-Lafontaine and Bajazet (2005) also reported high polarization (350–500 mrad) at relatively high frequencies (~2 kHz) for tomato ($Solanum lycopersicum$ L.) plants. Note that in that study, one current electrode was inserted to the stem and the potential electrode placed in the solution (stem-based method, see Kessouri et al., 2019).

The differences between the polarization and relaxation time of the two approaches can provide some insights about the mechanisms controlling the SIP signature of roots. We consider two different models for the low-frequency polarization of roots. In the cell model (Weigand & Kemna, 2019), the total surface area of the roots’ EDLs is polarized. Consequently, the magnitude of polarization should be related to the root biomass and the relaxation time to the length scale of the root cell. Assuming a diffusion coefficient of $10^{-9}$ m$^2$ s$^{-1}$, Weigand and Kemna (2019) calculate a polarization length scale ranging between 2 and 7 $\mu$m. Using the same diffusion coefficient, as an example, we obtained for Zone 1 in the first experiment a polarization length scale of 4.4 $\mu$m. Evidently,
**FIGURE 6** Relative changes in the (a) in-phase ($\sigma'$) and (b) quadrature ($\sigma''$) conductivities, and (c) phase shift ($\phi$, at 30 Hz) as a function of time for the control and poisoned treatments. The changes are shown with respect to the beginning of the experiment (time zero). Index “0” refers to the values at the beginning of the experiment. Note the $\sim$30% drop in quadrature conductivity for the poisoned treatment.

**FIGURE 7** Parameters from the Cole–Cole model as a function of time for the poisoned treatment. Cyanide (a) reduces the chargeability ($m$) and (b) increases the relaxation time ($\tau$) of the root. (c) The Cole–Cole exponent ($c$) increases in the first 45 min and then decreases slightly and fluctuates around 0.52. The shadow regions represent the uncertainty in the model parameters.

This is at the same scale as the size of the root cell diameter (Grierson et al., 2014). It is, however, important to note that there is uncertainty about the exact value of the diffusion coefficient, as it is given for Na in a free solution, and in our case, there are multiple chemical species near the cell membrane (each with a different diffusion coefficient). In addition, as suggested by Bücker et al. (2019), the relationship between the relaxation time and the polarization length scale also depends on the chemical properties of the electrolyte (the parameter $\alpha$ in Equation 5).
In the alternative model proposed here, polarization occurs at the external surface area of the root. Consequently, the relaxation time is related to the length scale of roots’ external structures such as root diameter and root hair. The magnitude of polarization is associated with the outer surface area of the root and not with the root biomass. Comparing the results from the two approaches to measure root polarization—stem-based (Ehosioke et al., 2018; Ozier-Lafontaine & Bajazet, 2005) and contact-free (this work and Weigand and Kemna, 2019)—supports the hypothesis that polarization is dominated by the external surface area of the root. First, using the same diffusion coefficient \(10^{-9} \text{ m}^2 \text{s}^{-1}\), the polarization length scale in the stem-based approach is much lower than in the contact-free approach \(0.17–0.4 \mu\text{m in the first case, and 2–7 \mu m in the second case}\). The ratio between the cell diameter and root diameter for wheat is \(\sim 1:40\) (Colombi, Herrmann, Vallenback, & Keller, 2019), meaning it is in the same order of magnitude as the ratio between the polarization length scale obtained by the two approaches. Second, the polarization is orders of magnitude higher in the stem-based method, which suggests that the polarized surface area in that method is larger than in the contact-free approach.

We suggest that in the stem-based approach, a direct connection between the root and the electrodes (even if through a conductive bridge) results in a passage of the electric current through the inner part of the root and, therefore, in the polarization of the entire root’s cells EDLs. In this case, the polarization length scale is the cell diameter. On the other hand, when the root system embedded in an electrolyte, we postulated that the electrical current does not penetrate through the highly resistive root surface, and polarization occurs only at the external surface area of the root. In that case, the polarization length scale is the root diameter. We note that at this stage, only indirect evidence supports our suggested conceptual model and that more experimental work and models on root polarization are needed.

5 CONCLUSION

In this study, we directly link the SIP signature of wheat roots to their surface area and biomass and demonstrate that the source of polarization is the cell membrane potential. Based on a comparison between the results from this study, and results from studies that used the stem-based approach, we suggest that in the contact-free approach the magnitude of polarization is related to the external surface area of the root and that the relaxation time is related to the root diameter.

Overall, the results from this study enhance our understanding of the relationships between root properties and their SIP signature. It appears that a severe limitation arose from the low polarization of roots measured with the contact-free approach. Although in the zero-polarization hydroponic medium we detect a clear polarization, it is questionable whether, in a polarizable medium such as soil, roots can be detected using the SIP method. This is especially true, as many soils have a higher magnitude of polarization than detected for roots. However, since root activity significantly changes the physical, chemical, and biological properties of the rhizosphere (the zone near the root), and because the SIP method is sensitive to these soil properties, it might be that the rhizosphere will generate a measurable electrical signature.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Nimrod Schwartz https://orcid.org/0000-0002-9350-2047

REFERENCES

Anderson, W. P., Robertson, R. N., & Wright, B. J. (1977). Membrane potentials in carrot root cells. Functional Plant Biology, 4, 241–252. https://doi.org/10.1071/pp9770241

Atkinson, D. (2000). Root characteristics: Why and what to measure. In A. L. Smit, A. G. Bengough, C. Engels, M. van Noordwijk, & S. Pellerin (Eds.), Root methods: A handbook (pp. 1–32). Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-662-04188-8_1

Bera, T. K., Bera, S., Kar, K., & Mondal, S. (2016). Studying the variations of complex electrical bio-impedance of plant tissues during boiling. Procedia Technol, 23, 248–255. https://doi.org/10.1016/j.protechnol.2016.03.024

Binley, A., Hubbard, S. S., Huisman, J. A., Robinson, D. A., Singha, K., & Slater, Lee D. (2015). Understanding of subsurface processes over multiple scales. Water Resources Research, 51, 3837–3866. https://doi.org/10.1002/2015WR017016

Bücker, M., Flores Orozco, A., Undorf, S., & Kemna, A. (2019). On the role of stem- and diffuse-layer polarization mechanisms in porous media. Journal of Geophysical Research: Solid Earth, 124, 5656–5677. https://doi.org/10.1029/2019JB017679

Cai, G., Vanderborght, J., Klotzsche, A., Van Der Kruk, J., Neumann, J., Hermes, N., & Vereecken, H. (2016). Construction of minirhizotron facilities for investigating root zone processes. Vadose Zone Journal, 15(9). https://doi.org/10.2136/vzj2016.05.0043

Cao, Y., Repo, T., Silvennoinen, R., Lehto, T., & Pelkonen, P. (2011). Analysis of the willow root system by electrical impedance spectroscopy. Journal of Experimental Botany, 62, 351–358. https://doi.org/10.1093/jxb/erq276

Chen, J., Kemna, A., & Hubbard, S. S. (2008). A comparison between Gauss-Newton and Markov-chain Monte Carlo-based methods for inverting spectral induced-polarization data for Cole-Cole
conduction in soil-root continuum: A virtual rhizotron study. Biogeo-
sciences Discussion. https://doi.org/10.5194/bg-2018-280
Revil, A., Coperay, A., Shao, Z., Florsch, N., Fabricius, I. L., 
Deng, Y., … Gunnink, J. L. (2017). Complex conductivity of soils. Water Resources Research, 53, 7121–7147. https://doi. 
org/10.1002/2017WR020655
Revil, A., Koch, K., & Holliger, K. (2012). Is it the grain size or the 
characteristic pore size that controls the induced polarization relax-
ation time of clean sands and sandstones? Water Resources Research, 48(2). https://doi.org/10.1029/2011WR011561
Rewald, B., & Ephrath, J. E. (2013). Minirhizotron techniques. In E. 
Amram & T. Beeckman (Eds.), Plant roots: The hidden half (4th ed., 
pp. 42.1–15). New York: CRC Press.
Ryan, P. R., Delhaize, E., Watt, M., & Richardson, A. E. (2016). 
Plant roots: Understanding structure and function in an ocean of 
complexity. Annals of Botany, 118, 555–559. https://doi.org/ 
10.1093/aob/mcw192
Schwartz, N., Shalem, T., & Furman, A. (2014). The effect of 
organic acid on the spectral-induced polarization response of 
soil. Geophysical Journal International, 197, 269–276. https://doi. 
org/10.1093/gji/ggt529
Tsukahara, K., Yamane, K., Yamaki, Y., & Honjo, H. (2009). A 
nondestructive method for estimating the root mass of young 
peach trees after root pruning using electrical capacitance mea-
surements. Journal of Agricultural Meteorology, 65, 209–213. 
https://doi.org/10.2480/agrmet.65.2.6
van Beem, J., Smith, M. E., & Zobel, R. W. (1998). Estimating 
root mass in maize using a portable capacitance meter. Agron-
omy Journal, 90, 566–570. https://doi.org/10.2134/agronj1998. 
00021962009000040021x
Vanderborght, J., Huisman, J. A., van der Kruk, J., Vereecken, H., 
Anderson, S. H., & Hopmans, Jan W. (2013). Geophysical meth-
ods for field-scale imaging of root zone properties and processes. In 
S. H. Anderson & J. W. Hopmans (Eds.), Soil–water–root processes: 
Advances in tomography and imaging (pp. 247–282). Madison, WI: 
SSSA. https://doi.org/10.2136/sssaspecpub61.c12
Weigand, M., & Kemna, A. (2017). Multi-frequency electrical 
impedance tomography as a non-invasive tool to characterize and 
monitor crop root systems. Biogeoosciences, 14, 921–939. 
https://doi.org/10.5194/bg-14-921-2017
Weigand, M., & Kemna, A. (2019). Imaging and functional char-
acterization of crop root systems using spectroscopic electrical 
impedance measurements. Plant and Soil, 435, 201–224. 
https://doi.org/10.1007/s11104-018-3867-3
Zarebanadkouki, M., Kim, Y. X., & Carminati, A. (2013). Where do roots 
take up water? Neutron radiography of water flow into the roots of 
transpiring plants growing in soil. New Phytologist, 199, 1034–1044. 
https://doi.org/10.1111/nph.12330
Zhang, C., Revil, A., Fujita, Y., Munakata-Marr, J., & Redden, G. 
(2014). Quadrature conductivity: A quantitative indicator of bac-
terial abundance in porous media. Geophysics, 79, D363–D375. 
https://doi.org/10.1190/GEO2014-0107.1
Zimmermann, E., Kemna, A., Berwix, J., Glaas, W., & Vereecken, 
H. (2008). EIT measurement system with high phase accuracy 
for the imaging of spectral induced polarization properties of 
soils and sediments. Measurement Science & Technology, 19. 
https://doi.org/10.1088/0957-0233/19/9/094010

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