

## Role of phase angle measurement in electrical impedance spectroscopy

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**A b s t r a c t.** Importance of phase angle measurement during the application of electrical impedance spectroscopy was studied by executing pot experiments with maize. Electrical impedance, phase angle (strength of capacitive character), and dissipation factor in the plant-soil system were scanned between 100 and 10 000 Hz current frequency. The frequency-dependent change in the phase angle could be described by optimum curves culminating within 920-3 650 Hz. Since the rate of energy dissipation is independent of root extent, the higher phase angle and lower energy dissipation were associated with the higher coefficient of determination achieved for the root electrical impedance – root system size (root dry mass and root surface area) regressions. The characteristic frequency selected on the basis of phase angle spectra provided a higher significance level at statistical comparison of plant groups subjected to stress conditions influencing root development. Due to the physicochemical changes observable in aging root tissue, the apex of phase angle spectra, thus the characteristic frequency, shifted continuously toward the higher frequencies over time. Consequently, the regularly repeated phase angle measurement is advisable in time-course studies for effective application of the electrical impedance method, and the systematic operation at the same frequency without determination of phase angle spectra should be avoided.

**K e y w o r d s:** dissipation factor, root capacitance, electrical impedance spectroscopy, phase angle, root surface area

### INTRODUCTION

Destructive root investigation methods, such as soil cores, in-growth cores, or monoliths are unsuitable for continuous monitoring of root development or activity in response to changing environmental conditions. The applicability of the non-destructive ground penetrating radar, radioactive tracers, MRI or X-ray imaging techniques is also strongly limited in many cases: providing little resolution of

root structure (detect clearly coarse roots only), these methods are not adapted for quantification of root surface area and examination of many plant phenomena related to root development (Cao *et al.*, 2010; Čermák *et al.*, 2006). Conversely, electrical impedance (EI) and electrical capacitance (EC) measurements in a plant-soil system offer good opportunities of rapid in situ investigation of the root system size and root activity without any intrusion into plant life function. By fixing an electrode at the plant stem and embedding the other one in the soil and connecting them by an LCR- instrument, the measured root EI and EC are directly correlated with root mass, root length, or root surface area (Chloupek, 1972; Ozier-Lafontaine and Bajazet, 2005; Rajkai *et al.*, 2002). EI and EC methods have been used for investigation of detached plant tissues and organs subjected to various stress conditions (cold acclimation, freeze-thaw injury, drought, nutrient deficiency, or pathogen infection) as well as for studying intact root systems of plants cultivated in soil or grown in hydroponic solution (Chloupek *et al.*, 2006; Cseresnyés *et al.*, 2012, 2013; Preston *et al.*, 2004).

Dalton (1995) developed a conceptual model for interpretation of the plant root-soil system in which root surface area was regarded to be the surface area of a group of cylindrical condensers having the same average diameter as the cellular system constituting the roots. The root-soil interface has a capacitance proportional to the charges accumulated on the active membrane surfaces. Thus, the polarized membrane plays the role of a dielectric in a capacitor, where plant solution provides the first plate and soil solution is the second one. Rajkai *et al.* (2005) recommended a two-dielectric (series-connected root and soil dielectric) capa-

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citor model, where the capacitive character of the soil solution was also considered. The impedance measurement is based on the physical principle of electrical current continuity. An applied electric current passes from the soil to the root (or vice versa) through the electrically conducting water and ion absorption zones, thus the magnitude of EI measured in a root-soil system correlates negatively with the active root surface area (Aubrecht *et al.*, 2006; Ozier-Lafontaine and Bajazet, 2005). Single and double distributed circuit element (DCE) models can be regarded as good physical approximations to electrical behaviour of plant tissues (Repo and Zhang, 1993).

The main limitation of the EI and EC method is the sensitivity of electrical conductance to the edaphic factors: temperature, soil properties (water saturation, ionic status, texture, clay and organic matter content) and the position of the plant electrode have been observed to exert an influence on the electrical measurements, chiefly on EI values (Beem *et al.*, 1998; Chloupek, 1977; Dalton, 1995). Nevertheless, under well-defined conditions (homogenized planting medium, constant moisture content, salinity and temperature, consistent electrode placement etc.) the method can provide a good estimation of the root system size (Beem *et al.*, 1998; Chloupek, 1977; Cseresnyés *et al.*, 2012).

The capacitive impedance is specified by its magnitude ( $Z$  in Ohms) and phase angle ( $\Phi$  in degrees); the latter shows the phase difference between current and voltage, indicating the tendency of the plant root to behave as a capacitor ( $\Phi$  is regarded as negative in capacitors and positive in inductors in which voltage lags and leads the current, respectively). The root-soil system is a relatively poor capacitor. The phase angle is  $-90^\circ$  in ideal capacitors irrespective of current frequency, but in the root-soil systems, the phase angle is much lower in general; furthermore, it depends strongly on the applied current frequency and also on the soil type and phenological phases of the plant (Aubrecht *et al.*, 2006; Greenham *et al.*, 1982). The phase angle is inversely proportional to the dissipation factor ( $D = 1/\tan \Phi$ ) and is used for measuring the tendency of dielectric materials to absorb and dissipate some energy without inducing charge alignment on the membrane surfaces.

According to several studies (Aulen and Shipley, 2012), a low correlation was found between EI and root extension under laboratory and field conditions. A great part of the previous investigations was executed either at the conventional current frequency of 1 000 Hz or at other arbitrarily chosen operating frequency without paying attention to measuring  $\Phi$  (Rajkai *et al.*, 2005). We hypothesized that – besides the edaphic factors described above – the frequency-dependent change in the capacitive strength (indicated by  $\Phi$  and  $D$ ) may also contribute to the weakening of the correlation between the EI and the root system size. For this reason, three pot experiments were conducted by growing maize plants under laboratory conditions in order to verify the usefulness of phase angle measurement during

the application of electrical impedance spectroscopy (EIS). Experiment 1 was designed to study the frequency-dependent change in  $\Phi$  as well as the coefficient of determination ( $R^2$ ) for both the EI – root dry mass and the EI – root surface area regression in order to show the necessity of an appropriate current frequency range for obtaining good fit at the regression analyses. The objective of Experiment 2 was to investigate whether the operating current frequency could influence the level of significance obtained by the statistical comparison of the EI values of untreated (control) plants with those of their counterparts treated with a growth-inhibiting herbicide active ingredient (acetochlor). By performing Experiment 3, we investigated the changes in  $\Phi$  and  $D$  related to both the applied current frequency and the age (phenology) of plants.

We aimed to prove that measuring of the phase angle in the course of the EIS application could promote selection of the characteristic current frequency (or frequency range) that is most efficient for root size estimation, resulting in improved data accuracy and statistical authenticity.

#### MATERIALS AND METHODS

Maize seeds (*Zea mays* L., DK-440 Hybrid) were germinated on a moistened paper towel. On experiment day 1, the germinated seeds were transplanted into 1.25 dm<sup>3</sup> plastic pots containing 1.8 kg of oven-dry Arenosol (FAO-UNESCO, 86.3% – sand, 8.3% – silt, 5.4% – clay) with pH<sub>H<sub>2</sub>O</sub> of 6.14 and pH<sub>KCl</sub> of 5.95, cation exchange capacity (CEC) of 7.50 mmol 100 g<sup>-1</sup>, 0% lime content, 1.18% humus content, 1.53 kg dm<sup>-3</sup> bulk density and 0.19 cm<sup>3</sup> cm<sup>-3</sup> water content at field capacity. Three germinated seeds were deposited in each pot and after emergence thinned to one. The pots were transferred to a growth chamber and maintained at 24/18°C and 16/8 h for day/night temperature and photoperiod respectively, photon flux density of 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and relative humidity of 50-80%. The soil moisture content was maintained near field capacity by daily irrigation. In order to keep up the optimal nutritional status, the plants were fertilized weekly (started on experiment day 1) with 200 cm<sup>3</sup> of Hoagland solution.

The EI, phase angle ( $\Phi$ ) and dissipation factor ( $D$ ) were measured with a HP 4284A LCR-bridge (Agilent Techn., Santa Clara, USA) in a parallel equivalent circuit at 55 logarithmically distributed, pre-programmed frequencies ranging from 100 to 10 000 Hz with 1 V terminal voltage. One terminal of the instrument was connected to the ground electrode *ie* a stainless steel rod (6.3 mm in diameter and 15 cm long) inserted to a depth of 10 cm into the potting soil and positioned 6 cm away from the stem base. The second terminal was attached to the plant stem with a spring tension clamp (Beem *et al.*, 1998; Kendall *et al.*, 1982; Rajkai *et al.*, 2005). Since plant electrode placement strongly affects electrical measurements, a distance of 20 mm was precisely maintained between the lower edge of the clamp and the soil

surface (root neck). A thin layer of electrocardiograph paste was smeared around the stem to keep up electric connection, and dry leaves that did not conduct current were removed from the basal part. Two hours before the measurement, the pots were brought into the laboratory (22°C) and watered to field capacity to avoid the moisture content effect of the soil (Dalton, 1995). The percentage water content of soil was verified by a Trime-FM3 TDR instrument (IMKO GmbH, Ettlingen, Germany). EI and  $\Phi$  of the soil were also measured using two ground electrodes attached to the LCR-bridge.

Altogether 18 maize plants were subjected to Experiment 1. During the early vegetative growth stage (between experiment day 10 and 27), one plant was selected daily, then the spectra of its root EI and  $\Phi$  were scanned. Immediately after the electrical measurement, the selected plant was destructively sampled and the root systems were washed thoroughly off the potting soil. Care was taken during washing to minimize the loss of fine roots. The harvested root systems were stained with methyl violet; afterwards, they were subjected to scanning and image analysis (Delta-T Devices Ltd., Cambridge, UK) for assessing root surface area. Thereafter the roots were oven-dried at 70°C for 4 days and their dry masses were precisely weighed on analytical scales ( $\pm 1$  mg). The correlation between the EI readings and root system size (root dry mass and root surface area) was determined for each of the 55 applied current frequencies separately by performing simple regression analyses (regression type with the greatest  $R^2$  was accepted). Changes in the mean value of  $\Phi$  and the coefficient of determination ( $R^2$ ) for the EI – root dry mass and the EI – root surface area regression were plotted against the current frequency.

Experiment 2 involved 16 maize plants. Half of the plants remained untreated (control group) while the others were treated with the herbicide active ingredient acetochlor. This pre- and early post-emergent total herbicide agent is widely used for the control of annual grasses and dicotyledonous weeds on maize fields. Acetochlor acts as a general growth inhibitor by suppressing the anabolic pathways of proteins, fatty acids, and gibberellic acids (Abu-Qare and Duncan, 2002). The growth-inhibiting effect prevails non-selectively both against the target weeds and against the crop plants including maize (Cseresnyés *et al.*, 2012). The herbicide treatment was executed in compliance with the required application rate of acetochlor (1 200 g ha<sup>-1</sup>) used in agricultural practice. The product applied was Harness® (Monsanto Eur. S.A., Antwerpen, Belgium), containing 900 g dm<sup>-3</sup> acetochlor [2-chloro-N-(etoxyethyl)-N-(2-ethyl-6-methyl-phenyl) acetamide]. 100 cm<sup>3</sup> of the aqueous solution of the herbicide was poured over the soil surface on experiment day 9. EI- and  $\Phi$ -spectra of the control and the treated plants were scanned 7 days after herbicide addition (on experiment day 16). The effect of herbicide addition on the EI of soil was also determined (using two ground electrodes) to eliminate the possibilities

of systematic errors in EI readings. The measured EI and  $\Phi$  values of the control and the treated plants were statistically compared for each applied current frequency separately. The normality of data groups was checked by the Kolmogorov-Smirnov test. The unpaired t test was used to compare means of data groups or the nonparametric Welch test was applied if the standard deviations of the compared groups (F test) were not identical. Computations were performed using Statistica software (ver. 9., StatSoft Inc., OK, USA).

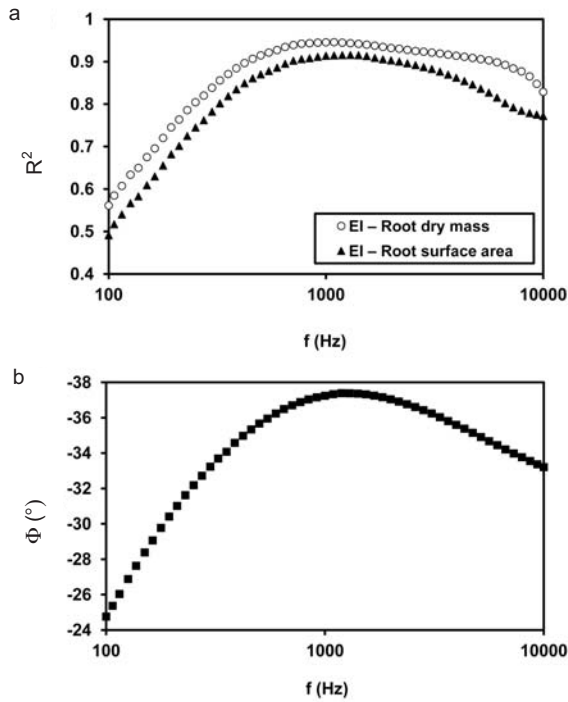
Twelve replicates of plants were sorted for Experiment 3 and each of them was grown until experiment day 74 (beginning of the flowering stage). Measurement of EI,  $\Phi$ , and D related to current frequency was executed on ten occasions, weekly from experiment day 11 to 74. Mean values of  $\Phi$  and D were calculated for each frequency; next, the frequency-dependent changes in the two electrical properties were plotted for different plant ages.

## RESULTS AND DISCUSSION

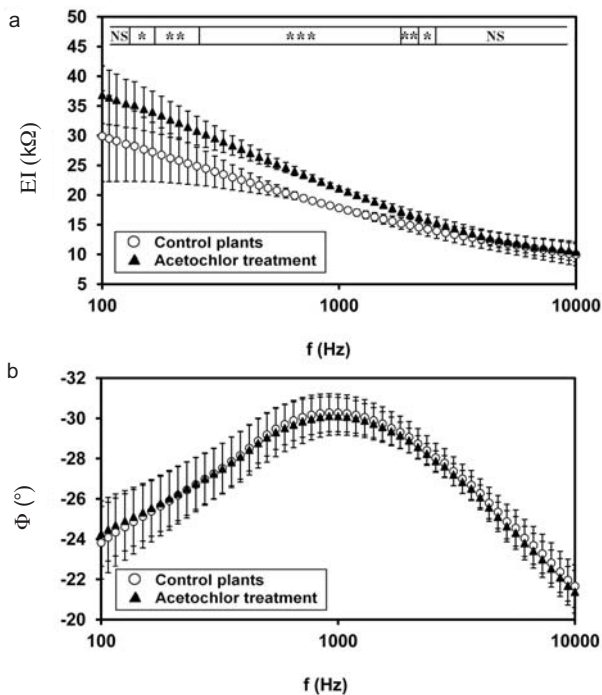
The preliminary measurements aimed directly at the soil showed the high conductance of the planting medium: EI in soil was only 120~160  $\Omega$  with a -0.4~ -0.6° phase angle. These values were insignificant in relation to the magnitude of EI (8~48 k $\Omega$ ) and  $\Phi$  (-22~ -38°) measured in the root-soil system. The root EI and  $\Phi$  were not influenced by the impedance and phase angle of soil; consequently, no correction of the results obtained was required.

The correlation analyses of root EI in the function of root dry mass and root surface area indicated negative logarithmic relationships between the impedance and root extent parameters (Experiment 1): the computed coefficient of determination ( $R^2$ ) was the highest using the  $y = -a \times \ln(x) \pm b$  regression equation type in the entire range of measuring frequency.  $R^2$  showed a frequency-dependent trend for both the EI – root dry mass and the EI – root surface area regression (Fig. 1a). As for the EI – root dry mass relationship,  $R^2 = 0.561$  was obtained at the lower end of the frequency range (100 Hz); then, it greatly increased along with the increasing frequency, reaching the maximum value of 0.946 at 1090 Hz. From this point, the coefficient decreased continuously with the increasing current frequency ( $R^2 = 0.823$  at 10 000 Hz). The strength of the correlation between EI and root surface area was similarly frequency-dependent at the applied frequency interval but the achieved coefficient of determination proved to be slightly lower than that calculated for the EI – root dry mass regressions (Fig. 1a). In this case,  $R^2 = 0.491$  was obtained at 100 Hz; it culminated at 1 300 Hz ( $R^2 = 0.916$ ) and finally reduced to the value of 0.772 at 10 000 Hz. The change in the average of  $\Phi$  as a function of current frequency (Fig. 1b) was observed to be perceptibly very similar to the trends of the  $R^2$ . Since a higher negative  $\Phi$  value indicates a higher phase angle in capacitors, phase angle graphs are shown with inverted vertical axis. Although the apex of the phase angle curve changes





**Fig. 1.** The coefficient of determination ( $R^2$ ) for the electrical impedance (EI):root dry mass and root surface area regressions obtained in maize plants ( $n = 18$ ) (a), as well as the mean value of the phase angle ( $\Phi$ , °) (b) related to the current frequency applied for electrical measurements.

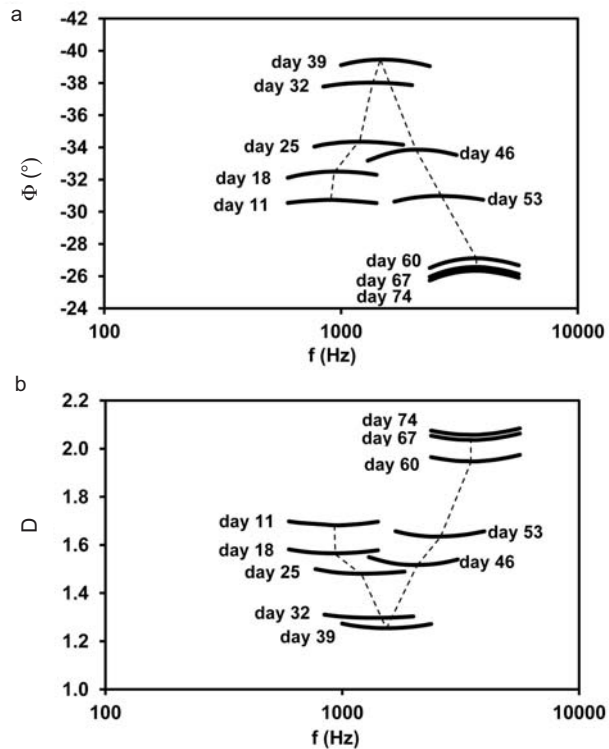


**Fig. 2.** Electrical impedance (EI,  $k\Omega$ ) spectra of the control and the acetochlor-treated plants with the significance levels (on top of graph) obtained by statistical comparison between the EI of the two plant groups ( $n = 8$ ) (a), as well as (b) the mean value of the phase angle ( $\Phi$ , °) related to the applied current frequency. \*\*\*, \*\*, \*  $p < 0.001$ , 0.01 and 0.05, respectively; NS – not significant.

with plant development (as will be shown by Experiment 3), the results of different measurements at different times could be pooled and averaged in this experiment limited to the early plant growth stage only (18 days). The phase angle had an upward trend from 100 Hz ( $\Phi = -24.8^\circ$ ) to 1190 Hz ( $\Phi = -37.4^\circ$ ) and then it decreased towards the higher current frequencies ( $\Phi = -33.2^\circ$  at 10 000 Hz). Thus, the apex of  $\Phi$  practically coincided with the culmination of  $R^2$  values considering the current frequency.

As for the herbicide treatment (Experiment 2), acetochlor addition did not induce any changes in the electric properties of soil. The root EI of the maize plants showed a downward trending curvilinear relationship along with the increasing current frequency (Fig. 2a). The inhibiting effect on plant root expansion produced by the acetochlor agent is clearly demonstrated by the EI-spectra: the mean EI of herbicide-treated plants exceeded the mean value of the control group at the entire frequency range. In turn, the statistical comparison of the two groups (by the unpaired  $t$  test or nonparametric Welch test in some cases) resulted in various significance levels for different parts of the frequency range. The highest level of significance ( $p < 0.001$ ) was obtained when current frequencies between 250 Hz and 1 830 Hz were applied (Fig. 2a). Outside this interval, the significance level decreased gradually with the decreasing or the increasing frequency until the difference between the groups became statistically insignificant ( $p > 0.05$ ) below 120 Hz or above 2 820 Hz. The acetochlor treatment had no effect on the phase angle:  $\Phi$  of the control and treated plants was statistically equal at the entire frequency range (Fig. 2b). The change in the magnitude of the phase angle as a function of the current frequency was somewhat similar to that in Experiment 1. The maximum  $\Phi$  value ( $-30.3^\circ$ ) occurred at the frequency of 920 Hz and decreased continuously towards the ends of the applied frequency range ( $\Phi$  was  $-23.6^\circ$  and  $-21.7^\circ$  at 100 Hz and 10 000 Hz, respectively). The highest significance level obtained at the comparison of the control and treated plants coexisted with the  $\Phi$  value more than approximately  $-27^\circ$  in this experiment, while the growth-impeding effect of acetochlor became undetectable at the frequency range corresponding to  $\Phi$  lower than about  $-24.5^\circ$ .

The effect of plant age on the magnitude and frequency-dependency of  $\Phi$  and  $D$  was clearly demonstrated by Experiment 3. The first electrical measurement carried out on experiment day 11 showed a  $-30.7^\circ$  maximal phase angle and 1.68 minimal  $D$  values (Fig. 3a and 3b). The capacitive character of the root was increasingly stronger up to experiment day 39, when the highest phase angle ( $\Phi = -39.5^\circ$ ) and inherently the lowest energy dissipation ( $D = 1.26$ ) were detected. Afterwards, the decreasing phase angle and the increasing rate of the dissipated energy definitely indicated the continuously reducing charge-storage ability of root cell membranes: the maximal  $\Phi$  and minimal  $D$  were  $-26.3^\circ$  and 2.06, respectively on experiment day 74. When



**Fig. 3.** Change in the: a – phase angle ( $\Phi$ , °) and b – dissipation factor ( $D$ ) related to current frequency applied for electrical measurements at different ages of maize plants ( $n = 12$ ). For better legibility of the results, only the apexes of the spectra were displayed and linked with dashed lines.

plants reached the flowering stage between experiment day 67 and 69, the reduction in capacitive strength lessened considerably: the  $\Phi$ - and  $D$ -spectra obtained on experiment day 67 and 74 nearly overlapped. The characteristic frequency, at which the strongest capacitive character was detected, also varied during the plant development. At the first two measurements, the application of 920 Hz current frequency produced the maximal  $\Phi$  (and minimal  $D$ ) reading. Subsequently, the characteristic frequency shifted continuously to 3 650 Hz obtained on experiment day 60, thereafter it remained constant until the end of the experiment.

Analyses of our measurement results confirm that the applied current frequency has a great effect on the phase angle and the extent of energy dissipation (goodness of the capacitor). Higher  $\Phi$  and consequently a lower  $D$  value are apparently associated with a stronger correlation observed between the magnitude of root EI and root system size as well as with the better distinctness of plant groups subjected to different environmental conditions. Thus, a characteristic current frequency or frequency range determined properly on the basis of  $\Phi$  measurement can clearly contribute to improved statistical authenticity of the results and to detection of different stress conditions having an influence on root development.

Regarding an ideal capacitor, the magnitude of impedance is inversely proportional to the applied current frequency: the EI value is infinity against the direct current and approaches zero with the increasing alternating current frequency with a constant phase angle ( $\Phi = -90^\circ$ ). In a plant-soil-electrode network, the frequency-dependency of EI is similar to those of ideal capacitors, but the change in  $\Phi$  related to current frequency can be described by an optimum curve. The capacitive character of the plant root-soil system proved to be relatively strong within the 700–4 000 Hz frequency range. At low frequencies (mainly below 100 Hz), the current flows through the extracellular (apoplastic) pathway, bypassing the cells: the high EI and low  $\Phi$  measured is mostly due to the high resistance and low capacitance of extracellular space, respectively (Ozier-Lafontaine and Bajazet, 2005; Repo *et al.*, 2000). At increasing frequencies, an alternating current starts to penetrate root cells: this intracellular (symplastic) pathway of current generating a capacitance effect is clearly reflected by the growing tendency of  $\Phi$ . The further increase in frequency first begins to reduce and then cancels the capacitance of cell membranes, leading to a continuously decreasing  $\Phi$  value. The characteristic change in the capacitive behaviour of cell membranes corresponds to the various frequency-dependent dispersion regions exhibited by biological materials. The  $\alpha$ -dispersion (100 mHz – 1 kHz) is due to surface admittance and depends principally on the external membrane surface and apoplastic structures (cell walls), while  $\beta$ -dispersion (1 kHz – 1 MHz) is mainly associated with the internal membrane and intracellular structures. Results of earlier studies also confirmed our findings that especially the upper part of the  $\alpha$ -dispersion and the lower part of the  $\beta$ -dispersion region (0.5–10 kHz) is suitable for the complex, extra- and intracellular electrical investigation of plant tissues and organs (Euring *et al.*, 2011).

Tending towards the lower (100 Hz) and upper (10 000 Hz) ends of the frequency range we applied, the declining capacitive character of root membranes (indicated by the decreasing phase angle and the increasing rate of energy dissipated) results in the decreasing coefficient of determination ( $R^2$ ) achieved for the different EI – root extent regressions. The reason is that the amount of energy dissipated is independent of the root system size, thus the measured EI also becomes increasingly independent of the root system size in these cases. Additionally, this phenomenon can be intensified by occurrence of two sources of systematic error such as electrode polarization impedance and parasitic (stray) capacitances apparent at low and high frequencies, respectively (Cao *et al.*, 2010; Ozier-Lafontaine and Bajazet, 2005). As for Experiment 2 (acetochlor treatment), the reduced charge-storage capacity of root membranes and the enhanced rate of energy dissipation caused an increase in the standard deviations of the data groups, leading to the lower significance levels at statistical comparison (Fig. 2).

As Experiment 3 confirmed, the frequency-dependency of  $\Phi$ , thus the characteristic frequency can be varied over time even in the same plant-soil system (Fig. 3). One presumable explanation for this phenomenon is that the physicochemical structure of root tissue undergoes characteristic changes during plant development. In aging roots, impermeable and electrically insulating suberin is deposited in the radial and transverse cell walls of the endodermal cells (this structure is known as the Casparian strip), altering the proportion of the electric current passing through the apoplastic and symplastic pathways to the root stele at a given current frequency. Owing to the insulating effect of suberin, principally inhibition of the apoplastic pathway occurs; therefore, the maximal value of  $\Phi$  shifts continuously to the higher frequency. At the second part of the vegetative stage (day 46), the magnitude of  $\Phi$  begins to decrease, probably as a consequence of the reduced root activity and decreased capacitance due to the age-dependent physicochemical changes of rhizodermal cell membranes, including altered permeability, ion conductivity, membrane potential, and structure of the electric double layer (Čermák *et al.*, 2006; Dalton, 1995). The declined root activity and the enhanced electrical resistance caused by suberin deposition are corroborated by the outcomes of our EI measurements: root EI showed a continuous decrease in relation to plant root expansion during the early vegetative stage and then began to increase from day 46 (data not shown). These observations suggest that – particularly in time-course studies – the regularly repeated phase angle measurement is advisable for appropriate application of electrical impedance spectroscopy (EIS), and the systematic operation at the same reference frequency (1 000 Hz or other) without determination of phase angle spectra should be avoided.

EI and EC measurement is a very promising, simple, rapid, and low-cost technique for non-intrusive studying of roots and their function. Against its disadvantages described previously, the method can be reliably applied in various fields of plant physiological, ecological and agricultural research, substituting at least partially the intrusive, complicated, as well as time- and cost-consuming techniques used commonly (Aubrecht *et al.*, 2006; Cao *et al.*, 2010; Chloupek *et al.*, 2006; Cseresnyés *et al.*, 2013). EIS, a general method as well, has been successfully employed for characterization and quality evaluation of various agricultural products such as fruits, vegetables, cereals, meats, raw and processed milk as well as dairy products without destroying the materials (Li *et al.*, 2011; Soltani *et al.*, 2011; Żywica *et al.*, 2012). Due to its versatile adaptability as well as its importance in scientific research and in agriculture and food industry, development of the EI method is a timely task. The present study has indicated that the phase angle measurement could contribute to greater accuracy and statistical reliability of EI results, thus the frequency- and time-dependent changes in  $\Phi$  should be considered during the

application of EIS. Further work is needed to conduct investigations aimed at determination of the effect of soil properties (*eg* soil type, moisture status, chemical composition, ionic strength) and plant characteristics (*eg* species, phenological stage, physiological status) on phase angle spectra.

## CONCLUSIONS

1. Our experimental results have highlighted the importance of phase angle measurement during the application of electrical impedance spectroscopy.
2. In a plant root-soil system, the frequency-dependent phase angle change can be described by an optimum curve.
3. The higher phase angle and lower rate of energy dissipation is associated with a higher coefficient of determination achieved for the electrical impedance – root dry mass and electrical impedance – root surface area regression as well as with better distinctness of plant groups subjected to different stress conditions.
4. The characteristic current frequency that is the most efficient for electrical impedance measurements should be chosen on the basis of phase angle spectra.
5. Since the frequency-dependency of the phase angle can be varied by plant aging due to the physicochemical changes observable in root tissue, regularly repeated phase angle measurement is advisable during the time-course experiments.

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