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Application of Impedance Spectroscopy and Conductometry for Assessment of Varietal Differences in Wheat

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The potentials of an electrochemical and a physical technique for detection of physiological differences in three wheat cultivars under optimal growth conditions were outlined in the study. Electrolyte leakage kinetics was established by continuous measurements of conductivity of solutions in which leaf pieces were incubated for 24 hours. Impedance spectra were obtained from intact leaves at frequency range from 7 to 2010 Hz and 250 mV measuring voltage applied between two gold plated silicon substrates serving as electrodes. The obtained spectra were approximated by a model employing two ARC elements connected in series. Parameters of the previously described diffusion model based on time course conductivity measurements were inversely correlated with electrical impedance spectroscopy data, thus the genotype with highest ion leakage (cultivar Prelom) exhibited lowest impedance magnitude. It was concluded that the two methods were able not merely to distinguish the three studied cultivars but also to rank them in the same order based on their electrical properties.

Keywords: diffusion model, electrolyte leakage kinetics, impedance, *Triticum aestivum*

Introduction

Thus far, a large body of experimental techniques has been employed to discriminate between intact and stressed plant species (Bajji et al. 2002; de Faria et al. 2013; Jócsák et al. 2009; Mancuso et al 2004; Repo et al. 2000). However, the question of distinguishing untreated samples of different varieties is challenging in no lesser extent. Undoubtedly, protocols giving possibilities for differentiating plants characteristics under optimal conditions could significantly donate to our knowledge of such important phenomena as acclimation, cold and drought tolerance, yields, field performance, etc. As far as plants (like all living systems) are abundant of water and ions, electrochemical approaches seem to be the most promising ones in this direction. Indeed, to date many routine tests on electrochemical basis have been developed and successfully used for various assays in laboratory and in the field. To mention few of them, these are amperometric devices for estima-

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tion of oxygen evolution rate and gas exchange in photosynthesis (Blinks and Skow 1938; Delieu and Walker 1981) mostly employing rapid or Clark electrode (Clark 1956), conductometric tools for assessment of electrolyte leakage from different parts of the plant (Murray et al. 1989; Radoglou et al. 2007), devices measuring dielectric parameters of plant tissues (Cole 1968; Ackmann and Seitz 1984; Zhang and Willison 1991; Repo and Zhang 1993) and so on.

In this work we explored the potentials of two frequently used techniques, for elucidation of possible differences in wheat cultivars under optimal conditions. First of them – conductometric measurement of electrolyte leakage from plant tissues – was so long widely accepted for assessment of cell membrane injury as consequence of drought, salinity and low temperatures (Bajji et al. 2002; Saneoka et al. 2004; Farooq and Azam 2006). Moreover, some authors introduced the time course observation of ion efflux (Whitlow et al. 1992; Prášil and Zámečník 1998). Recent improvements of this approach revealed new perspectives to collect more information about the specific stress response of cell walls and membranes (Kocheva et al. 2005, 2014) caused by the movement of ions between two distinct spaces defined on the outer side (apoplast) and on the inner side (symplast) of the plasmalemma.

The second technique, concerning the evaluation of dielectric properties of investigated species by impedance measurements, could be regarded as an extension of conductometry over the complex plane of the electrical variables, i.e. the material response would content not only the real conductance, but an imaginary reactance as well. This is usually achieved with the aid of time-dependent external potentials which cause polarization and relaxation in the sample leading to changes in amplitude and phase of the measured alternating current (AC) response signal. Furthermore, by applying an alternating electromagnetic field on the probe at different frequencies, some kind of spectroscopy can be realized. This method, known as Electrical Impedance Spectroscopy (EIS), allows an impedance spectrum to be obtained, hence passive dielectric properties to be investigated (Barsoukov and Macdonald 2005).

Because of enormous complexity of living matter however, it is almost obvious that explicit generalized theory of bioimpedance can not be completed. Instead, empirical models, expressing semi-quantitatively the main features of objects under observation, could be constructed and this approach is well accepted. Actually, attempts for impedance measurements of plant samples at limited frequencies have been made ever since the early seventies (for review see Wilner and Brach 1979). With the employment of larger electromagnetic spectrum of excitation, however, much more information was gained about the mechanisms of reaction of different cell compartments. This allowed the uniform plant tissue to be represented by specific models including extracellular resistance, plasma membrane capacitance, cytoplasmic resistance, tonoplast capacitance, and vacuole interior resistance (Zhang and Willison 1991). The impedance data were used to characterize plant tissues and their changes during stress (Zhang et al. 2002; Jócsák et al. 2009). EIS was also employed to monitor moisture content and growth of tea leaves (Mizukami et al. 2006, 2007). Many researchers were interested in using impedance spectroscopy to better understand dielectric properties of plant tissues as seen from the evolution of models used to describe the passive electrical characteristics of plant samples. Markedly, they expand from equivalent circuits comprising only ideal frequency independent constituents (Zhang and Willison 1992) to schemes consisting of distributed elements (Repo and Pulli 1996; Zhang et al. 2002).

The aim of the present study was to assess the potentials of EIS and conductometry for distinguishing electrochemical properties of three wheat cultivars possessing different genotypic background and grown under optimal conditions.

Materials and Methods

Plant material and growth conditions

Two Bulgarian (Prelom and Katya) and one Slovak (Ilona) wheat (*Triticum aestivum*, L.) cultivars were used in the experiments. Seeds were soaked for 4 h in tap water and were subsequently sewn in 1 kg pots filled with alluvial meadow soil (pH 6.2). Plants were grown for 14 days in a climatic chamber with 22/18 °C day/night temperature, 14 h photoperiod, irradiance of 250 μmol m^{-2} s^{-1} and 70% relative humidity. Tap water was supplied daily sustaining 60% of full soil moisture capacity estimated according to Evett (2008). All measurements were performed on the first leaf.

Electrolyte leakage measurements

For determination of electrolyte leakage 15 leaf pieces (2 cm in length) were cut from different plants separately for each variant. They were briefly washed with distilled water to remove the solution from injured cells and were then submerged in 15 ml of distilled water for 24 h at constant room temperature (20 °C). During this incubation period conductivity of the solutions was measured at multiple time points with a conductometer (Elwro 5721, Poland). For obtaining the total electrolyte content of tissues, they were killed by boiling for 30 min at 100 °C and conductivity of the solutions was read for the last time. The results were normalized, i.e. expressed as relative conductivity ratio κ/κ_{max} where κ is conductivity of samples at a particular moment and κ_{max} is the conductivity of total electrolyte content. Thus a multiple-point kinetics curve was obtained. Fitting of experimental data was performed by the Exponential Associate function of Origin 5.0 software:

$$\kappa/\kappa_{max} = C_o(t) \approx A_1(1 - e^{-t/t1}) + A_2(1 - e^{-t/t2}) + C_o^o$$

From this kinetics, which is obviously biphasic, four main parameters were derived describing the ion efflux from the leaves. These are: the amplitude A_1 and time constant t_1 of the first phase, as well as the amplitude A_2 and time constant t_2 of the second phase. C_o^o is the initial leakage which can easily be neglected since its value is very low compared to the maximal leakage (Kocheva et al. 2014). The physical meanings of these parameters are defined more thoroughly in the previously introduced diffusion model (Kocheva et al. 2005). Briefly, according to the model assumptions, A_1 and A_2 are dimensionless coefficients connected with the volumes of apoplast (V_e) and symplast (V_i) and the concentration of ions contained in these compartments. If $\alpha = V_e/V_o$ and $\beta = V_i/V_o$, are the ratios of the respective volumes to the volume of the external solution V_o, in which the measurement is carried out, then A_1 and A_2 can be represented as:

$$A_1 = C_e^o\alpha/(C_e^o\alpha + C_i^o\beta)(1 + \alpha)$$
$$A_2 = C_i^o\beta/(C_e^o\alpha + C_i^o\beta)(1 + \beta)$$

Here C_e^o and C_i^o are the initial ion concentrations in the apoplast and symplast. Thus parameters A_1 and A_2 reflect the maximal capacity of each compartment donating ions to the overall efflux. Correspondingly, time constants t_1 and t_2, representing the rate of efflux from the individual compartment, are given by:

$$1/t_1 = (1 + \alpha)(P_w A_w/V_e)$$
$$1/t_2 = (1 + \beta)(P_m A_m/V_i)$$

Here P_w and P_m are the permeabilities of apoplast (wall) and symplast (plasmalemma), while A_w and A_m are their surfaces.

The impedance spectroscopy and acquisition of electrical parameters of the samples

The most common way to perform EIS is by applying sinusoidal AC voltage to a simple two-electrode electrochemical cell, to monitor its reaction in the form of current passing through it. From the theory of electrical circuits, it is well known, that depending on the components of the impedance, the current is also sinusoidal, exhibiting certain phase shift with positive or negative angle θ. Biological material rarely shows any inductive component, so the equivalent impedance can be represented as a combination of resistances R and capacitances C, connected in a proper fashion. However, in most cases the equivalent impedance is a complex function of the frequency and distributed elements should be used either (Bard and Faulkner 1980). In the present work we have employed a model comprising two so called ARC elements connected in series (see inset in Fig. 3). The impedance Z_{ARC} of such an element consists of parallel coupled ideal resistor R and Z_{CPE} of Constant Phase angle Element (CPE) defined as: $Z_{CPE} = (i2\pi f)^{-P}/T$, where T and P are constants and f is frequency (Cole and Cole 1941). Here the parameters of CPE are denoted according to the software ZView2.1 in order to avoid confusion with the parameters of the leakage model. Obviously, for $P = 0$ the impedance Z_{CPE} represents ideal resistor $R = 1/T$ and for $P = 1$ it is an ideal capacitor $C = T$. It should be said, that similar equivalent circuit has been used earlier by other authors for determination of the impedance of other plant species (Repo et al. 2000). All impedance measurements were performed using a set up described in details elsewhere (Simeonov et al. 2013). It consisted of two identical gold plated silicon substrates (produced by MICROSENS SA, Neuchâtel/Switzerland), serving as electrodes. The electrodes were attached to a micrometer screw, so the distance between them can be precisely defined, depending on the leaf thickness.

Electrode surface was 6 × 6 mm. The measuring device was accomplished by the PC sound card (SC) and additional high input buffer preamplifier with unity gain (Klaper and Mathis 2008) for overcoming the relatively low input impedance of the card. Open-short correction was not performed. Voltage applied to specimens was U_r = 250 mV and operating frequency ranged between 7 to 2010 Hz. As software for the SC we have used the specialized program PhysLab 5.0, developed by prof. S. P. Palto, Shubnikov Institute of Crystallography, Russian Academy of Sciences, Moscow, Russia (Palto 1998). The impedance of the cell Z_x was measured by the aid of a reference resistor with resistance R_{ref} = 100 kΩ connected in series with the cell (Simeonov et al. 2013). As an input signal the reference sine voltage U_r was applied to the series $R_{ref} + Z_x$, and the voltage drop U_x across the cell impedance Z_x was measured as output signal. Using the lock-in function of the PhysLab 5.0 software, the amplitudes $|U_r|$ and $|U_x|$ of the two signals and the phase angle difference θ between them were measured. The impedance of the cell was derived starting from the equation:

$$\frac{|U_r|}{|U_x|}e^{i\theta} = \frac{Z_x + R_{ref}}{Z_x} = 1 + R_{ref}Y_x$$

where $|U_r|$ and $|U_x|$ are the amplitudes of the two signals, θ is the phase angle between them, R_{ref} is the reference resistance, Z_x is the impedance of the cell, and $Y_x = 1/Z_x$ is the admittance of the cell. From this equation the real and imaginary parts of the admittance $\mathrm{Re}(Y_x)$ and $\mathrm{Im}(Y_x)$ could be easily derived:

$$\mathrm{Re}(Y_x) = \frac{1}{R_{ref}}\left(\frac{|U_r|}{|U_x|}\cos\theta - 1\right)$$

$$\mathrm{Im}(Y_x) = \frac{1}{R_{ref}}\frac{|U_r|}{|U_x|}\sin\theta$$

The real and imaginary parts of the impedance $\mathrm{Re}(Z_x)$ and $\mathrm{Im}(Z_x)$ were obtained according to:

$$\mathrm{Re}(Z_x) = \frac{\mathrm{Re}(Y_x)}{[\mathrm{Re}(Y_x)]^2 + [\mathrm{Im}(Y_x)]^2}$$

$$\mathrm{Im}(Z_x) = \frac{-\mathrm{Im}(Y_x)}{[\mathrm{Re}(Y_x)]^2 + [\mathrm{Im}(Y_x)]^2}$$

The samples electrical parameters were obtained with the aid of the program ZView2.1, Scribner Associates, Inc., which allowed a complex fitting of the experimental data with the chosen equivalent circuit (Fig. 3). Thus the parameters (Fig. 4) of model impedance were deduced.

Statistical analysis

Two independent experiments were carried out with three replications for each set of conductivity and impedance measurements. Results were presented as means ± standard error (SE), n = 6. The significance of differences in the pairwise comparisons was analyzed by Mann–Whitney U-test using STATISTICA 7 software package (StatSoft 2005).

Results

Electrolyte leakage kinetics

Typical time course of electrolyte leakage from leaves of three wheat cultivars (Prelom, Katya and Ilona) was represented in the form of exponential curve with two distinct phases (Fig. 1). Prelom was characterized by the highest flowing out of ions in comparison with the other genotypes under study. Katya and Ilona clearly showed lower amount of efflux assessed by the relative conductivity values κ/κ_{max}. Thus the three cultivars were naturally ranked according to the magnitude of their ion leakage in the following order: Prelom-Katya-Ilona.

After fitting the experimental data with a model, comprising the Exponential Associate function, four basic parameters were obtained: amplitude of the prompt phase A_1 and its corresponding time constant t_1, amplitude of the slower second phase A_2 and its corresponding time constant t_2. The parameters values for each of the three studied wheat genotypes were presented in Fig. 2. Amplitude of the first phase, A_1, did not differ between the cultivars (Fig. 2a), while t_1 had highest values for Ilona and lowest for Prelom

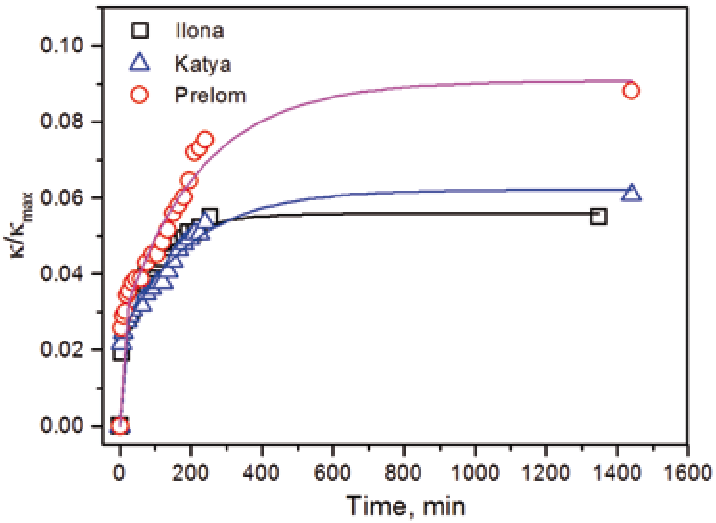

Figure 1. A typical ion leakage kinetics from three wheat cultivars. Symbols represent experimental data, and curves are the corresponding best nonlinear fits

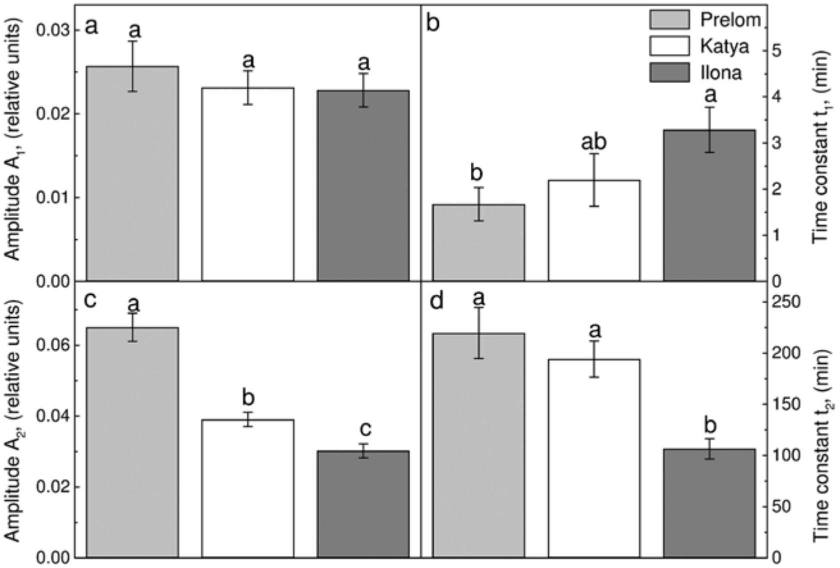

Figure 2. Parameters of the diffusion model for ion leakage from leaves of three wheat cultivars. (a) amplitude of the prompt phase A_1; (b) time constant of the prompt phase t_1; (c) amplitude of the slow phase A_2; (d) time constant of the prompt phase t_2. Data are means ± SE (n = 6). Different letters indicate significant differences (p<0.05) according to Mann–Whitney U test

(Fig. 2b). Parameters A_2 and t_2 characterizing the second phase had concordant behavior and both decreased in the order Prelom-Katya-Ilona, although regarding t_2 values there were no significant ($p < 0.05$) differences between Katya and Prelom (Fig. 2d).

Electrical impedance spectroscopy, EIS

The results of EIS measurements corresponded to Nyquist dependence (i.e. imaginary vs. real part) of the impedance in a frequency range of 10 Hz to 2 kHz. Evidently, under optimal physiological conditions all three wheat cultivars exhibited well distinguished impedance patterns, characteristic for equivalent schemes containing ARC element, i.e. semicircle curves slightly displaced versus abscissa (Fig. 3). An overall increasing magnitude of impedance in the sequence Prelom-Katya-Ilona was clearly observed which was inversely correlated with the decreasing ion efflux from the cultivars in the same order (Fig. 2). This naturally demonstrated arrangement of the three genotypes seemed convenient for comparing the specific parameters of the models engaged in the two approaches used in the study. An EIS analysis was made with the employment of the above mentioned equivalent circuit (inset of Fig. 3). In principle, there should be three independent parameters describing each ARC element, namely *R*, *T* and *P*. After approximation very similar values of parameter *P* for the three genotypes were obtained which were not significantly different at $p < 0.05$. P_1 of CPE1 was *ca.* 0.718 and P_2 of CPE2 was *ca.* 0.97 for

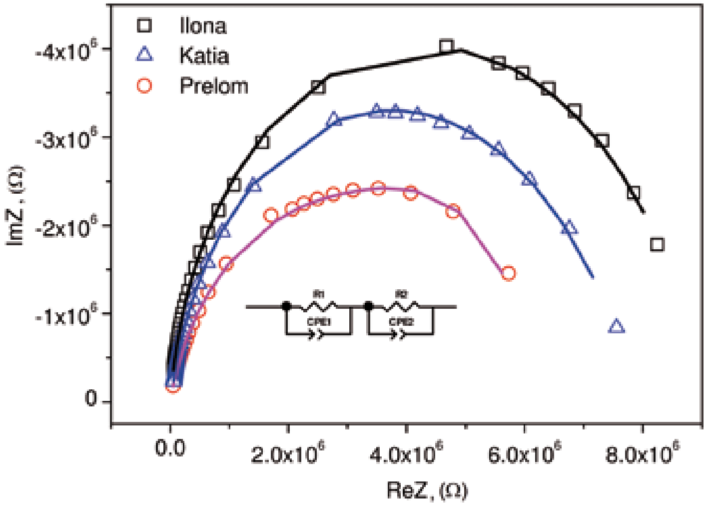

Figure 3. Impedance behavior of three wheat cultivars represented in the form of Nyquist plot. Symbols are illustration of typical examples of experimental data and curves are the best fit with the equivalent model (shown on the inset)

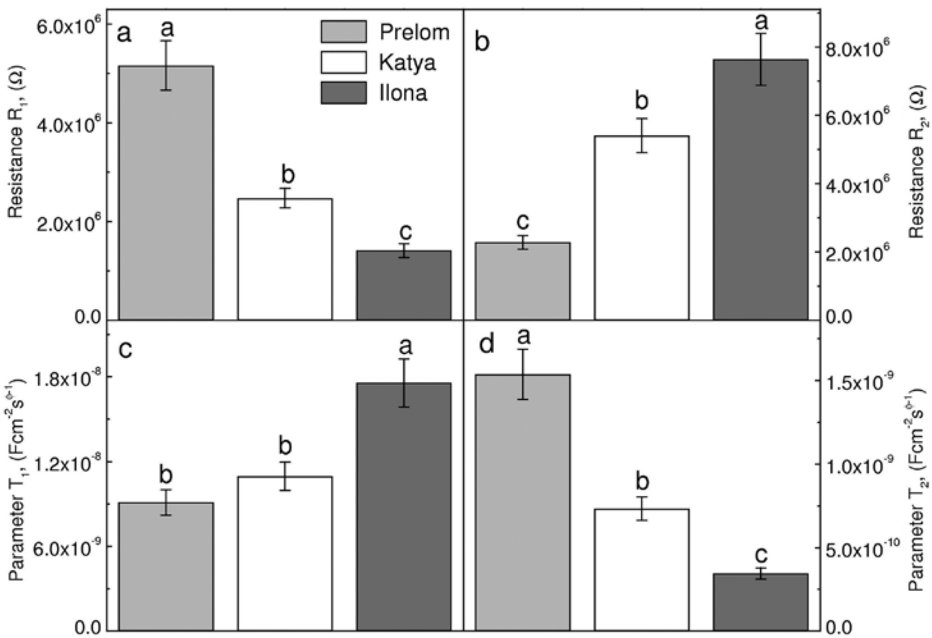

Figure 4. Electrical impedance parameters of the leaves of three wheat cultivars. (a) resistance R_1 of the ARC1; (b) resistance R_2 of the ARC2; (c) parameter T_1 of the CPE1; (d) parameter T_2 of the CPE2. Values are derived from the EIS data by fitting with the equivalent circuit from Fig. 3. Data are means ± SE ($n = 6$). Different letters indicate significant differences ($p < 0.05$) according to Mann–Whitney U test

the three studied cultivars. Obviously P could not be used as a characteristic parameter for proper differentiation of various genotypes. For this reason, the set of two parameters, R and T, was chosen in further analysis.

Thus, four impedance parameters (two for each ARC element) were compared in the studied wheat cultivars. The resistance R_1 of ARC1 was found to decrease in the sequence Prelom-Katya-Ilona, while R_2 of ARC2 increased in the same order (Fig. 4a and b). Similar values of the parameter T_1 of CPE1 were observed for Prelom and Katya which were significantly different (at $p < 0.05$) from Ilona, whereas parameter T_2 of CPE2 decreased in the order Prelom-Katya-Ilona (Fig. 4c and d).

Discussion

Although conductometric measurement of electrolyte leakage from plant tissues has been frequently used by many authors for assessment of cell membrane injury caused by various abiotic stress factors, only few of them have employed time course recording of the ion efflux (Whitlow et al. 1992; Prášil and Zámečník 1998; Roy et al. 2009; de Faria et al. 2013). The latter approach is advantageous because it provides a larger amount of data for characterizing the electrolyte flow from the studied tissue. In earlier works the kinetics of leakage has not been thoroughly analyzed, with the exception of Whitlow and coauthors (1992) who offered an interesting interpretation of their data by defining a novel parameter more directly evaluating ion efflux which explicitly includes chemical driving force and tissue surface area. The recently proposed diffusion model and its improvements (Kocheva et al. 2005, 2014) suggest that the kinetics of electrolyte leakage from various plant tissues could be represented by an exponential curve with two phases revealing the movement of ion fluxes from the apoplast and the symplast.

The parameters derived from the first leakage phase (A_1 and t_1) describe processes of ion efflux through the cell wall (apoplast) while the pair of parameters characteristic for the slower second phase (A_2 and t_2) are indicative of electrolyte leakage through cellular membranes (symplast). Higher values of the time constants t_2 of the second phase in comparison with t_1 of the three wheat cultivars studied could reflect the capability of the plasmalemma to actively regulate processes of ion movement through it and thus to determine the slower rate of their efflux. As expected, cell walls were more pervious and thus ions passage through them was faster. Three of the model parameters (t_1, A_2, and t_2) supported ranking of the tested cultivars in the order Prelom-Katya-Ilona and could clearly distinguish the genotypes based on their cell wall and membrane ion permeability. Prelom exhibited fastest efflux through the apoplast (lowest t_1) and slowest one through the membranes (highest t_2) although reaching highest amounts of ions effused. It was contrasted by Ilona which had the least quantity of electrolytes leaked with greatest time constant t_1 and smallest t_2. Katya took intermediate position as regards of all leakage parameters values.

EIS has been widely used for various purposes in life sciences, since this method was able to adequately assess the status of biological tissues (Kell 1987). Impedance data were readily used for characterization of changes in plant tissues under stress conditions

(Zhang et al. 2002; Jócsák et al. 2009). Lately, impedance spectroscopy was applied by Mizukami et al. (2007) to monitor leaf growth. In similar way equivalent circuits were used for determination of the impedance in Scots pine shoots during cold acclimation (Repo et al. 2000). Some authors have tried even more sophisticated approach to this problem. For example, Mancuso et al. 2004 used three independent techniques (two of them electrochemical) for more satisfactory estimation of cold tolerance.

Our modest efforts were oriented towards the employment of the same electrochemical methods which we have used recently for investigation of drought tolerance in plants (Kocheva et al. 2014; Simeonov et al. 2013). Here, the idea was to apply them for determination of varietal differences in three wheat cultivars grown under optimal conditions. We have attempted to compare EIS data obtained from untreated wheat leaves with data gathered by a previously established technique for assessment of electrolyte leakage in order to extract some peculiar features allowing adequate discrimination of the cultivars. An overall increasing magnitude of the impedance in the sequence Prelom-Katya-Ilona was clearly observed which was inversely related to the decreasing ion efflux from the cultivars in the same order. Definitely, this naturally demonstrated arrangement seemed convenient and we were motivated to use it in comparing the specific parameters of the models engaged in these two different techniques. In comparison with the parameters of ion leakage, the pattern revealed by impedance parameters appeared to be superior in distinguishing the three wheat cultivars under study. In our opinion, this result leaves no doubt about the potential of EIS approach for describing specific electrical properties of samples, thus differentiating plant genotypes. It is a matter of future work, however, to find precise correlation between the parameters of EIS model and that of the ion leakage model. In this way, it would be possible to reveal the explicit relation between them and the physiological status of symplast and apoplast in particular.

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References

Ackmann, J.J., Seitz, M.A. 1984. Methods of complex impedance measurement in biological tissue. CRC Crit. Rev. Biomed. Eng. **11**:281–311.

Bajji, M., Kinet, J.M., Lutts S. 2002. The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul. **36**:61–70.

Bard, A.J., Faulkner, L.R. 1980. Electrochemical Methods: Fundamentals and Applications. 1st ed. John Wiley & Sons. New York, USA.

Barsoukov, E., Macdonald, J.R. (eds) 2005. Impedance Spectroscopy. Theory, Experiment, and Applications. 2nd ed. John Wiley & Sons. Hoboken, New Jersey, USA.

Blinks, L.R., Skow, R.K. 1938. The time course of photosynthesis as shown by a rapid electrode method for oxygen. Proc. Natl. Acad. Sci. USA **24**:420–427.

Clark, Jr. L.C. 1956. Monitor and control of blood and tissue oxygen tension. Trans. Am. Soc. Artif. Intern. Organs **2**:41–48.

Cole, K.S., Cole, R.H. 1941. Dispersion and absorption in dielectrics. I. Alternating current characteristics. J. Chem. Phys. **9**:341–351.

Cole, K.S. 1968. Membranes, Ions and Impulses, University of California Press. Berkley, Los Angeles, USA.

de Faria AP, Lemos-Filho J, Modolo LV, França MGC. 2013. Electrolyte leakage and chlorophyll *a* fluorescence among castor bean cultivars under induced water deficit. Acta Physiol. Plant. **35**:119–128.

Delieu, T.J., Walker, D.A. 1981. Measurement of O_2 evolution by leaf discs. New Phytol. **9**:165–178.

Evett, S. 2008. Gravimetric and volumetric direct measurements of soil water content. In: Field estimation of soil water content. A practical guide to methods, instrumentation and sensor technologies. IAEA-TCS 30. Vienna, Austria. pp. 23–38.

Farooq, S., Azam, F. 2006. The use of cell membrane stability (CMS) technique to screen for salt tolerant wheat varieties. J. Plant Physiol. **163**:629–637.

Jócsák, I., Droppa, M., Horváth, G., Bóka, K., Vozáry, E. 2009. Cadmium- and flooding-induced anoxia stress in pea roots measured by electrical impedance. Z. Naturforsch. **65c**:95–102.

Kell, D.B. 1987. The principles and potential of electrical admittance spectroscopy, In: Turner, A.P.F., Karube, I., Wilson, G.S. (eds), Biosensors: fundamentals and applications. Oxford University Press. New York, USA. pp. 427–469.

Klaper, M., Mathis, H. 2008. 2-Pound RLC Meter – impedance measurement using a sound card. Elektor. **6**:64–68.

Kocheva, K.V., Georgiev, G.I., Kochev, V.K. 2005. A diffusion approach to the electrolyte leakage from plant tissues. Physiol. Plant. **125**:1–9.

Kocheva, K.V., Georgiev, G,I., Kochev, V.K. 2014. An improvement of the diffusion model for assessment of drought stress response in plants. Physiol. Plant. **150**:88–94.

Mancuso, S., Nicese, F.P., Masi, E., Azzarello, E. 2004. Comparing fractal analysis, electrical impedance and electrolyte leakage for the assessment of cold tolerance in *Callistemon* and *Grevillea* spp. J. Hort. Sci. Biotechnol. **79**:627–632.

Mizukami, Y., Sawai, Y., Yamaguchi, Y. 2006. Moisture content measurement of tea leaves by electrical impedance and capacitance. Biosyst. Engin. **93**:293–299.

Mizukami, Y., Yamada, K., Sawai, Y., Yamaguchi, Y. 2007. Measurement of fresh tea leaf growth using electrical impedance spectroscopy. Agricultural J. **2**:134–139.

Murray, M.B., Cape, J.N., Flower, D. 1989. Quantification of frost damage in plant tissues by rates of electrolyte leakage. New Phytol. **113**:307–311.

Palto, S.P. 1998. D. Sci. Thesis, Inst. Crystallogr. Ros. Acad. Nauk. Moscow, Russia.

Prášil, I., Zámečník, J. 1998. The use of a conductivity measurement method for assessing freezing injury. I. Influence of leakage time, segment number, size and shape in a sample on evaluation of the degree of injury. Environ. Exp. Bot. **40**:1–10.

Radoglou, K., Cabral, R., Repo, T., Hasanagas, N., Sutinen, M.-L., Waisel, Y. 2007. Appraisal of root leakage as a method for estimation of root viability. Plant Biosyst. **141**:443–459.

Repo, T., Zhang, M.I.N. 1993. Modeling woody plant tissues using a distributed electrical circuit. J. Exp. Bot. **44**:977–982.

Repo, T., Pulli, S. 1996. Application of impedance spectroscopy for selecting frost hardy varieties of english ryegrass. Ann. Bot. **78**:605–609.

Repo, T., Zhang, G., Ryypö, A., Rikala, R. 2000. The electrical impedance spectroscopy of Scots pine (*Pinus sylvestris* L.) shoots in relation to cold acclimation. J. Exp. Bot. **51**:2095–2107.

Roy, R., Agarwal, V., Gupta, S.C. 2009. Comparison of drought-induced polypeptides and ion leakage in three tomato cultivars. Biol. Plant. **53**:685–690.

Saneoka, H., Moghaieb, R.E.A., Premachandra, G.S., Fujita, K. 2004. Nitrogen nutrition and water stress effects on cell membrane stability and leaf water relations in *Agrostis palustris* Huds. Environ. Exp. Bot. **52**:131–138.

Simeonov, F., Kocheva, K., Georgiev, G.I., Kochev, V.K. 2013. A simple impedimetric device for in situ analysis of plant tissues. Compt. Rend. Acad. Bulg. Sci. **66**:969–974.

StatSoft. 2005. STATISTICA v. 7. StatSoft Inc., Tulsa, OK, USA. Available at www.statsoft.com/textbook.

Whitlow, T.H., Bassuk, N.L., Ranney, T.G., Reichert, D.L. 1992. An improved method for using electrolyte leakage to assess membrane competence in plant tissues. Plant Physiol **98**:198–205.

Wilner J, Brach EJ. 1979. Utilization of bioelectric tests in biological research. Engineering and Statistical Research Institute, Ottawa, ON, Canada.

Zhang, G., Ryyppö, A., Repo, T. 2002. The electrical impedance spectroscopy of Scots pine needles during cold acclimation. Physiol. Plant. **115**:385–392.

Zhang, M.I.N., Willison, J.H.M. 1991. Electrical impedance analysis in plant tissues: a double shell model. J. Exp. Bot. **42**:1465–1475.

Zhang, M.I.N., Willison, J.H.M. 1992. Electrical impedance analysis in plant tissues: The effect of freeze-thaw injury on the electrical properties of potato tuber and carrot root tissue. Can. J. Plant Sci. **72**:545–553.