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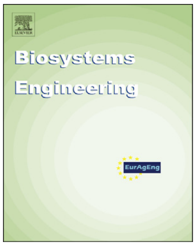

Research Paper

Detecting mycorrhizal colonisation in Scots pine roots using electrical impedance spectra

Tapani Repo [a,*], *Anna Korhonen* [a,b], *Miikka Laukkanen* [c], *Tarja Lehto* [b], *Raimo Silvennoinen* [c,d]

[a] *Finnish Forest Research Institute, PO Box 68, FI-80101 Joensuu, Finland*
[b] *School of Forest Sciences, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland*
[c] *Department of Physics and Mathematics, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland*
[d] *Institute of Photonics, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland*

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To investigate whether root colonisation of Scots pine (*Pinus sylvestris* L.) seedlings with symbiotic mycorrhizal fungi (*Hebeloma* sp. and *Suillus luteus*) could be detected *in situ*, classification analysis of the electric impedance spectra (IS) of the root system was carried out. The seedlings were inoculated either with *Hebeloma* or *Suillus* with some left as controls. The seedlings were firstly cultivated in long-day and high temperature (LDHT) conditions. Half of the seedlings remained in LDHT and half were moved to short-day and low temperature conditions (SDLT) to acclimatise to the cold. The electrical impedance spectra of the root systems were measured at a frequency range of 5 Hz–100 kHz. The results of principal component analysis (PCA) showed that current delivery through root system, sensed by real and imaginary parts of IS, depended upon the cold acclimation and mycorrhizal treatment. Comparison of SDLT to LDHT via correlation analysis indicated a 13% and a 27% change in PCA responses for the real and imaginary parts of the impedance, respectively. When the mycorrhizal treatments were compared with a non-mycorrhizal treatment, the respective changes in the correlation coefficients were 30% for *Hebeloma* sp. and 39% for *S. luteus* in the real part and 28% and 38% in the imaginary part, respectively. These changes in the correlation coefficients appear to indicate physicochemical changes (e.g. ionic behaviour) in the roots as a result of fungal colonisation.

1. Introduction

Fine roots, with their symbiotic fungi, form an essential component in the growth and well-being of plants. To match the changes in the soil regimes they sense soil conditions and adjust the physiology and growth not only of the roots, but also of the shoots. In the boreal zone, soil temperature and moisture have strong seasonal variation and the roots and symbiotic mycorrhizas of perennial plants have to acclimatise themselves to these variations in order to survive. A number of physiological changes take place in plant cells with cold acclimation, especially in the composition of cell membranes and content of soluble sugars (Sakai & Larcher, 1987; Zwiazek, Renault, Croser, Hansen, & Beck, 2001). The complexity of the root systems and mycorrhizas, their interfaces and their

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[*] *Corresponding author*. Tel.: +358503913136; fax: +358 29 532 3113.
E-mail address: tapani.repo@metla.fi (T. Repo).

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Nomenclature

Symbols

$C(i,j)$	covariance matrix
f	frequency
$\{f_1, f_2, \ldots, f_n\}$	set of n frequencies
Im	imaginary part
L	k-dimensional subspace
M	correlation matrix
N	number of vectors
N_H	number of impedance spectra of *Hebeloma* seedlings
N_O	number of impedance spectra of non-mycorrhizal seedlings
N_{SL}	number of impedance spectra of *Suillus* seedlings
r	mutual correlation coefficient
r_{Im}	correlation coefficient of imaginary parts for seedlings in LDHT and SDLT
r_{Re}	correlation coefficient of real parts for seedlings in LDHT and SDLT
$r(i,j)$	correlation coefficient matrix
Re	real part
X_j	jth normalised vector of real or imaginary part of impedance
$\{X_1(f), X_2(f), \ldots, X_N(f)\}$	set of N normalised vectors
X^N	data vector
$\{Y_1(f), Y_2(f), \ldots, Y_k(f)\}$	linear combinations of k linearly independent basis vectors ($k < N$)
Y'	covariance matrix
Z	complex impedance

Abbreviations

A	cold acclimation treatment
AM	arbuscular mycorrhiza
CLAFIC	Class-Featuring Information Compression
d/n	day/night
EI	electrical impedance
EIS	electrical impedance spectroscopy
EM	ectomycorrhiza
H	*Hebeloma* sp.
H_{LDHT}	*Hebeloma* in long-day and high temperature
H_{SDLT}	*Hebeloma* in short-day and low temperature
LDHT	long-day and high temperature
M	mycorrhizal treatment
N	nitrogen
O	non-mycorrhizal
O_{LDHT}	non-mycorrhizal in long-day and high temperature
O_{SDLT}	non-mycorrhizal in short-day and low temperature
PCA	Principal Component Analysis
RH	relative humidity
SDLT	short-day and low temperature
s.d.	standard deviation
SL	*Suillus luteus*
SL_{LDHT}	*Suillus* in long-day and high temperature
SL_{SDLT}	*Suillus* in short-day and low temperature
TCP	Three-Class Problem

interactions with the soil make studying them challenging. There are several methods for obtaining information on roots based on their physiology, morphology, growth and biomass, but they have limitations (Costa, Dwyer, & Hamilton, 2000; Hirano et al., 2009; Majdi, 1996; Samson & Sinclair, 1994; Smit et al., 2000). The methods are destructive, laborious and may not consider the physiological activity of fine roots and the role of mycorrhizas in the properties of the root–soil interface. Therefore, new methods are needed in order to obtain more information about the functioning of roots in various growing regimes.

Since the initial studies on electrical capacitance of roots by Chloupek (1972, 1977), a number of more recent studies has been published concerning the electrical properties (impedance, capacitance, resistance) of roots and how properties change according to different cultivation treatments (Cao, Repo, Silvennoinen, Lehto, & Pelkonen, 2010, 2011; Cseresnyés, Takács, Végh, Anton, & Rajkai, 2013; Dalton, 1995; Ellis et al., 2013; Ozier-Lafontaine & Bajazet, 2005; Rajkai, Vegh, & Nacsa, 2005; Vozáry, Jócsák, Droppa, & Bóka, 2012). The electrical impedance method is based on driving an alternating electric current into the root/soil system and measuring the changes in amplitude and phase as the signal passes through the system (Repo, Cao, Silvennoinen, & Ozier-Lafontain, 2012). These changes are due to polarisation and relaxation phenomena and the dissipation of energy at different interfaces (e.g. cell membranes, root/soil interfaces) and compartments (e.g. apoplast, symplast, soil pores, soil particles) when alternating current is passed through the specimen. There are two main study approaches to using this method for roots, i.e. single- and multi-frequency measurements. With single-frequency measurements, the most promising results have been obtained by root capacitance at a low frequency (e.g. 1 kHz) (Cao et al., 2010; Cseresnyés et al., 2013; Ellis et al., 2013; Ozier-Lafontaine & Bajazet, 2005).

However, more information about the root system can be obtained by adopting a multi-frequency rather than single-frequency approach. With a multi-frequency approach, complex impedance (real and imaginary part) is measured at a wide range of frequencies, e.g. from a few Hz upto 1 MHz. The method is termed electrical impedance spectroscopy (EIS), and it has been used to study various aspects of different organs, such as freezing and heating stress, cold acclimation, root hypoxia, root biomass and growth, and the decay of wood and seed germination (Ozier-Lafontaine & Bajazet, 2005; Repo, Laukkanen, & Silvennoinen, 2005; Repo, Paine, & Taylor, 2002; Repo, Zhang, Ryyppö, & Rikala, 2000; Repo, Zhang, Ryyppö, Vapaavuori, & Sutinen, 1994; Tiitta, Repo, & Viitanen, 2001; Vozáry et al., 2012; Zhang & Willison, 1992). Electrical circuit models (lumped or distributed) are commonly used to characterise the properties of plant organs. For example, in a recent study with willows, the capacitance for the interface between roots and the cultivation solution correlated with the root surface area (Cao, Repo, Silvennoinen, Lehto, & Pelkonen, 2011). Strong interference was caused by the stem coming into contact with the solution. To date there are no previous multi-