Exploration of Induced Polarization as a Non-Invasive Method to Quantify Herbaceous Root Traits

Nick Chou

ABSTRACT

This study evaluated the potential of using induced polarization to quantify herbaceous crop root traits. The target crop species was wheat (*T. aestivum*) grown in a lab-scaled hydroponic system. The magnitude of induced polarization was measured from the fixed electrodes in six vertical subzones (0-30cm) near the root on a Rhizotron. The magnitude was expressed in terms of resistance under two current injection scenarios: injection into the background aqueous medium versus into the plant via lower stem. The difference of the resistance between the two injection scenarios corresponded to the change in polarization response from the root exterior at which the measurements were acquired. We examined the correlation between the resistance contrast and the five root traits measured ex situ in the root image analysis software WinRhizo (length, surface area, volume, dry and wet biomasses). We selected root volume as our target root trait for quantification because it had the most similar vertical pattern and highest correlation with resistance data. Then, we created a two-step regression model combining a three-parameter logistic growth with a linear model to mathematically link resistance to the global root volume. We observed a general decrease in polarization response from the root exterior between the two injection scenarios, and we examined its relationship with the increase in sub-cellular and tissue-scaled capacitance within the root interior. Our regression model showed significant relationship between resistance and global root volume especially in the lower range of the values.

KEYWORDS

Root resistance, Root capacitance, Regression modeling, WinRhizo, Root volume, Hydroponic
INTRODUCTION

Climate change and rising food demand bring up new challenges for crop management in the conventional agriculture. The increases in temperature, precipitation intensity and drought duration have accelerated wind and rain erosion of the topsoil rich in organic matter (SOM) (Lee et al. 1996, Pimentel 1996). Because SOM strongly affects a soil’s capability of retaining water (Marchner 1995), the loss of SOM reduces soil moisture content and thus the moisture-sensitive microbial SOM decomposition that provide crops organic nutrients (Dakora and Keya 1997, Lal 2006). Moreover, intensified rainstorms have accelerated leaching of essential minerals such as nitrogen and phosphate (Zougmore et al. 2009). The degradation of bioavailable soil organic and inorganic nutrients has negatively impacted crop yields (Smith et al. 2009). In response to a series of climate-driven soil degradation, optimization of fertilizer application to ensure nutrient supply in the root zone has become pivotal. While inadequate fertilizer application hinders crop growth in mineral depleted fields (Sanchez 1976), excessive fertilization exacerbates mineral leaching and cause soil salinity stress (Flowers et al. 1997). Both fertilizer mismanagements are unsustainable and would further reduce crop yields in the long term.

As crop yields heavily depend on root development (St.Clair and Lynch 2010), and root development is constrained by soil nutrients, certain root morphological traits could potentially be used as the predictors for both soil quality and crop performance. Studies have found that increasing nitrogen fertilization below an optimal application level promotes wheat yields and wheat root development such as root length density (Ehdaie et al. 2010), surface area (Xue et al. 2014), weight density (Hossain et al. 2008) and dry biomass (Sinha 2015). On the other hand, decreasing nitrogen fertilization led to lower wheat yields (Wang et al. 2014), but it could also promote wheat root length (Sinha 2015), biomass and weight density (Elazab et al. 2016). While soil nitrogen availability and wheat yields were found to be positively correlated, there are still discrepancies on how this positive correlation manifests itself in the root morphology. To better understand the linkages encrypted in the root, non-destructive methods to quantify root traits are desirable. However, because of the root’s belowground location, not only were the studies on roots limited, but the sampling methods were usually destructive.

To tackle the root’s hidden nature and minimize physical disturbances during sampling processes, different geophysical methods have been explored as non-destructive approaches to
study root morphology in forestry and agriculture. For instance, electrical resistivity imaging was successfully applied to distinguish woody tree roots from soil and moisture (Ain-Lhout et al. 2016), and electrical impedance tomography also showed the potential in spatially characterizing crop roots physiological properties in both lab and field scale under nutrient stressed conditions (Weigand et al. 2017). In addition, induced polarization (IP) methods measure the media’s electrical responses such as the resistance and phase shift (Revil et al. 2012). In forestry, IP has been applied to spatially construct tree root architecture by differentiating roots from soil, and the resistivity data acquired from IP were also found to be correlated with root traits such as root volume and root growth direction (Mary et al. 2017), suggesting the potential of using IP to quantify root morphology. However, to the state of the art, IP has been more widely tested on woody roots, while few studies used herbaceous roots as their study samples. Therefore, studies that focus on the correlation between IP measurements and crop root morphology are needed.

The objective of our study is to address the following question: can we use IP as a non-destructive method to quantify root traits of hydroponically-grown wheat? Wheat is one of the major staple crops around the world, and hydroponic systems allow easier and less destructive harvesting and measuring processes. In our study, we will use a frequency-domain IP system to acquire the root’s electrical responses and WinRhizo image analytical software to analyze wheat root morphology. To identify the root traits that we aim to quantify, we will investigate the vertical patterns of the electrical responses and five root traits (length, surface area, volume, dry and wet biomasses) along the root depth and the correlation between the two datasets. Based on our data, we will construct a mathematical model that uses IP measurements as inputs and predicts the values of the target root trait. Finally, we will evaluate the model’s performance and IP as a non-destructive method to quantify herbaceous root morphology.

METHODS

Growth

We surface disinfected wheat seeds with 0.5mM NaOCl and rinsed them thoroughly with deionized water. The seeds were germinated with moist paper towel in Petri-dishes under dark conditions for 3 days. After they sprouted, we transferred the seedlings into a hydroponic
container filled with Hoagland solution (citation). The solution temperature was in the range of 25-28C, and the solution pH was in the range of 5.5-6.5. We refilled the container with tap water daily and renewed it biweekly with new Hoagland solution. The solution was constantly aerated to ensure oxygen supply to the roots.

The hydroponic container was inside a chamber (200x50x150cm) with direct lighting (1500µmol/m²/s) of 16 photoperiod hours. The relative humidity in the chamber was in the range of 40-60%, and the ambiance temperature was in the range of 28-32C during the light period and 24-28C during the dark period. After 8 weeks, we gradually harvested the wheat for measurements.

**Data collection**

*Frequency domain induced polarization (FDIP)*

Upon harvest, we performed FDIP measurement (MPT-DAS-1, USA) on the wheat (n=27) in a rhizotron (PCB, 53x53x2cm). On the Rhizotron’s surface, 64 potential electrodes (Ag/AgCl) spaced out evenly in an 8x8 (35x35cm) mesh grid. For each electrode, half of it was penetrated through the Rhizotron and immersed in the solution (2.5mM NaCl), while the other half was outside and connected to the FDIP measurement system. We placed the wheat in the center and aligned the stem-root interface with the second row of electrodes (Figure 1).

![Figure 1. Setup of FDIP measurements in a rhizotron.](image-url)
During FDIP measurement, the system delivered alternating current that cyclically polarized the root-water continuum. It measured the built-up and decay of the potential field ($\Delta V_P$) in the medium due to polarization using a reference resistor and a pair of potential electrodes on the Rhizotron (Figure 2). The system’s outputs were apparent resistance ($R_{app}$) values, which were in direct relationship with the normalized maximum magnitude of the induced polarization between each pair of potential electrodes (Equation 1). For our measurements, we specified the current source’s alternating frequency at 1Hz and targeted magnitude at $200\text{V}/20\text{mA}$. Lower frequency allowed enough time for the medium to be maximally polarized, while higher voltage and lower current intensity produced data ($R_{app}$) in the desirable magnitude without permanently damaging the wheat.

\[
I = \frac{V_{ref}}{R_{ref}} \quad \& \quad R_{app} = \frac{\Delta V_P}{I} \quad (\text{Equation 1})
\]

**Figure 2.** (a) FDIP system setup. Current was injected into the medium and received back to the system with two current electrodes (I+ and I-). Polarization was measured with two potential electrodes (P+ and P-). (b) A diagram of induced polarization. The measured potential difference ($\Delta V_P$) was the maximum magnitude of induced polarization between two potential electrodes due to current injection.
To measure the polarization in the root-water continuum, we injected current into the lower stem near the root-stem interface and measured the resistance ($R_p$) (Figure 4a). We also measured the background polarization ($R_w$) by injecting current into the solution near the previous injection point on the stem (Figure 4b). By subtracting $R_p$ from $R_w$, we calculated the portion of resistance ($R_{diff}$) contributed by the polarization due to the current traveling in the root system. To correlate resistance measurements with root traits, we measured $R_p$ and $R_w$ in the near-root zone using diagonal pairs of potential electrodes along the major current pathway (Figure 4c). Our study design was exempt from the heterogeneity caused by variable soil moisture and texture in the fields (Cao et al. 2010), and the polarization effect on the electrodes especially at a lower frequency was also reduced by using the same reversible Ag/AgCl electrodes with fixed location on the Rhizotron (Ozier-Lafontaine and Bajazet 2005), while only the current electrode ($I^+$) was switched in between measurements.

![Electrode configuration](image)

**Figure 4. Electrode configuration.** (a) $R_p$: current injection into the lower stem. (b) $R_w$: current injection into the solution in proximity to the injection point of $R_p$ measurements. (c) Diagonal pairs of potential electrodes in the near-root zone.

**Root Data**

**WinRhizo.** To obtain root morphological data, we scanned root images (EPSON 11000, 2016, USA) after FDIP measurements and performed image analyses in a root image analytical software (WinRhizo, Pro 2017 Version, Canada). We cut the roots from the wheat and spread
them out in a transparent tray filled with shallow water, maintaining their positions in the tray relative to their original depths in the Rhizotron. We then acquired 1200 dpi high-resolution black-and-white root images (Figure 5) with the scanner and performed grey analyses in WinRhizo. In WinRhizo, we divided the roots into six 5-cm vertical subzones (0-5cm, 5-10cm, etc.) corresponding to the second to eighth rows of electrodes on the Rhizotron. For each subset, WinRhizo calculated the local root length (RL), surface area (RSA) and volume (RV). For each individual wheat, we summed the local data and obtained global root data.

Figure 5. Scanned black-and-white root images.

**Biomass.** To acquire root biomass data, we towel-dried the roots and measured their global wet biomass (RWM), then we oven-dried the roots under 60°C for 48 hours and measured their global dry biomass (RDM). To calculate local root biomass, we estimated the biomass allocated in each subzone based on the corresponding local RV.

**Statistical analysis**

**Resistance and Root Data**

To determine the root trait that we would aim to predict from FDIP measurements, we performed statistical tests between the resistance and root data. Firstly, to compare the vertical patterns of resistance and each root trait, we performed Welch F-tests using the 6 vertical
subzones as categories. Secondly, we calculated Pearson correlation coefficients between resistance and each local root trait both measured in the same vertical subzone. Based on the statistical results, we selected the target root variable that fulfilled three criteria: (1) the target root variable showed similar vertical pattern with resistance, (2) the target root variable’s local values were strongly correlated to the resistance data. Lastly, to identify in which subzone was the local root data most strongly correlated with the global root data, we performed Pearson correlation tests between local root data in each subzone and global root data.

Model fitting and testing

To evaluate the potential of FDIP method in quantifying root morphological traits, we formulated a two-step mathematical model that used local resistance data to predict global values of the target root trait. The reason for creating a two-step model rather than a single-step one was because our resistance data were collected with respect to different depths instead of spanning through the entire root zone. Therefore, the first-step of our model used resistance data as the predictor and the target root trait’s local values in a specific subzone as the response variable. Because the root data were positively skewed among our samples, which was a common observation for root measurements (Pielou 1969, Amato et al. 2011), we decided to test statistical regression models that were also suitable for non-normally distributed data: generalized linear models (GLM) and logistic models. We examined several univariate models under the GLM and logistic model family (including Gaussian GLM, Gamma GLM, inverse Gaussian GLM and logistic growth model) with different polynomial degrees (1-3) and link functions (for GLM) and raw and log-transformed root data (to correct for non-normal data). We decided our final model based on the minimization of the root mean square error (RMSE) and Akaike information criterion (AIC, for GLM). The second step of our linear model used the estimated local values to predict the target root trait’s global values as the final output. To test the performances of our models, we performed linear regression between the measured and predicted local root data from the first-step of our model, and between the measured and predicted values from the second-step of our model. All statistical analyses were performed in R studio software (Version 3.4.2).
RESULTS

Resistance and Root Data

Among the 27 wheat samples, the resistance ($R_{\text{diff}}$, $R_w - R_p$) was significantly higher in 0-5 cm, while no differences were observed across the subzones beneath 5 cm. Similarly, root volume (RV), wet mass (RWM) and dry mass (RDM) were also significantly higher only in the top subzone. Meanwhile, root surface area (RSA) was significantly higher in 0-10cm, and root length (RL) was highest in 5-10cm and gradually decreased as the depth increased (Table 1). In agreement with the similar vertical pattern found among resistance, RV, RWM and RDM, the resistance measured in each subzone was strongly correlated ($r \geq 0.7$) with local RV, RWM and RDM in the corresponding depth, while it was moderately correlated ($0.7 > r \geq 0.5$) with local RSA and weakly correlated ($0.5 > r$) with local RL (Table 2).

Table 1. Average values of resistance and local root trait in 6 subzones.

<table>
<thead>
<tr>
<th>Vertical subzones</th>
<th>Welch statistic</th>
<th>0-5cm</th>
<th>5-10cm</th>
<th>10-15cm</th>
<th>15-20cm</th>
<th>20-25cm</th>
<th>&gt;25cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance data</td>
<td>$R_{\text{diff}}$ (Ω)</td>
<td>$F_{5,70.12} = 6.215$ ***</td>
<td>3.617 b</td>
<td>1.206 a</td>
<td>0.631 a</td>
<td>0.386 a</td>
<td>0.277 a</td>
</tr>
<tr>
<td>Local root data</td>
<td>RL (cm)</td>
<td>$F_{5,66.32} = 28.018$ ***</td>
<td>202.30 cd</td>
<td>230.87 d</td>
<td>137.81 bc</td>
<td>75.38 ab</td>
<td>40.78 a</td>
</tr>
<tr>
<td></td>
<td>RSA (cm²)</td>
<td>$F_{5,65.39} = 26.255$ ***</td>
<td>33.12 b</td>
<td>23.75 b</td>
<td>11.50 a</td>
<td>5.85 a</td>
<td>3.09 a</td>
</tr>
<tr>
<td></td>
<td>RV (cm³)</td>
<td>$F_{5,61.09} = 13.704$ ***</td>
<td>0.58 b</td>
<td>0.21 a</td>
<td>0.08 a</td>
<td>0.04 a</td>
<td>0.02 a</td>
</tr>
<tr>
<td></td>
<td>RWM (mg)</td>
<td>$F_{5,61.12} = 11.946$ ***</td>
<td>704.80 b</td>
<td>250.73 a</td>
<td>91.91 a</td>
<td>47.46 a</td>
<td>27.51 a</td>
</tr>
<tr>
<td></td>
<td>RDM (mg)</td>
<td>$F_{5,61.13} = 15.711$ ***</td>
<td>111.30 b</td>
<td>38.02 a</td>
<td>13.82 a</td>
<td>7.26 a</td>
<td>4.35 a</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001

Table 2. Pair-wise correlation coefficient ($r$) between resistance and local root data (n=137).

<table>
<thead>
<tr>
<th>Local root data</th>
<th>RSA</th>
<th>RV</th>
<th>RWM</th>
<th>RDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance data</td>
<td>$R_{\text{diff}}$</td>
<td>0.335 ***</td>
<td>0.606 ***</td>
<td>0.751 ***</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001
The local values of each root trait showed the strongest correlation with its global values in either the first (for RV, RWM and RDM) or second (for RL and RSA) subzone. With the exceptions of local RL and RSA in 0-5cm, the strength of the correlation dwindled as the depth increased. The correlation became weak for all the root traits beneath 20cm (Table 3).

Table 3. Pair-wise correlation coefficient (r) between global and local root data in each subzone (n=27).

<table>
<thead>
<tr>
<th>Global root data</th>
<th>0-5cm</th>
<th>5-10cm</th>
<th>10-15cm</th>
<th>15-20cm</th>
<th>20-25cm</th>
<th>&gt;25cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL</td>
<td>0.417</td>
<td>0.933</td>
<td>0.839</td>
<td>0.796</td>
<td>0.543</td>
<td>0.252</td>
</tr>
<tr>
<td>RSA</td>
<td>0.802</td>
<td>0.961</td>
<td>0.868</td>
<td>0.807</td>
<td>0.512</td>
<td>0.116</td>
</tr>
<tr>
<td>RV</td>
<td>0.971</td>
<td>0.945</td>
<td>0.870</td>
<td>0.756</td>
<td>0.488</td>
<td>-0.028</td>
</tr>
<tr>
<td>RWM</td>
<td>0.975</td>
<td>0.959</td>
<td>0.858</td>
<td>0.692</td>
<td>0.492</td>
<td>0.094</td>
</tr>
<tr>
<td>RDM</td>
<td>0.968</td>
<td>0.939</td>
<td>0.779</td>
<td>0.546</td>
<td>0.401</td>
<td>-0.128</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001

The statistical analyses showed that RV, RWM and RDM had vertical patterns similar to that of the resistance, and the local values of these three root traits were strongly correlated with the resistance measured in the same depth (Table 4). However, rather than being directly measured, local RWM and RDM in each subzone were calculated based on local RV, with the assumption of a constant root mass density. Therefore, we selected root volume over biomasses as the target root trait we would try to estimate in a two-step mathematical model. Because local RV most strongly correlated with global RV in 0-5 cm, the model would first use the resistance measured in 0-5 cm to predict the local RV in the top subzone, and it would use the predicted local RV to estimate the global RV of the wheat samples.

Table 4. Summary of the three criteria of target root trait selection.

<table>
<thead>
<tr>
<th>RL</th>
<th>RSA</th>
<th>RV</th>
<th>RWM</th>
<th>RDM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Is the vertical pattern of this root trait similar to that of the resistance (Table 1)?</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>How strongly are the local values of this root trait correlated with the resistance measured in the same depth (Table 2)?</td>
<td>Weak</td>
<td>Moderate</td>
<td>Strong</td>
<td>Strong</td>
</tr>
<tr>
<td>In which subzone was the local values of this root trait correlated to its global values the most (Table 3)?</td>
<td>5-10cm</td>
<td>5-10cm</td>
<td>0-5cm</td>
<td>0-5cm</td>
</tr>
</tbody>
</table>
Mathematical Model

Based on the minimization of RMSE and AIC values, we found that a three-parameter logistic regression model best fitted the $R_{\text{diff}}$ – local RV relationship in the first part of the model (Figure 7), and a linear regression model showed high significant relationship between the local RV (0-5 cm) – global RV (Figure 8). We tested the overall performance of our $R_{\text{diff}}$ – local RV – global RV model by comparing the predicted local RV and global RV from our models with the actual measured values (Figure 9). Our model showed a underestimation of the local RV and the global RV, and the major contribution to such deviation was due to the first step of the logistic regression model. However, the measured and predicted global RV value was in a high linear relationship especially in the lower root volume range.

$$Local\ RV = \frac{11.0831}{1 + e^{\frac{17.9450 - R_{\text{diff}}}{3.8445}}}$$

Figure 7. Logistic model: local root volume (RV) as a function of resistance (R) (n=134). Filled circles, measured local RV; open triangles, predicted local RV; dotted line, fitted curve; inset plot, residuals of the logistic regression model as a function of the fitted values.
Figure 8. Linear Model: global RV as a function of RV in 0-5cm (n=27). Filled circles, measured global RV; open triangles, predicted global RV; dotted line, fitted line: global RV = 1.53943 local RV + 0.03243; inset plot, residuals of the linear regression model as a function of the fitted values.

Figure 9. Model testing (n=27). Left, measured RV in 0-5cm versus predicted values using logistic model; right, measured global RV versus predicted values using the linear model and the predicted local RV from the logistic model.
DISCUSSION

Relationship between FDIP and local root trait

Contrasting the two current injection scenarios (Figure 4a & 4b) allows us to investigate how the root conducted the current and how the current polarized the root. To visualize such comparison, we divided the plant-water continuum into three types of resistor analogues in an electrical circuit (Cao et al. 2010): the root interior (\(R_{\text{root}}\)), the root-solution interface in the near root zone (\(R_{\text{rsi}}\)), and other aqueous passages not in proximity to the potential electrodes (\(R_{\text{sol}}\)). Between the two injection scenarios, these three resistor analogues remained approximately constant because the root was fixed at the same position in the same solution. The distinction between the two scenarios was due to the accessibility of the three types of resistor analogues. During the water injection scenario (Figure 10), the current traveled across the root-solution interface and the solution; while during the plant injection scenario, in addition to the previous two aqueous passages, the root interior became passable for the current. Since that the IP measurements were acquired from the root-solution interface (\(R_{\text{rsi}}\)) between the same pairs of potential electrodes under both scenarios, the differences in the output resistance were due to the change in the polarization response from the root-solution interface. From our results, we observed decreases in the output resistance in all subzones as we moved the current injection location from water onto the plant (Table 1). Such decreases indicated weaker polarization effect on the root-solution interface (Figure 3), which further implied that the current (\(I_{\text{rsi}}\)) passing through the root-solution interface also decreased across all depths (Equation 1).

We hypothesized that the decrease in the amount of current (\(I_{\text{rsi}}\)) passing through root-solution interface (\(R_{\text{rsi}}\)) under the plant injection scenario was due to the combination of (1) root capacitance. Rather than being pure resistors, root cells were combinations of small resistors and capacitors. Therefore, as the current traveled in the root interior (\(R_{\text{root}}\)), it charged the root cells, and the current was stored as electrical potential in the cells. This capacitance effect in the root’s interior led to an overall decrease in the amount of current passing through the root-solution interface; (2) root conductance. Before leaking from the root’s interior into the root-solution interface, the current traveled exclusively in the root, which also caused decreases in the current passing those non-leakage zones in the root-solution interface; or (3) stem effect. Because a
portion of the lower stem was also immersed in the solution during the measurements, the current started to leak from the lower stem even before reaching the root because the stem-solution interface was a less resistive pathway comparing to the root interior (Cao et al. 2010). The stem effect thus led to a redistribution of the current in the root-solution interface. Because we observed consistent decreases in the resistance values along the entire root system regardless of depths, our results supported the first root capacitance and the third stem effect hypotheses. However, because we did not observe increases in the resistance values at any depth and thus identify the potential leakage zones, it was unclear if our results support the root conductance hypothesis.

**Figure 10. Segmentation of plant-water continuum under two current injection scenarios.**

Meanwhile, root cellular capacitance depended on the dielectric property (McBride et al. 2008), which we would explore at both the tissue and cellular scale. At the tissue scale, each root segment could be modeled as a cylinder consisting of dielectric ionic fluids, the sap solution in the center vascular system (stele) and the exterior solution in the Rhizotron, separated by the cells predominantly in the root cortex (Dalton 1995). Based on this cylindrical geometry, the capacitance was directly proportional to the root surface area in contact with the dielectric fluids (McBride et al. 2008). At the cellular scale, the capacitance occurred at the membranes. During
current injection, the applied electric field charged the root cell membranes and disturbed the ionic equilibrium. To reach a new electrical dynamic across the membrane, ion movements were induced in the apoplasmatic and symplastic fluids, and such fluxes led to the formation of the electrical double layers (EDL) at the cell membrane to neutralize the charges (Weigand 2017). If the membranes were strongly charged, the equilibrium EDL would be thicker to offset the imbalanced charge distribution and high ion gradient across the membrane. Also, if there were more membranes involved, the equilibrium EDL would also have larger surface area. The overall process, from the beginning of EDL formation to the attainment of equilibrium, was described as the polarization magnitude, which was a quantifiable measurement of the total thickness and the area of the EDL.

Combining the tissue and cellular scales, our plant injection scenarios led to the EDL formation and the polarization of the root interior via two types of cellular ion movement: (1) radial movement. In a root cross-section, the sap solution was bounded with the cortex by the Casparian band, while the exterior solution was bounded with the cortex by the epidermis. The oppositely charged fluids (sap and exterior solution) excited the membranes of the inner cortex and the outer epidermis, which first induced the formation of EDL at these boundary cell membranes. This ionic imbalance led to a spatially continuous formation of EDL from inner cortex to the outer epidermis and created the electrolytic current flow. Because the root epidermis was physiologically responsible for ions and water absorption from the surrounding medium, the current flow across the epidermis was not inhibited. Thus, the root resembled a leaky cable and allowed the current to exit into the exterior solution toward the negative current electrode in the Rhizotron (Anderson et al. 1976), and (2) vertical movement. The formation of EDL could also occur vertically, and the ionic current traveled along the stele (xylem, phloem, endodermis) or the cortex cells. This stele-cortex exclusion was due to the Casparian band that prevented the current from crossing between the stele and the cortex (Amato et. al 2010). For the vertical movement, Dalton (1995) argued that stele tissue, responsible for the transportation of ionic solution, was the least resistive vertical cellular pathway for current flow, while Anderson et al. (1976) identified the cortex was even less resistant because of its higher cross-sectional area.

The polarization response from the root interior became more relevant in our plant injection scenario when the external electric field created the dielectric fluids that sandwiched
the root (Weigand 2017). The formation of the EDL at the cellular scale due to the presence of the dielectric fluids could be summed up to the total root capacitance. Based on the theoretical approximation with a cylindrical geometry, the magnitude of total capacitance was proportional to the contact area with the sap fluid at the inner cortex (Weigand et al. 2017) and the external fluid at the root-solution interface (Amato et al. 2010). Empirically, Cao et al. (2010) also identified the relationship between the root electrical property and the root trait. They observed an overall decrease in the root resistance when they gradually immersed the willow root into the water and increased the contact area between the root and water. Here, we further hypothesized that root capacitance could also be manifested in root morphology. As discussed above, the polarization magnitude and root capacitance depended upon the overall surface area covered by the EDL. Since that the increase in root surface area was in parallel with the increase in root volume (Dalton 1995), the larger root volume also meant a higher EDL surface area and thus a higher capacitance.

In general, studies agreed that the older portion of the root was less resistive than the younger portion. As known, the current preferred to travel in the least resistive pathway. For cellular paths, it was typically via the liquid symplasm with the fewest crossings of membranes at apoplast (cell walls), symplast (plasma membranes), and tonoplast (vacuole membranes) (Anderson et al. 1976). The younger portion of the root, closer to the root tip and the elongation zone, consisted of a larger number of root cells and functional membranes, which increased the resistance of the pathways. Whereas the aging portion was undergoing both the subsidization of the root uptake activities and the decay of root cells (Amato et al. 2010, Weigand 2017), which led to the decrease of the electrical resistance (Dalton 1995). Experimentally, although Anderson et al. (1976) argued that cortex was generally less resistive than stele, they also found that older stele was the least resistant cellular pathways for current flow.

Our results showed that the greater the local root volume was, the greater the decrease in the polarization response from the root-solution interface ($R_{rsi}$) would be (Table 2). Moreover, we also observed a stronger decrease in the top subzone with significantly high local root volume. Such decreases could be explained with the relative increase in the polarization response from the root interior. In the topmost layer, the strongest decrease in the polarization effect from the root-solution interface was due to the greatest decrease of the current ($I_{rsi}$) flowing in the region. Being the oldest, the root stele cells in the top subzone were less resistive cellular pathways for
current flow, which promoted the vertical current flow and EDL formation inside the Casparian band. The greater root volume in this subzone corresponded to the higher EDL surface area and root capacitance, which led to a higher current storage of the root interior and a stronger decrease in the polarization effect from the root-solution interface. As the current traveled downward into the subzones with younger roots, the number of active cells and membranes in the stele increased, which raised the resistance of this vertical pathway. To overcome the resistance, radial EDL formation was prompted, and the current started to traveled from the cortex, to the epidermis, and finally leaked out from the root-solution interface. Meanwhile, because of the smaller root volume in the younger region, the total EDL surface area, the overall root capacitance, and the current storage were also smaller in the deeper subzones. Comparing to the top subzone, the decrease in the polarization response from the root-solution interface was less prominent because of the current leakage into the root-solution interface from the less resistive radial pathways and the smaller area of EDL in this region due to the smaller root volume. Our findings agreed with the previous discussion on how root tissue, cell types, cell ages and cellular passages affected the root resistance, capacitance and the subsequent polarization response from both the root interior and the root-solution interface. Furthermore, our results also supported the hypothesis that root volume could be positively related to root capacitance.

However, we also acknowledged that even with the least resistant root cellular pathway via the symplasm with the least membrane crossings, it was still much more resistant to current flow in comparison with the electrolytic solution (McBride et al. 2008). Therefore, the aqueous vertical pathway along the ionic sap solution was in theory the least resistant pathway, followed by the pathway in the exterior aqueous solution. This limitation was reflected in the relative small magnitude (Ohm) of our observations.

**Model Performance**

Regression models between root electrical properties and root traits have been examined in few studies, and to our knowledge, there still is not any model that focused on either traits of the herbaceous roots or the electrical properties of the root interior. Past studies that investigated the electrical-trait relationship delved into the coarse tree root traits, the soil properties and their relationship with the soil electrical resistivity measurements. Amato et al. (2008) found that a
univariate four-parameter logistic regression model best fitted the relationship between soil resistivity ($\rho$) and root mass density (RMD), and they also found the model’s high predictability of RMD using the $\rho$ measurements. On the other hand, Rossi et al. (2011) identified that a univariate gamma generalized linear model (GLM) best described the $\rho$-RMD relationship. Both studies concluded that sigmoid growth curve could very well describe the $\rho$-RMD relationship, while root length density (RLD) neither strongly correlated to RMD nor fitted in any statistically significant $\rho$-RLD models. Our findings agreed with theirs because we also found (1) strong positive correlation between the resistance measurement ($R_{\text{app}}$) and local root dry biomass (RDM), wet biomass (RWM) and volume (RV) but a weak positive correlation between $R_{\text{app}}$ and root length (RL), and (2) moderate correlation between local and global root length and dry/wet biomass (Table 2). Our model showed a moderate predictability of global root volume in the smaller end of observations using the resistance contrast between the two injection scenarios, which also agreed with both the models proposed by Amato et al. (2008) and Rossi et al. (2011).

Both Amato et al. (2008) and Rossi et al. (2011) acknowledged common constraints of the univariate $\rho$-RMD regression models: the models fitted the data better and generated more reliable prediction when (1) the electrical measurements ($\rho$) were mainly contributed by the root rather than by the highly resistive surrounding soil, and (2) $\rho$ were in the lower range because of the positively skewed nature of the data and thus the lack of observation in both the higher $\rho$ and RMD values. However, they also recognized that low $\rho$ measurements ($<40$ $\Omega$ m) tended to be shadowed by the background soil $\rho$, which thus led to a less significant $\rho$-RMD relationship and hindered the detection of the low and fine root biomasses. With their soil resistivity measurements on the coarse tree roots, both studies aimed to delineate the roots from the soil medium and to quantify the root exterior’s contribution to the high contrast of resistivity. In comparison, our study focused on the root interior’s contribution to the change in the polarization response, and the passage of current in the root interior was highly desirable. Therefore, rather than the high resistive background medium, high conductive (low resistive) pathways such as stem-solution interface and the aqueous solution were our major concerns. These more conductive pathways curtailed the current’s tendency to flow within the root interior, and the smaller amount of current lowered the overall magnitude of the contrast between the two scenarios. Such effect was analogous to Amato et al. (2008) concern about how small resistivity measurements could be masked by the high background resistivity. Moreover, although our
herbaceous root data were in the relatively smaller end of observations comparing to their coarse tree root data, our root data were still highly positively skewed and did not include ideal number of data points on the higher end of both root volume and resistance. Such data distribution also limited our model’s explanation and predictability of the root volume on the larger end of observation.

CONCLUSIONS

The two-step model combining a resistance – local root volume three-parameter logistic regression model and a local – global root volume linear regression model showed significant relationship between the measured and predicted global root volume especially in the lower root volume range. To improve the model performance, a single-step regression model between resistance and global root volume could help reduce the error introduce in the two-step model, and thus an electrode configuration that would account for the entire root system is needed. The resistance difference between our two current injection scenarios, which entailed decreases in the polarization response from the root exterior, could be explained by the additional capacitance effects contributed by the root interior as the current started to flow among the root cells. The difference in root capacitance effect across the rooting depths could be related to the sub-cellular scale of electrical double layers formation within the tissue scale of the current flow direction, which were tied back to the amount of local root volume present in the vertical subzones. To further investigate how the root interior’s current conductance affected the polarization response from the root exterior, several data collection method revision could be considered: (1) removal of the undesirable stem effects by minimizing the stem portion in the solution and maintaining the stem current electrode at a similar distance from the root, and (2) promotion of the current flow inside the root by increasing the aqueous solution resistivity and highlighting the contrast between the root and the background. Induced polarization is a promising non-invasive in situ method for measuring and quantifying important herbaceous root metrics such as volume and potentially biomasses in a hydroponic system. Future research that examines the change in polarization responses using our two injection scenarios and electrical survey setup with the herbaceous roots in a field, soil setting is needed to incorporate the soil heterogeneity and dynamics in the measurement and quantification processes.
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