

Measurement of the Tree Root Growth Using Electrical Impedance Spectroscopy

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The non-destructive evaluation of plant root growth is a challenge in root research. In the present study we aimed to develop electrical impedance spectroscopy (EIS) for that purpose. Willows (*Salix myrsinifolia* Salisb.) were grown from cuttings in a hydroponic culture in a growth chamber. Root growth was monitored at regular intervals by a displacement method and compared with the EIS parameters of the plants. To measure its impedance spectrum (IS) (frequency range from 40 Hz to 340 kHz) each plant was set in a measuring cell filled with a solution of the hydroponic culture. The IS was measured using a two-electrode measuring system. A silver needle electrode was connected to the stem immediately above the immersion level and a platinum wire was placed in the solution. The measurements were repeated twice weekly for a root growth period of one month. The IS of the entity consisting of a piece of stem, roots and culture solution were modelled by means of an electric circuit consisting of two ZARC-Cole elements, one constant-phase element, and a resistor. On the plant basis, an increase in root volume by growth correlated with a reduction in the sum of resistances in the ZARC-Cole elements (mean Pearson's correlation coefficient $r = -0.70$).

Keywords CNLS-curve fitting, displacement method, distributed electric model, hydroponics, impedance analysis, willow

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1 Introduction

Roots of plants, especially the dynamics of root growth, have not been studied as much as shoots, largely due to the technical problems. Several methods are used to study root growth, e.g. core sampling, in-growth cores, monoliths, excavation of the root system, ground-penetrating radar, isotopes, X-ray imaging, magnetic resonance imaging, electrical capacitance, and rhizotrons and minirhizotrons (Chloupek 1972, Prokushkin 1982, Vogt and Persson 1991, van Beem et al. 1998, Hruska et al. 1999, Smit et al. 2000). These methods have provided information about the various properties of roots but all have limitations in their application (Atkinson 2000). Minirhizotron imaging is the only non-destructive method for effective monitoring root growth in situ (e.g. Richner et al. 2000).

Recently new developments in the determination of root mass have been obtained by using electrical capacitance (Dalton 1995, van Beem et al. 1998, Ozier-Lafontaine et al. 2001, Matsumoto et al. 2001, Rajkai et al. 2002). The method is based on the assumption that the capacitance of the root/soil-system changes when the contact surface area between roots and the soil increases with growth (Chloupek 1977, Dalton 1995). Typically, the measurements are limited to a measurement at a single 1 kHz frequency. However, more comprehensive information of the root system may be obtained by multi-frequency measurement and by using the approach of Electrical Impedance Spectroscopy (EIS) but this technique has not used to quantify root growth. In a recent study multi-frequency response of capacitance and resistance for sunflower plants was measured but the data was not analyzed in relation to an electric circuit model of the system (Rajkai et al. 2002).

In EIS, a wide frequency range is used for measuring an impedance spectrum (IS) which is comprised of real and imaginary parts, with frequency as an intrinsic variable. An electric circuit model is formulated for the system and the parameters are estimated by means of Complex Nonlinear Least Squares (CNLS) curve fitting. For plant tissues the best fitting results can be obtained with a model consisting of distributed circuit elements (DCE) (Repo and Zhang 1993,

Repo et al. 1994, 2000). By using distributed models it is possible to take detailed IS-features into account across a wide frequency range, and in that way to obtain mathematically accurate estimates of the model parameters.

The root-soil system is a challenging study object for EIS since it includes several polarisation interfaces with conductive mediums in-between. A detailed electrical model for such a system consists of several linear circuit elements (resistors and capacitors) (Dalton 1995). In addition to roots themselves, the EIS properties of the system are affected by several experimental factors such as soil type and soil moisture content as well as type, spacing and position of electrodes. In a simplified system of hydroponic culture it is possible to standardise some of the confounding factors of soil, and thus to gain knowledge of the change in root system itself on the EIS parameters.

The aim of the present study was to find an EIS parameter that would correlate best with the root growth of willows. We hypothesized that when the contact area of roots with the substrate increases by growth, the resistances in the distributed electric circuit models decrease as a result of a facilitated passage of the current through the sample.

2 Materials and Methods

Willow cuttings (*Salix myrsinifolia* Salisb.) were selected for this study because they have no roots when started and raise new roots soon after immersion into the hydroponic culture. The cuttings (diameter 5–11 mm, length 200 mm) were sampled from a plantation in Kaavi, Central Finland (62°95'N, 28°40'E) at the beginning of June 2002. The cuttings were set in an aerated hydroponic culture as three container replicates (size of a container 600 mm × 400 mm × 300 mm) in a growth chamber (PGW36, Conviron, Canada). The conductivity of the hydroponic solution was adjusted to 100 µS by adding Superex-6 fertilizer (N 22%, P 4%, K 19% and micronutrients, Kekkilä Co., Finland). During the growing period the chamber temperature was 20 °C, the photon flux density 320 µmol s⁻¹ m⁻², the photoperiod 18/6h

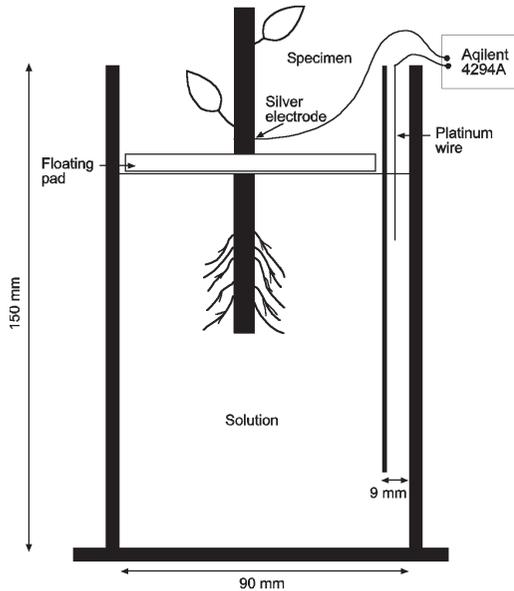


Fig. 1. The measuring cell used for studying root growth by electrical impedance. The impedance spectra are measured using a LCR analyser (Agilent 4294A).

(day/night) and the air relative humidity 80%. The cuttings were mounted on floating pads in hydroponics with 22, 22 and 20 plants in each of the containers 1, 2 and 3 respectively. The immersion depth of the plants in the containers was kept constant (approximately 10 cm) throughout the study. The hydroponic solution was replaced at one-week intervals.

The impedance spectra of the plants were measured twice weekly for one month using an impedance analyser (Agilent 4294A Precision Impedance Analyzer, Palo Alto, CA, U.S.A.) at 488 frequencies between 40 Hz and 340 kHz. A custom-designed measuring cell (transparent plastic) was used for the IS measurements (Fig. 1). The cell was filled with a similar solution to that used in the containers. The IS were measured using a two-electrode system. Prior to the start of the hydroponic culture, one of the electrodes (Ag-wire, \varnothing 0.5 mm) was pushed through the stem just above the floating pad. The Ag-electrode was kept in this position throughout the study. Another electrode (Pt wire, \varnothing 0.5 mm) was immersed in the solution in one of the pipes of the measuring

cell (Fig. 1). Thirty minutes before the start of the IS measurements the containers were brought into the conditions prevailing in the laboratory. The immersion depth of the plant in the measuring cell was kept constant during the subsequent measurements. The effect of the solution and the Pt-wire/solution interface was examined by measuring the IS of the measuring cell without the plant, i.e. the system function, and then subtracted from the IS with the plant.

The circuit between the electrodes consisted of a piece of stem, an interface between roots and a shoot in a culture solution, the culture solution, and the contact layers on the surface of the electrodes. The IS of this entity was modelled by means of a circuit consisting of a resistor, two ZARC-Cole elements and a constant-phase element (CPE) in series (for ZARC-Cole and CPE see Macdonald 1987). The complex impedance (Z) of the circuit is provided by the equation

$$Z = R + \frac{R_1}{1 + (j\tau_1\omega)^{\psi_1}} + \frac{R_2}{1 + (j\tau_2\omega)^{\psi_2}} + \frac{1}{\tau_3(j\omega)^{\psi_3}} \quad (1)$$

where R is the value of the series resistor, R_i , τ_i and ψ_i , ($i=1, 2$) are the resistor, relaxation time and distribution coefficient of the relaxation time in the ZARC-Cole elements, and τ_3 and ψ_3 are the values of the parameters in the CPE. The CPE was included in the model in order to take into account the polarization impedance of the plant electrode at low frequencies.

Estimation of the model parameters was done using a Complex Nonlinear Least Squares (CNLS) curve fitting program LEVM 7 (J. R. Macdonald, University of North Carolina, U.S.A.). The program minimizes the sum

$$S(P) = \sum_{j=1}^M w_j [Y_j - YC_j(P)]^2 \quad (2)$$

where M is the total number of data points, w_j is the weight associated with the j th point, Y_j is the j th data point value to be fitted and $YC_j(P)$ is the value of the calculated fitting function involving the set of parameters P . The CNLS-curve fitting of the model (Eq. 1) produces estimates for a total of nine parameters. Two of the parameters in the CPE take into account the low frequency part of

the spectrum, i.e. the ‘elbow’. The parameters of the two ZARC-Cole elements describe the two overlapping arcs of the spectrum. The series resistor in the model estimates the high-frequency end of the spectrum.

The root fresh mass was determined by the mass of water displaced by the roots at the same time as IS measurements were made (Burdett 1979). In the displacement method the root zone area of the plant was immersed in a water solution at a constant depth, and the weight was determined (Sartorius 1265MP, accuracy ± 1 mg). At the end of the study the roots were severed, and the fresh and dry mass was determined.

Statistical Analysis

Pearson’s correlation analysis was applied to find out which of the EIS parameters or their derivatives would best predict the root mass. The correlation coefficient (r) between the root fresh mass and the EIS parameters was calculated for each plant over the whole period of study. The mean and the frequency distributions of the correlation coefficient were calculated.

3 Results

The root mass increment, as determined by the displacement method, commenced after one week in hydroponics and ceased within three weeks (Fig. 2). At the end of the study, the root mass as determined by the displacement method corresponded well with the root fresh and dry mass (Pearson’s correlation coefficients of 0.88 and 0.93 respectively, $n=64$). The mean water content (\pm sd) of roots (on fresh weight basis) was 97% (± 1.7) at the end of the experiment.

A typical impedance spectrum of willow had two overlapping impedance arcs and an ‘elbow’ at low frequencies (Fig. 3). Two impedance arcs appeared in the early stages of the experiment. The overlap of the arcs increased and the magnitude of the real and imaginary parts of the spectra decreased during the period of study. The same distributed model could be used for the CNLS curve fitting of all of the measurements.

Based on the mean of the Pearson’s correlation coefficient between the EIS parameters and the root mass increment, the highest (negative) correlation was found for the sum of the resistances ($R_1 + R_2$) of the ZARC-Cole elements (Table 1).

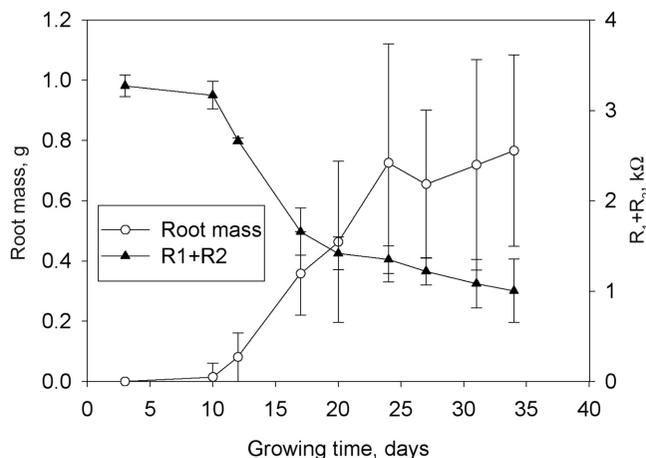


Fig. 2. The root fresh mass and the sum of resistances R_1 and R_2 (see Eq. 1) for the willows grown in hydroponic culture. The mean and standard deviation for three containers is indicated. Fresh mass was determined by water displacement method and the resistances by electrical impedance spectroscopy.

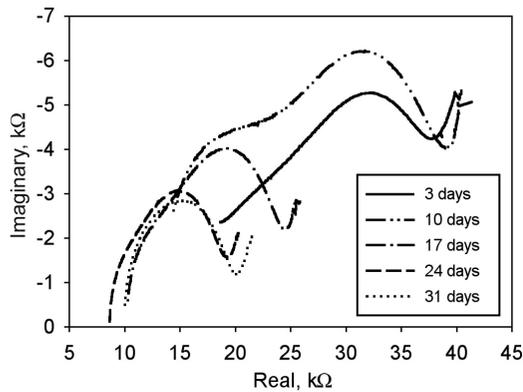


Fig. 3. The impedance spectra of a willow as determined at different times during the hydroponic culture. In each curve low frequency (40 Hz) is on the right and high frequency (340 kHz) on the left.

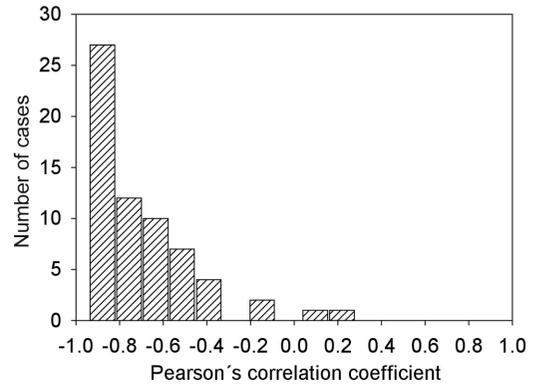


Fig. 4. The frequency distribution of the Pearson's correlation coefficient (r) for the relation between $(R_1 + R_2)$ in Eq. 1 and the root fresh mass ($n=64$).

The resistance sum decreased with the increase in the root mass and stabilized when the root mass increment ceased (Fig. 2). As determined by plants, the correlation coefficient was between -0.8 and -0.9 in most cases (Fig. 4). In four out of 64 cases the correlation was close to zero.

4 Discussion

EIS approach was applied to measure root growth. The most important finding was that the sum of the resistances R_1 and R_2 in the distributed electric model decreased with an increase in root mass. The mean Pearson's correlation coefficient by plants was moderately high ($r=-0.70$) raising for several plants up to -0.9 . This compares well with results from minirhizotron imaging and capacitance measurements (Upchurch 1987, Dalton 1995, Beem et al. 1998, Ozier-Lafontaine et al. 2001). We do not know which proportion of the change in the resistances is due to the increase of root mass or root surface area and which, if any, is due to the change in stem or stem/solution interface during the growing period.

The overall system used for the IS measurements consists of several components, i.e. an electrode/stem interface, a piece of stem, a root system, a root/solution interface in parallel with

Table 1. Pearson's correlation coefficient (r) for the EIS parameters and their derivatives with the root fresh mass of willows grown for 34 days in hydroponic culture. The correlation was calculated for each plant and then averaged ($n=64$).

EIS parameter or its derivative	r
R	-0.12
R_1	-0.65
τ_1	0.11
ψ_1	0.33
R_2	-0.58
τ_2	-0.20
ψ_2	-0.28
τ_3	0.29
ψ_3	-0.23
$R_1 + R_2$	-0.70
$R + R_1 + R_2$	-0.63

a stem/solution interface, a solution (growing medium) and a solution/electrode interface, each of the components having its own frequency dependent impedance. The effects of the solution and the electrode/solution interface on the IS were eliminated by measuring the system function and then by subtraction. One part of the remaining spectrum is due to the polarization impedance of the electrode/stem interface, appearing as the 'elbow' at low frequencies (Repo 1994). The rest

of the spectrum originates of stem, roots and their interfaces with the solution.

Due to the complex structure of the system, we may assume that the measurement at a single frequency may not be sufficient to obtain comprehensive information, particularly of root growth. A detailed electrical model of the root system, after subtraction of the system function and considering the electrode polarization impedance in the model, is still complicated since each of the major components of the circuit is made up of a distribution of resistors and capacitors, and thus also of an array of time constants (Starzack 1984, Madonald 1987, Repo and Zhang 1993, Zhang et al. 1995). In mathematical terms, the model consisting of distributed circuit elements may be in good accordance with this kind of data. Such models have been used in previous studies of woody plants (Repo and Zhang 1993, Repo et al. 1994, Repo et al. 2000). An inconvenience is encountered with this type of models; while the mathematical accuracy of the estimation is improved, the exact physicochemical and biological interpretation of the model parameters may decline.

The distributed model consisting of one Constant Phase Element (CPE), two ZARC-Cole elements and one resistor all in series fit well in the IS of the root/stem/electrode-system. The polarization impedance of the stem/electrode interface appearing at low frequencies was modelled with the CPE. When the electrode polarization impedance was considered in the model, the biological system could also be modelled more accurately. We may conclude that the two overlapping arcs in the IS are due to the roots and stem and their interfaces with the solution. The change over time in that system appeared in several EIS parameters from which the sum of the resistances ($R_1 + R_2$) correlated most clearly with the root fresh mass. The pseudo-capacitances C_1 and C_2 , as calculated according to the distributed elements (Repo et al. 2000, 2002), had a low correlation with root mass ($r = 0.23$ and -0.11 respectively). However, these pseudo-capacitances are not directly comparable with the capacitance at a single frequency (e.g. 1 kHz) (cf. Chloupek 1977, Ozier-Lafontaine et al. 2001).

The growth rate and the final fresh mass of the roots varied considerably between plants. We may

expect the same for root surface area too, whose determination by plants would have required measurement of root diameter distribution and corresponding root lengths. If we approximate average root diameter of 1 mm at the end of the experiment we get for mean root surface area per plant 25.7 cm² with a large variation from 1.2 cm² to 98 cm² between plants (Pietola and Smucker 1998). With the same assumption, the mean specific root area was 1470 cm²/g DW varying from 270 to 4400 cm²/g DW between plants. Large between-plant variation in root size and probably root vitality may partly explain low correlation between resistance ($R_1 + R_2$) and root fresh weight in the pooled data. In the time series by plants the correlation was quite high, however, which deserves further studies (Fig. 4). No common factor in the growth rate, in the condition of the root system or the whole plant could be found to explain the low correlations ($r > -0.4$) for some plants.

The water content of roots was high (97%) at the end of the experiment. Thus we may conclude that a reasonable approximation of the root fresh mass was obtained according to the mass of water displaced by the roots. Consideration of the root dry matter in the fresh mass would have required knowledge of the amount and density of dry matter with time. Such a correction would have had minor effects on the results.

In earlier studies, the moisture content of the root/soil system was seen to have a strong effect on the capacitance (Chloupek 1977, Dalton 1995). The moisture content had also a strong effect on certain EIS parameters of bean seeds (*Phaseolus vulgaris* L.) and stems of Scots pine (*Pinus sylvestris*) (Repo et al. 2000, 2002). In this study, however, its impact was eliminated by using hydroponics where root water content was high. Therefore, variation of EIS parameters by tissue water content or culture medium would be negligible. The effect of the electrode position and the content of the solution in the measuring cell were also standardized.

In conclusion, this study showed that the EIS has developmental potential for monitoring root growth. The method is non-destructive to the plant, and minor tissue damage occurs only in the process of inserting a single needle electrode into the stem. In situ measurements are fast and

easy to perform. In the further developments, more studies are needed for plants growing in a soil medium in order to find out the effects of soil type, soil moisture content and the position of electrodes on the EIS parameters.

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