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Research Note

Electrical impedance phase angle as an indicator of plant root stress

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This study aimed to demonstrate that single-frequency (1 kHz) measurement of impedance phase angle (Φ) in root–soil systems is suitable for monitoring plant responses to environmental stresses. Potted wheat, soybean and maize plants were exposed to cadmium contamination, alkaline stress, drought or weed competition. Φ was detected at regular intervals between a ground and a plant electrode during plant development, at the end of which root and shoot biomass were measured. Each type of stress significantly reduced both Φ and the root and shoot dry mass, to an extent proportional to the stress level. The decrease in Φ was attributed to various physicochemical changes in root cell membranes, the accelerated maturation of the exo- and endodermis and altered root morphology. These stress responses modified the dielectric properties of the root tissues, influencing the apoplast and symplast pathways of the electrical current inside the roots. The stress-induced increase in the amount of electrically insulating lignin and suberin in root tissues was considered to be an influential factor in decreasing Φ. These results show that in pot experiments the measurement of the impedance phase angle in intact root systems is a potentially useful *in situ* method for detecting plant responses to stresses affecting roots.

1. Introduction

Methodological constraints strongly limit access to the plant root system in undisturbed soil, so developments in non-invasive investigation techniques have been the subject of renewed interest in recent years. One such method is based on the dielectric characterisation of roots. The measurement of electrical capacitance in plant–soil systems, first proposed by Chloupek (1972), is considered a promising technique for studying root growth and function *in situ* (Postic & Doussan, 2016). Using this approach, significant correlations were found between various root traits (mass, length or surface area) and the electrical capacitance measured at a low (~1 kHz) frequency between a ground electrode inserted into the rooting medium and a plant electrode fixed on the stem base. Several studies demonstrated the successful application of this simple, rapid capacitance method for root size estimation in pot-grown and field-grown plants, involving both crops and native species (Cseresnyés, Rajkai, & Takács, 2016a; Cseresnyés, Takács, Füzy, Végh, & Lehoczky, 2016b; Ellis et al., 2013; Heřmanská, Středa, & Chloupek, 2015).

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The electrical capacitance method is based on measuring changes in the amplitude and phase of an alternating current (AC) signal driven into the root–soil system (Repo, Zhang, Ryyppö, & Rikala, 2000). These changes are due to the dielectric polarisation and relaxation of root cell membranes and compartments, *e.g.* apoplast and symplast. Conceptual models consider the root system as a group of imperfect cylindrical capacitors: the conductive xylem and phloem sap and the conductive soil solution are separated by root membranes, which, acting as a dielectric, exhibit a capacitance proportional to the area of the root–soil interface (Dalton, 1995; Dietrich, Bengough, Jones, & White, 2013; Ellis et al., 2013).

Due to membrane polarisation, plant tissues present an electrical impedance (Z) to the AC signal. Impedance is specified by both its magnitude |Z|, *i.e.* the ratio of the voltage applied and the current measured, and its phase angle, Φ, which expresses the phase shift between current and voltage (Fig. 1). In complex notation, impedance can be dissected into a real part or resistance, $R = |Z| \times \cos\Phi$, and an imaginary part or reactance, $X = |Z| \times \sin\Phi$. $\Phi = 0^\circ$ for purely ohmic resistances and -90° in ideal (lossless) capacitors, irrespective of the AC frequency (conventionally, Φ is negative in capacitors and positive in inductors where voltage lags or leads the current, respectively). In biological tissues the value of the phase angle is $0^\circ \geq \Phi \geq -90^\circ$. If Φ is near to 0° or near to −90°, then the tissue has mainly ohmic or capacitive character, respectively.

The root–soil system is a lossy capacitor, in which Φ is substantially smaller (in absolute value) than −90° and, furthermore, is strongly influenced by AC frequency, plant phenology and soil type (Aubrecht, Staněk, & Koller, 2006; Cseresnyés, Rajkai, & Vozáry, 2013). Φ depends on the dielectric component of the plasma membrane and apoplast, and is thus basically determined by the physicochemical properties of the root tissue. Various environmental stressors induce changes in the composition, structure and function of cell membranes and cell walls, affecting the electrical behaviour

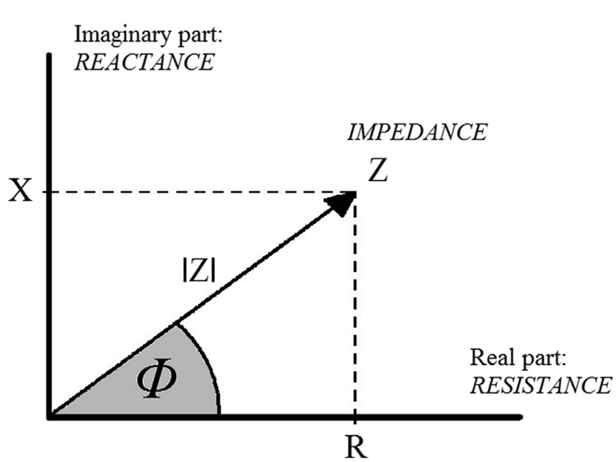

Fig. 1 – Graphical representation of the complex impedance (Z) in terms of the real component or resistance (R) and the imaginary component or reactance (X). Φ symbolises the impedance phase angle.

of the plant tissue (Jócsák, Droppa, Horváth, Bóka, & Vozáry, 2010; Suhayda, Giannini, Briskin, & Shannon, 1990). Electrical impedance spectroscopy has been used to evaluate the response of detached plant organs or tissues to *e.g.* freeze–thaw injury, cold acclimation, osmotic stress, root hypoxia or nutrient deficiencies (Hamed, Zorrig, & Hamzaoui, 2016; Jócsák et al., 2010; Li, Li, Wei, & Zhu, 2017; Repo et al., 2000). These studies focused on the use of a precision laboratory instrument to analyse the spectral measurements of reactance and extra- or intracellular resistance over a wide frequency range (from 5 Hz to 1 MHz). However, there is no information in the literature concerning the detection of root stress responses with measurements in intact root–soil systems.

The objective of this paper was to report experiments demonstrating that Φ of impedance, detected *in situ* between a ground and a plant electrode at a single low frequency (1 kHz), could be a simple indicator of stress-induced changes in root tissue, thus providing a potentially useful, rapid comparative method for studying roots grown under stress.

2. Materials and methods

The study comprised four pot experiments to show the effect of various environmental stressors, namely cadmium (Cd) contamination (*Exp.* 1), alkaline stress (*Exp.* 2), drought (*Exp.* 3) and weed competition (*Exp.* 4), on the impedance phase angle, Φ. *Exp.* 1 and 2 were carried out with spring wheat, *Exp.* 3 with soybean, and *Exp.* 4 with maize and the weed *Echinochloa crus-galli*. The trials were originally designed and carried out to evaluate the effect of stress factors on root growth and activity by monitoring electrical capacitance in the plant–substrate system (which is why species and growth conditions are different in each experiment). It was formerly shown that root capacitance was a useful indicator for the loss of root biomass caused by drought or weed interference (Cseresnyés et al., 2016a; Cseresnyés et al., 2016b). However, the present work focused solely on the relationship between stress and detected Φ. The specifics of the experiments are summarised in Table 1. First a general description is given of plant cultivation, electrical measurements and statistical methods, followed by the details of the treatments applied.

2.1. *Plant cultivation*

Seeds were germinated on moistened paper towels in Petri dishes in darkness, and were then planted into plastic pots filled with growth medium. The substrate was irrigated with tap water to field capacity (pumice: 0.21 $cm^3\ cm^{-3}$; arenosol: 0.19 $cm^3\ cm^{-3}$) before planting by placing the pots on a balance (±1 g). The plants were cultivated in a random arrangement in a growth chamber at 28/20 °C day/night temperature and 16/8 h photoperiod, PAR of 600 $\mu mol\ m^{-2}\ s^{-1}$ (300 $\mu mol\ m^{-2}\ s^{-1}$ in *Exp.* 1) and relative humidity of 50–80%. Except for plants subjected to drought stress (*Exp.* 3), the substrate was watered daily to field capacity, and the pumice medium was fertilised weekly with Hoagland's solution. The volumetric water content was measured using a TDR device (Trime-FM3; IMKO GmbH., Ettlingen, Germany).