

THESES OF THE DOCTORAL (PhD) DISSERTATION

Flórián Kovács

Budapest

2026



Hungarian University of Agriculture and Life Sciences

The applicability of bioimpedance spectroscopy for monitoring the nitrogen status of plants

Flórián Kovács

Budapest

2026

Doctoral School:	Doctoral School of Agricultural and Food Sciences
Discipline:	Horticulture and Crop Science
Head:	Prof. Dr. Kovács Melinda University professor, full member of Hungarian Academy of Sciences
Supervisor(s):	Katalin Juhos, PhD. Associate Professor Hungarian University of Agriculture and Life Sciences, Institute of Environmental Science, Department of Agro Environmental Studies Zoltán Vizvari, PhD. Associate Professor Óbuda University, John von Neumann Faculty of Informatics, Institute of Biomaterials and Applied Artificial Intelligence, University Research and Innovation Center, Bioimpedance Technologies Research Centre

.....

Head of Doctoral School

.....

Supervisor

Katalin Juhos, PhD

.....

Supervisor

Zoltán Vizvari, PhD

1. INTRODUCTION AND OBJECTIVES

In intensive vegetable production, nitrogen (N) fundamentally determines plant growth, photosynthetic activity, and yield formation. However, the amount of applied N often exceeds the actual demand of plants, which may cause not only economic losses but also environmental risks, particularly through nitrate (NO_3^-) leaching into groundwater. Therefore, rapid, reliable, and preferably non-destructive determination of plant N status is a key requirement for efficient N management. Currently used analytical methods are accurate but destructive and time-consuming, whereas optical sensors usually estimate N status indirectly, mainly based on chlorophyll content. Consequently, there is a need for measurement approaches that are more directly related to cellular-level physiological processes.

Bioimpedance spectroscopy (BIS) investigates the electrical properties of plant tissues and enables the separate interpretation of electrical components associated with extracellular, intracellular, and vacuole compartments. BIS parameters may provide information on cell hydration, ion distribution, and membrane status; therefore, they may be suitable for detecting N deficiency at the cellular level. However, the diagnostic value of this method for nutrient status, particularly under N deficiency, remains insufficiently clarified. A particularly important question is to what extent extracellular resistance reflects apoplastic NO_3^- concentration, how vacuole resistance is related to NO_3^- storage, and how cell membrane capacitance is associated with membrane stability. The physiological interpretation of BIS parameters is further complicated by the fact that tissue water status also strongly affects ion movement and electrical conductivity; therefore, separating the effects of N status and water status is a fundamental methodological issue.

The aim of my research was to develop a minimally invasive, *in vivo* diagnostic approach that combines BIS with optimized impedance modeling to reveal cellular-level electrical changes associated with plant N status. My basic hypothesis was that cell-compartment-specific BIS parameters are quantitatively related to plant N status, particularly to the extracellular and intracellular distribution of NO_3^- .

For the preparation of my thesis, I formulated the following objectives:

1. To develop and validate a bioimpedance spectroscopy (BIS) measurement protocol suitable for the rapid and minimally invasive assessment of plant N status.
2. To investigate and quantitatively characterize the relationship between extracellular NO_3^- content and extracellular fluid resistance.
3. To analyze changes in resistance parameters associated with the extracellular, intracellular, and vacuole compartments under different nitrogen supply levels.
4. To examine changes in cell membrane capacitance under different nitrogen supply conditions and interpret them in relation to membrane status.
5. To reveal the relationships between BIS parameters and classical plant physiological indicators, including NO_3^- content, total nitrogen content, chlorophyll content, and relative water content, in order to support the physiological interpretation and diagnostic potential of the method.
6. To evaluate the suitability of the BIS method for detecting early N deficiency and to assess the combined effects of water status and N supply through changes in BIS parameters.

2. Materials and methods

2.1. Experimental Conditions and Plant Material

Several consecutive experimental systems were used to evaluate the plant physiological applicability of BIS. The aims of the experiments were, first, to validate the measurement procedure and modeling approaches, and second, to reveal the biophysical and plant physiological effects of N supply, water deficit, and their combined influence. Plant cultivation and measurements were carried out under controlled environmental conditions, mainly in growth chambers and greenhouse systems.

In the first phase, pepper (*Capsicum annuum* L.) plants were used to validate the BIS measurement procedure and the ZFIT- and PSO-based Double-Shell modeling approaches under water stress conditions.

Subsequently, the focus of the research shifted to the bioimpedance-based interpretation of N supply, for which lettuce (*Lactuca sativa* L.) plants were used. The lettuce experimental system enabled the investigation of relationships among BIS parameters, plant N status, and leaf NO_3^- content.

In further experiments, cucumber (*Cucumis sativus* L.) plants were used to evaluate whether the relationships identified in lettuce could also be detected in another plant species and in different genotypes. The cucumber experiments specifically focused on the early detection of N deficiency and on the combined effects of N form and water status. Thus, the experimental design followed a stepwise structure: pepper served methodological validation, lettuce served the investigation of N-specific physiological relationships, and cucumber served the evaluation of the diagnostic value and general applicability of the method.

2.1.1. Experimental setup of bioimpedance measurements under water deficit conditions

The effect of water deficit on bioimpedance parameters was investigated in sweet pepper (*Capsicum annuum* L. ‘Redskin’) plants. The plants were grown in a growth chamber under controlled environmental conditions. The temperature was maintained at 24 ± 1.5 °C, relative humidity at $75 \pm 1\%$, CO₂ concentration at 380–420 ppm, and the photoperiod was set to an 18/6 h light/dark cycle. Illumination was provided by full-spectrum LED lamps, with a PPFD of $500 \mu\text{mol m}^{-2} \text{s}^{-1}$. The plants were grown in 1 L pots containing a 2:1 mixture of peat and perlite and were irrigated with half-strength Hoagland solution.

The effect of water deficit was examined in three complementary experimental phases. In the first phase, nine-week-old pepper plants were subjected to three water supply levels: normally watered plants irrigated daily, moderately water-stressed plants irrigated every three days, and severely water-stressed plants exposed to complete water withdrawal for 10 days. The corresponding soil moisture values were 68.70%, 53.00%, and 39.80%, respectively, and were monitored using a PMS710 soil moisture sensor.

In the second and third experimental phases, detached 5×5 cm pepper leaf samples were allowed to dry under growth chamber conditions. In the second phase, BIS measurements were performed at three-hour intervals, whereas in the third phase, measurements were recorded hourly. In the third phase, both mass loss and impedance spectra were recorded at each measurement point.

2.1.2. Experimental setup for different nitrogen doses in two lettuce cultivars

The bioimpedance-based investigation of different N supply levels was carried out on lettuce (*Lactuca sativa* L.) plants grown under controlled growth chamber conditions. The main aim of the lettuce experiments was to reveal the relationship between BIS parameters and leaf NO_3^- content.

The lettuce experiments were performed in two consecutive stages. The aim of the preliminary experiment was to identify the frequency ranges that most sensitively follow NO_3^- dynamics in leaves. In this experiment, the ‘Kobak’ cultivar was used and grown under growth chamber conditions with Hoagland nutrient solution. At the four-true-leaf developmental stage, the plants were transferred to N-free nutrient solution for five days, after which 5 mM NO_3^- was added to the nutrient solution in the form of $\text{Ca}(\text{NO}_3)_2$. BIS measurements were performed before NO_3^- resupply and during the early period after NO_3^- addition, within a 1–6 h measurement interval. Impedance spectra were recorded between 1 Hz and 100 kHz and evaluated using Bode diagrams.

Based on the results of the preliminary experiment, the main lettuce experiment was established using two lettuce cultivars, ‘Kobak’ and ‘Május királya’, under different N supply levels. The selection of the two cultivars was justified by their different NO_3^- accumulation characteristics: ‘Kobak’ is characterized by higher NO_3^- accumulation, whereas ‘Május királya’ shows lower NO_3^- accumulation.

The plants were sown in rockwool cubes and then grown in pots containing perlite. After germination, the plants were transferred to a growth chamber, where the temperature was maintained at 20 ± 1.5 °C, relative humidity at $67 \pm 1\%$, CO_2 concentration at 380–420 ppm, and the photoperiod was set to an

18/6 h light/dark cycle. The PPFD was $170 \mu\text{mol m}^{-2} \text{s}^{-1}$. The different N supply levels were established using five modified Yamazaki nutrient solutions corresponding to 25, 50, 75, 100, and 125% of the standard N content. The treatments were designated as N0, N1, N2, N3, and N4. Nutrient solution was applied every two days, and the pH of the solutions was checked on each fertilization day and adjusted to 5.8–6.0.

2.1.3. Experimental setup for different nitrogen doses in cucumber plants under greenhouse conditions

The effect of different N doses was also investigated in cucumber (*Cucumis sativus* L.) plants under greenhouse conditions, using the 'ES2217 F₁' genotype. The experiment was established in Pusztaföldvár with a total of 20 plants and five replicates. Seeds were sown in rockwool cubes, and after the two-true-leaf developmental stage, the plants were further grown under greenhouse conditions.

During the experiment, the maximum daytime PPFD reached $1520.9 \mu\text{mol m}^{-2} \text{s}^{-1}$. Daytime temperature ranged between 25 and 33 °C, with an average of 28 °C, while night temperature did not fall below 20 °C. Daytime relative humidity ranged from 30 to 93.1%, with an average value of 63.8%.

Nutrient solution application was started after transplanting. The initial nutrient solution was prepared using Solinure 5 General Purpose 20-20-20+TE fertilizer, with a total N concentration of 6.94 mM and an estimated total N content of 97 mg L^{-1} . After the two- to three-true-leaf developmental stage, increased N levels were applied by adding KNO₃ solution. The treatments included four N levels: N1: 1.64 mM; N2: 3.29 mM; N3: 4.92 mM; and N4: 6.56 mM. These treatments ranged from relatively low to higher N supply levels, allowing the evaluation of plant physiological and

bioimpedance responses of cucumber plants to N supply. During irrigation, the applied nutrient solution volume was adjusted to plant development, gradually increasing from 70–80 mL per application to 150–200 mL per application.

2.1.4. Experimental setup for the investigation of early nitrogen deficiency

The experiment aimed at detecting early N deficiency was established using two cucumber (*Cucumis sativus* L.) genotypes, ‘ES2217 F₁’ and ‘Nairobi F₁’. The inclusion of two genotypes enabled the evaluation of genotype-dependent responses of BIS parameters. Seeds were sown in 75 × 75 × 65 mm rockwool cubes, and the plants were grown under controlled growth chamber conditions. The photoperiod was set to a 16/8 h light/dark cycle, illumination provided 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR, temperature was maintained at 25 °C, and relative humidity was kept between 60 and 70%.

The plants were irrigated with distilled water until the appearance of the first true leaf, after which half-strength Hoagland solution was applied. When the first true leaves had developed, the plants were divided into two N supply groups: optimal N supply and low N supply. The optimal treatment contained 11 mM total N, whereas the low-N treatment contained 2.2 mM total N. This low N level provided a limiting but not extreme deficiency condition, making it suitable for the bioimpedance-based detection of early N deficiency. Each treatment group included 25 randomly selected plants. For plant physiological and BIS measurements, three technical replicates were performed per plant, and their average was used for statistical evaluation. Altogether, data from 100 plants formed the basis of the analysis. The aim of the experiment was to

determine whether BIS parameters could distinguish between low and optimal N supply even at an early developmental stage.

2.1.5. Experimental setup for the combined investigation of water deficit and different nitrogen forms

The combined effect of water deficit and N form was investigated in cucumber (*Cucumis sativus* L.) plants using the ‘ES2217 F1’ genotype. The aim of the experiment was to determine how different N forms modify the bioimpedance response of plants to water deficit and whether treatment-specific electrical patterns can be distinguished under stress conditions.

Seeds were germinated and grown in 1 L pots filled with soil. A calcareous loam soil with a slightly alkaline pH and adequate macronutrient supply was used. The plants were grown in a growth chamber under a 16/8 h light/dark photoperiod, with a PAR of 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Four treatments were applied: control (CTRL), nitrate treatment (NO_3^-), combined ammonium-nitrate treatment (NH_4NO_3), and wool pellet treatment (WP). The effects of the treatments were monitored across four consecutive experimental phases: maximum nutrient supply (S1), reduced nutrient supply (S2), reduced nutrient supply combined with water deficit (S3), and rehydration (S4).

2.2. Applied plant physiological and soil analytical methods

2.2.1. Determination of Relative Water Content

Relative water content (RWC) was determined according to the method of Karim et al. (2025), with five replicates per treatment. The fresh weight, saturated weight, and dry weight of the leaf samples were measured, and RWC was calculated based on these values.

2.2.2. Determination of pigment content and chlorophyll fluorescence

Chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoid contents were determined from fresh leaf tissue after extraction with 80% acetone, using a spectrophotometric method based on Mackinney (1941). Leaf chlorophyll content was also measured non-destructively using an Apogee MC-100 instrument.

Chlorophyll fluorescence parameters were determined using a portable FluorPen FP 110-LM/D fluorometer according to the protocol of Li et al. (2024), following dark adaptation. The parameters F_v/F_m , F_o/F_m , $Y(II)$, ETR_{II} , qP , and NPQ were used for evaluation.

2.2.3. Determination of total nitrogen and nitrate content

Total N content was determined using the Kjeldahl digestion method according to Nelson and Sommers (1980), and the results were expressed on a dry matter basis. Leaf NO_3^- content was measured using the salicylic acid–sulfuric acid method according to Hesari et al. (2023), with spectrophotometric detection based on a $NaNO_3$ standard curve.

2.2.4. Determination of apoplastic nitrate concentration

Apoplastic NO_3^- concentration was determined according to the method of Nikolic et al. (2012). Leaf blades without the main vein were subjected to vacuum infiltration and centrifugation to obtain apoplastic washing fluid, after which its NO_3^- content was measured spectrophotometrically. This analysis was performed in the ‘Kobak’ lettuce cultivar under the N_0 – N_4 treatments.

2.2.5. Determination of antioxidant capacity and total polyphenol content

Total polyphenol content was determined using the Folin–Ciocalteu reagent, based on a gallic acid standard curve, according to the method of Singleton (1981). Antioxidant capacity was measured using the FRAP method according to Benzie and Strain (1996), following the procedure adapted for plant samples by Ficzek et al. (2025). Ascorbic acid standard solutions were used for calibration.

2.2.6. Determination of Membrane stability index

The membrane stability index (MSI) was determined using an electrolyte leakage-based method according to Khanna-Chopra and Selote (2007) and the protocol of Czékus et al. (2023). Leaf discs were incubated in distilled water, and membrane stability was calculated based on the initial electrical conductivity and the conductivity measured after heat treatment.

2.2.7. Determination of plant biomass

At the end of the experiments, fresh and dry biomass of the plants were determined. The whole plant material was harvested, its fresh weight was measured immediately, and then the samples were dried in a drying oven until constant weight was reached. The fresh-to-dry biomass ratio was calculated according to Gao et al. (2023).

2.2.8. Determination of soil nitrogen content and pH

In the experiment investigating the combined effects of water deficit and different N forms, soil samples were collected at the end of the experiment. Fresh soil samples were stored at +4 °C according to the protocol of Kabalan et al. (2024). Among the mineral N forms, NO_3^- content was determined from a 0.1 M CaCl_2 extract using a spectrophotometric method according to

Monteiro et al. (2003). Soil pH was determined as part of the soil chemical evaluation.

2.3. Bioimpedance spectroscopy measurements and data processing

2.3.1. Characterization of the bioimpedance measurement device

BIS measurements were performed using a custom-developed, four-channel, battery-powered bioimpedance analyzer operating on a digital *lock-in* principle. The measurement system was specifically developed for the investigation of biological and plant tissues, and its detailed technical description is provided by Vizvári et al. (2020) and Odry et al. (2021). The aim of the four-channel measurement configuration was to reduce errors arising from electrode polarization and contact impedance (Jinyang et al., 2016). The measurement system can operate in three modes: frequency-sweep impedance spectroscopy, FFT-based spectral measurement, and fixed-frequency bioimpedance measurement. The device generates a monochromatic sinusoidal signal and determines the amplitude and phase of the measured signal using a digital lock-in algorithm. Four 24-karat gold-coated needle electrodes were used for plant measurements. The measurement setup can be considered mechanically minimally invasive and electrically non-destructive.

2.3.2. Validation of the measurement system using a Double-Shell phantom

The accuracy and reliability of the BIS measurement system were verified using a Double-Shell physical phantom with known parameters. The phantom enabled validation of the measurement system independently of biological samples, as its theoretical impedance spectrum and equivalent circuit

parameters were predefined. During validation, the measured and theoretical impedance values, as well as the recovered DBS model parameters, were compared. Measurements were performed in the frequency range of 0.01 Hz to 100 kHz with different frequency resolutions, using ten replicates for each setting. The aim of the validation was to confirm that the measurement system is suitable for reliably recording impedance spectra from plant tissues and for accurately estimating model parameters.

2.3.3. Bioimpedance measurement setup and Double-Shell Model

Plant BIS measurements were performed in the frequency range of 1 Hz to 100 kHz. During the measurements, a 25 dB attenuated excitation signal was applied, and the electrodes were connected to the BIS analyzer in a tetrapolar configuration. The four gold electrodes were fixed at a distance of 1.5 cm from each other according to Li et al. (2017). To reduce contact impedance, a small amount of ECG gel was applied according to Serrano-Finetti et al. (2023). Measurements were performed on healthy, undamaged leaves, on leaf areas away from the main vein, based on Meiqing et al. (2016) and Li et al. (2017). Each measurement was repeated five times.

The Double-Shell model was used to interpret the BIS data. The model describes the plant cell through two main membrane interfaces, the plasma membrane and the tonoplast, thereby enabling the separate interpretation of electrical properties associated with the extracellular, intracellular, and vacuole compartments. In the model, R_1 represents the resistance of the extracellular fluid, R_2 the resistance of the intracellular fluid, and R_4 the resistance of the vacuole fluid, while Q_3 and Q_5 denote the constant phase elements associated with the cell membrane and the tonoplast, respectively.

The α and β parameters characterize the non-ideal capacitive behavior of the membranes (López-Villanueva and Rodríguez Bolívar, 2022).

2.3.4. Extraction of bioimpedance parameters

The parameters of the DBS model were determined by numerical optimization. A particle swarm optimization-based algorithm was used for parameter extraction in MATLAB R2023a. The aim of the optimization was to minimize the relative error between the measured and simulated impedance spectra, while a regularization term helped stabilize parameter estimation. The optimized parameters were the R_1 , R_2 and R_4 resistances, the Q_3 and Q_5 CPE parameters, and the α and β exponent parameters. The optimization was performed in the frequency range of 1 Hz to 100 kHz, using a resolution of 10 frequency points per decade. This method enabled quantitative estimation and plant physiological interpretation of cellular-level electrical parameters

2.3.5. Statistical evaluation

Statistical evaluation of plant physiological and BIS parameters was performed in R 4.2. Treatment effects were evaluated using MANOVA based on Wilks' λ , and the assumptions were checked using Box's M test, the Shapiro–Wilk test, and Levene's test. Effect sizes were estimated using partial eta squared (Levine and Hullett, 2002), and group means were compared using Tukey's post hoc test. Relationships between BIS parameters and plant physiological indicators were examined using Pearson correlation and linear regression, while the diagnostic separation of early N deficiency was evaluated using ROC analysis, AUC values, and threshold values determined based on the Youden index (Youden, 1950).

3. Results and Discussion

3.1. Effects of water stress and leaf drying using a bioimpedance-based approach

Water deficit significantly affected the physiological status of pepper plants. In plants exposed to severe water stress, RWC, leaf thickness, CCI, and F_v/F_m decreased. MSI also showed a decreasing tendency, although the difference among treatments was not significant in this case.

During BIS data modeling, ZFIT- and PSO-based fitting procedures were compared. The PSO-based fitting of the Double-Shell model using a combined objective function provided more stable parameter estimation than the conventional PSO- or ZFIT-based Double-Shell fitting. Therefore, the PSO-based Double-Shell approach was considered the most suitable for further evaluation, as it provided physiologically more interpretable results with lower parameter variability.

3.2. Effects of different nitrogen supply levels in two lettuce cultivars

In the short-term NO_3^- resupply experiment, after five days of N deprivation, the impedance modulus ($|Z|$) of ‘Kobak’ lettuce leaves increased, particularly in the low-frequency range. After the addition of $\text{Ca}(\text{NO}_3)_2$, $|Z|$ decreased, indicating that the low-frequency range sensitively followed rapid changes in the ionic status of leaf tissue. This is consistent with the fact that, at low frequencies, the cell membrane acts as a capacitive barrier; therefore, the current mainly passes through the extracellular, apoplastic space (Prasad and Roy 2020). Accordingly, the low-frequency BIS response may primarily reflect changes in R_1 resistance and apoplastic ion concentration, in which NO_3^- plays a key role (Nikolic et al. 2012; Li et al. 2017).

The results showed that significantly lower apoplastic NO_3^- concentrations accumulated under low N supply treatments than under the higher N3–N4 levels ($p < 0.05$). The lowest extracellular NO_3^- concentration was measured in the N0 treatment, which differed significantly from the other N treatments ($p < 0.05$; **Figure 1A**). Furthermore, a significant relationship was found between apoplastic NO_3^- concentration and R_1 resistance ($R^2 = 0.67$, $p < 0.01$; **Figure 1B**), confirming that changes in the ionic composition of the extracellular space directly and sensitively influence R_1 resistance.

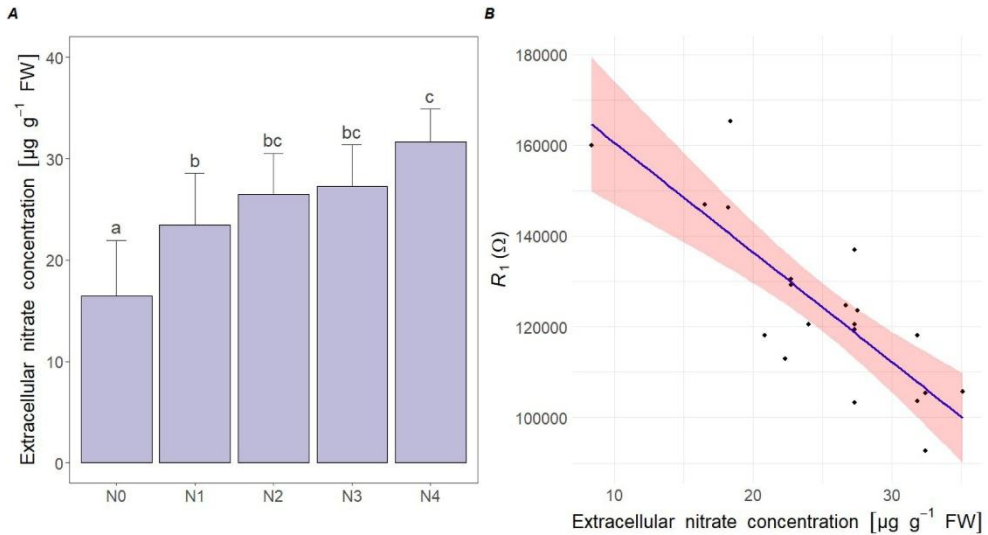


Figure 1. Effect of nitrogen treatments (N0–N4) on extracellular nitrate concentration in the leaves of the ‘Kobak’ genotype. Bars represent mean $\pm$ SD. Different letters above the bars indicate statistically significant differences among treatments based on Tukey’s post hoc test ($p < 0.05$) (A). Linear relationship between extracellular nitrate concentration and extracellular fluid resistance measured in the ‘Kobak’ genotype. The blue line represents the linear regression fit, and the shaded area shows the 95% confidence interval (B).

N supply had a significant effect on total leaf N and NO_3^- content, aboveground biomass, chlorophyll and carotenoid contents, and the

membrane stability index. These results are consistent with previous studies showing that N supply fundamentally determines chlorophyll synthesis and plant productivity (Huang et al. 2014; Zhao et al. 2017).

Under low N supply, R_1 resistance increased in both cultivars (**Figure 2A**), which can be explained by the decrease in apoplastic ion concentration, particularly the amount of NO_3^- ions. This is consistent with the fact that, in the low-frequency range, the current mainly passes through the extracellular/apoplastic space (Prasad and Roy, 2020). In the ‘Kobak’ cultivar, strong negative correlations were found between leaf NO_3^- content and R_1 ($r = -0.76$), as well as between total leaf N content and R_1 ($r = -0.73$). In ‘Május királya’, the relationship between NO_3^- content and R_1 was weak ($r = -0.23$), whereas the correlation between total leaf N content and R_1 remained strongly negative ($r = -0.77$). This is consistent with the different NO_3^- accumulation characteristics of the two cultivars (Yaseen and Takács-Hájos, 2022). R_2 resistance changed to a lesser extent (**Figure 2B**) and did not show a strong relationship with leaf NO_3^- content, which may be explained by the rapid regulation of cytoplasmic NO_3^- levels (Masclaux-Daubresse et al. 2010; Xu et al. 2012). In contrast, R_4 resistance responded more sensitively (**Figure 2C**): a moderately strong negative correlation was found between leaf NO_3^- content and R_4 in ‘Kobak’ ($r = -0.60$), whereas the relationship was weaker in ‘Május királya’ ($r = -0.44$). This suggests that, in ‘Kobak’, the NO_3^- pool in the vacuole contributed more strongly to intracellular ion conduction.

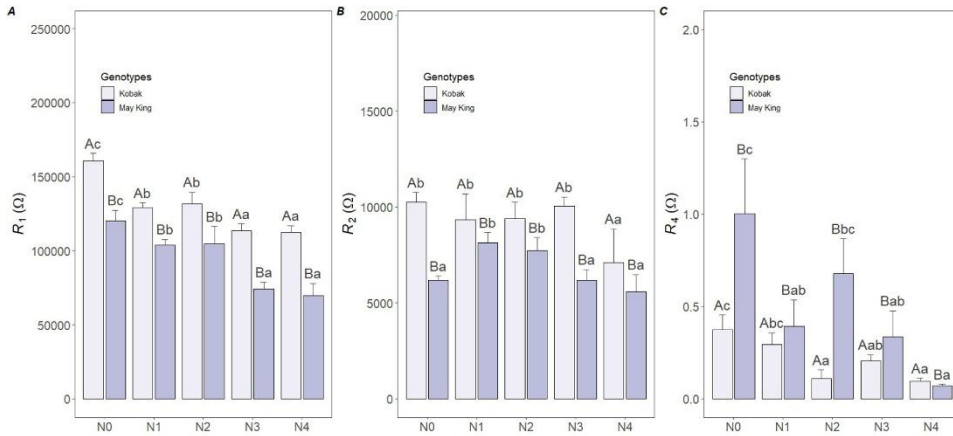


Figure 2. Effect of N treatments on cell electrical resistance parameters: A) extracellular fluid resistance (R_1), B) intracellular fluid resistance (R_2), and C) vacuole fluid resistance (R_4) for the N-treatments (N0-N4) on two lettuce genotypes. Capital letters indicate significant differences between genotypes under the same nitrogen treatment. Lowercase letters indicate significant differences between treatments with different nitrogen levels within the same lettuce genotype. (Tukey's post hoc test, $p < 0.05$).

Low N supply reduced C_m (**Figure 3A**). In the 'Kobak' cultivar, a positive correlation was observed between MSI and C_m ($r = 0.68$), indicating a relationship between membrane integrity and charge storage capacity. Previous studies have shown that N deficiency can modify membrane fluidity and lipid composition, thereby reducing C_m (Azzarello et al. 2012; Liu et al. 2020).

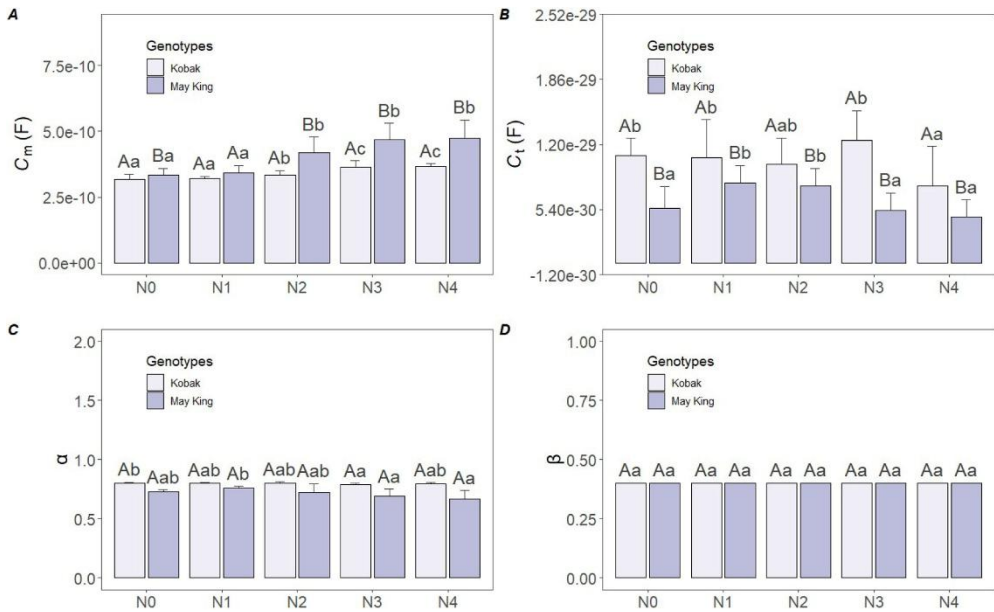


Figure 3. Effect of N treatments on cell electrical capacitance parameters: A) cell membrane capacitance (C_m), B) vacuole membrane capacitance (C_t), C) α associated with the vacuole membrane, D) β associated with the cell membrane. Capital letters indicate significant differences between genotypes under the same nitrogen treatment. Lowercase letters indicate significant differences between treatments with different nitrogen levels within the same lettuce genotype. (Tukey's post hoc test, $p < 0.05$).

3.3. Effects of different nitrogen supply levels on bioimpedance and plant physiological parameters in cucumber plants under greenhouse conditions

In the greenhouse cucumber experiment, N treatments significantly affected the plant physiological parameters at the 6–7-leaf developmental stage ($F_{(3,32)} = 28.03$; $p < 0.001$; Wilks' $\lambda = 0.27$; $\eta^2 = 0.72$). The lowest CCI, total leaf N, and leaf NO_3^- content were detected in the N1 treatment, whereas the highest values were observed in the N3 and N4 treatments. For several parameters, no significant difference was found between the N3 and N4 treatments, indicating a saturation response in the higher N supply range. This is consistent with the

regulation of N uptake and NO_3^- homeostasis (Li et al. 2022; Hesari et al. 2023).

Based on the BIS spectra, the impedance modulus increased under low N supply treatments (N1–N2), whereas higher N levels (N3–N4) resulted in lower impedance, which can be explained by increased ion conductivity (Li et al., 2017). Among the DBS parameters, R_1 , R_2 , R_4 and C_m all responded significantly to changes in N supply (**Figure 4**). R_1 indicated reduced apoplastic NO_3^- availability, R_2 reflected a decrease in the intracellular ion pool, and R_4 indicated changes in the NO_3^- pool of the vacuole (Cookson et al., 2005; Nouaze et al., 2022; Prasad and Roy, 2020). The decrease in C_m under low N supply (**Figure 5**) may indicate reduced membrane charge storage capacity and impaired dielectric stability (Azzarello et al. 2012; Liu et al. 2020).

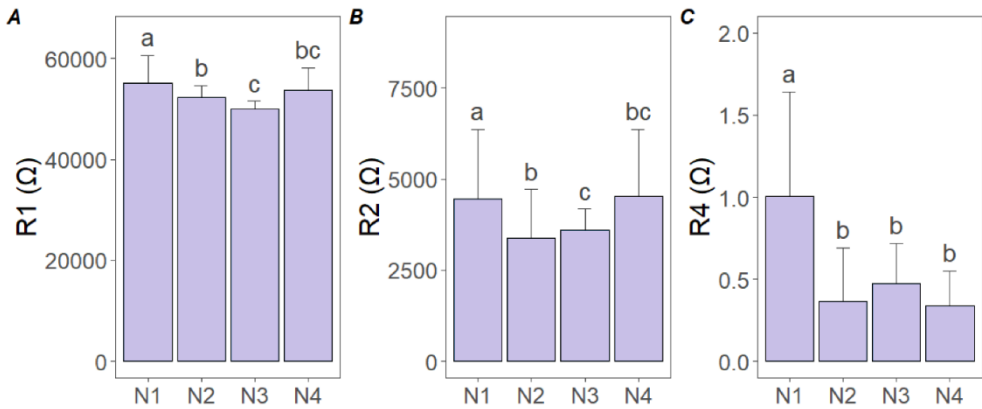


Figure 4. Results of the Double-Shell model with PSO optimization algorithm for the average of all four treatments on cucumber plants. (A) Extracellular fluid resistance (R_1); (B) intracellular fluid resistance (R_2); (C) vacuole resistance (R_4). Values are means $\pm$ standard deviation ($n = 4$). Lowercase letters indicate significant differences between treatments according to Tukey's HSD, $p < 0.05$.

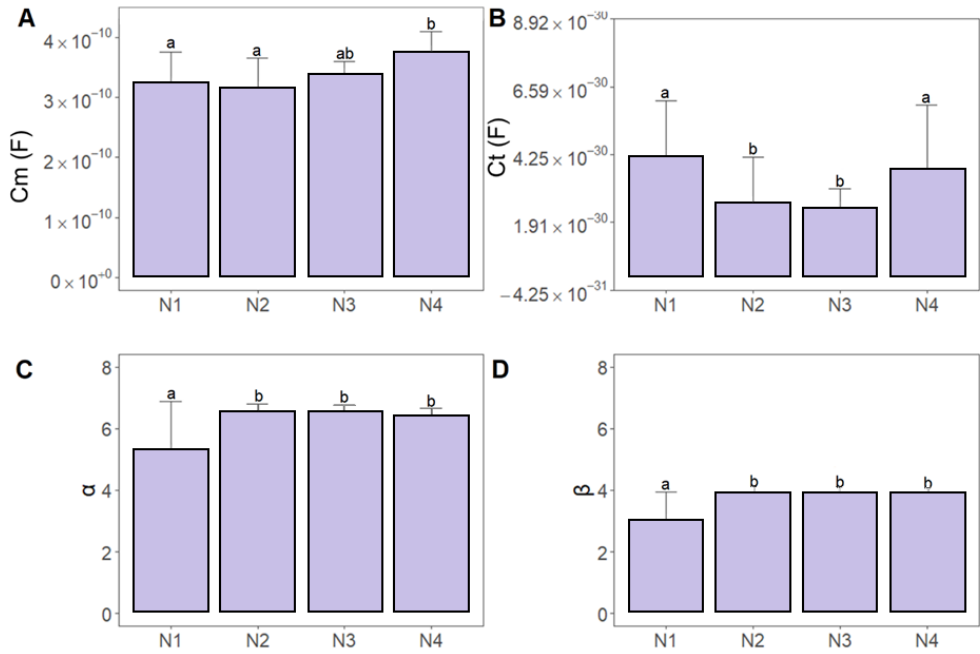


Figure 5. Results of the Double-Shell model with PSO optimization algorithm for the average of all four treatments on cucumber plants. (A) Cell membrane capacitance (C_m); (B) vacuole membrane capacitance (C_t); (C) α associated with the vacuole membrane; (D) β associated with the cell membrane. Lowercase letters indicate significant differences between treatments according to Tukey's HSD, $p < 0.05$.

3.4. Effects of early nitrogen deficiency on bioimpedance and plant physiological parameters

Based on ROC analysis, the BIS parameters were suitable for distinguishing early N deficiency, although their diagnostic performance was genotype-dependent. In 'ES2217 F₁', R_1 and C_m showed the strongest discriminatory ability (AUC = 0.91) (**Figure 6A**), whereas in 'Nairobi F₁', C_m proved to be the best predictor (AUC = 0.92), followed by R_2 (AUC = 0.86) (**Figure 6B**). The β parameter showed weak diagnostic value in both genotypes.

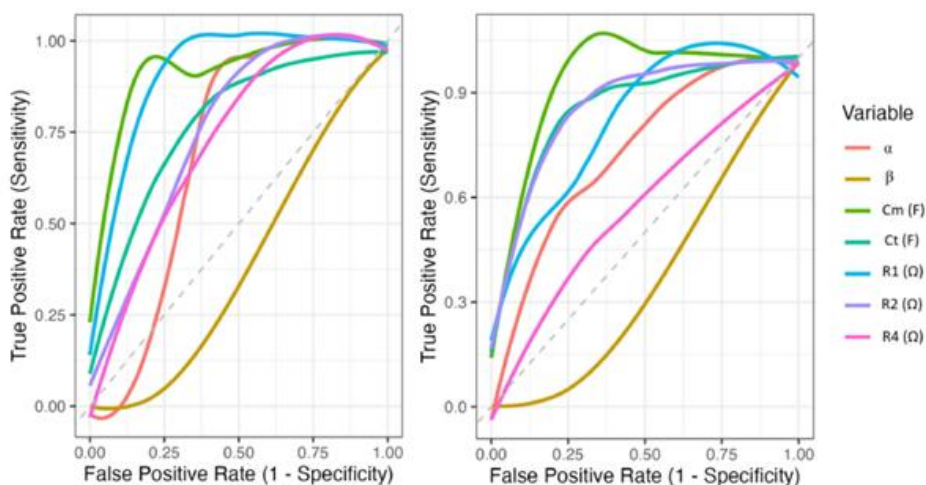


Figure 6 A) Discriminatory performance of the BIS model parameters in distinguishing between different nitrogen supply levels (optimal N and low N) in the ‘ES2217 F1’ cucumber genotype based on ROC analysis. B) Discriminatory performance of the BIS model parameters in distinguishing between different nitrogen supply levels (optimal N and low N) in the ‘Nairobi F1’ cucumber genotype based on ROC analysis. Note: extracellular fluid resistance (R_1), intracellular fluid resistance (R_2), vacuole fluid resistance (R_4), cell membrane capacitance (C_m), vacuole membrane capacitance (C_t), alpha (associated with the vacuole membrane), beta (associated with the cell membrane).

Overall, the results confirmed that BIS may be suitable for the *in vivo* detection of early N deficiency. However, the interpretation of the method should take into account genotype-dependent electrophysiological responses, as well as the fact that BIS is not an ion-specific method.

3.5. Combined effects of different nitrogen supply and drought stress on bioimpedance and plant physiological parameters

Among the DBS parameters, R_1 , R_4 , and C_m proved to be the most informative. R_1 resistance was higher in the control and water-deficit treatments, indicating reduced apoplastic ion conduction. R_4 resistance responded particularly sensitively to changes in N and water supply: higher R_4 values may indicate reduced ion content in the vacuole, whereas lower values may suggest more

intensive NO_3^- storage, since the vacuole is the main storage site of leaf NO_3^- (Crawford and Glass, 1998; Masclaux-Daubresse et al. 2010). Based on **Figure 7**, R_1 and R_4 clearly followed the differences among treatments; therefore, these parameters can be considered important biophysical indicators of combined N and water stress.

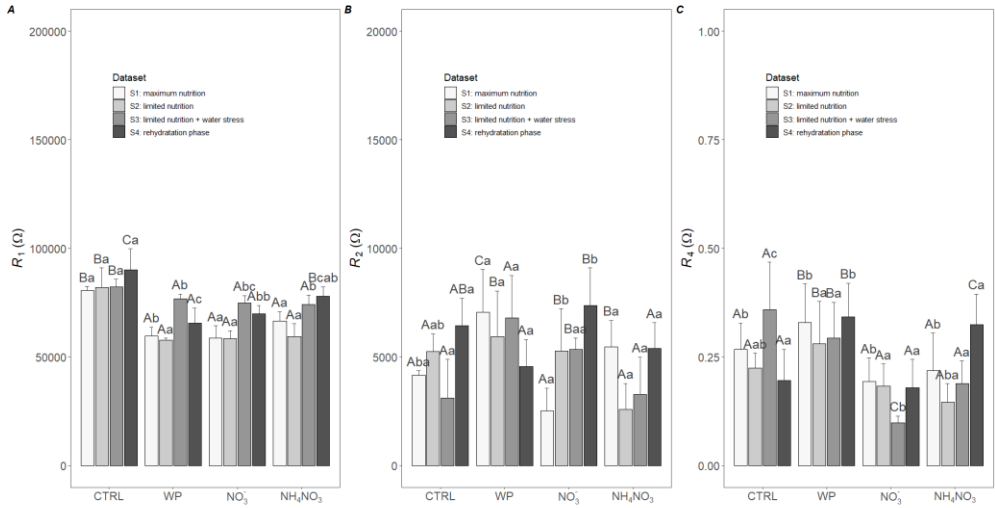


Figure 7. Effect of N treatments on cell electrical resistance parameters of cucumber leaves across physiological phases: A) extracellular fluid resistance (R_1), B) intracellular fluid resistance (R_2), and C) vacuole fluid resistance (R_4). Uppercase letters indicate significant differences among nitrogen treatments within a given dataset phase, whereas lowercase letters indicate significant differences among dataset phases within a given nitrogen treatment (Duncan's post hoc test, $p < 0.05$). Error bars represent standard deviation. Note: CTRL = control plants; WP = wool pellet treatment; NO_3^- = nitrate supply; NH_4NO_3 = combined ammonium–nitrate treatment.

C_m changed significantly as a function of N supply and stress phase (**Figure 8**). Higher C_m values indicate better membrane status and greater charge storage capacity, whereas lower values suggest alteration of the dielectric properties of the membrane (Jamaludin et al., 2015; Jinyang et al., 2016; Zhang et al., 2024). At the end of the experiment, leaf NO_3^- content showed a

strong negative relationship with R_4 ($r = -0.74$), while a more moderate negative correlation was observed with R_1 ($r = -0.32$).

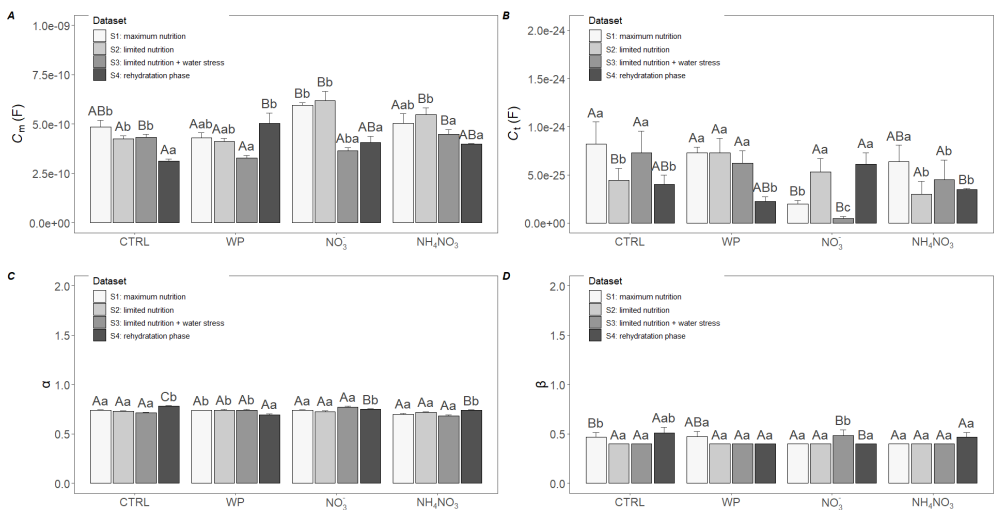


Figure 8. Effect of N treatments on cell electrical capacitance parameters of cucumber leaves across physiological phases: A) cell membrane capacitance (C_m), B) vacuole membrane capacitance (C_t), C) α associated with the vacuole membrane, D) β associated with the cell membrane. CTRL = control plants; WP = wool pellet treatment; NO₃⁻ = nitrate supply; NH₄NO₃ = combined ammonium–nitrate treatment. Uppercase letters indicate significant differences among nitrogen treatments within a given dataset phase, while lowercase letters indicate significant differences among dataset phases within a given nitrogen treatment (Duncan's post hoc test, $p < 0.05$). Error bars represent standard deviation.

4. Conclusions and Recommendations

In summary, four-electrode bioimpedance spectroscopy (BIS), suitable for in vivo plant measurements, combined with the Double-Shell (DBS) cell model and particle swarm optimization (PSO)-based parameter extraction, proved suitable for the minimally invasive, cellular-level investigation of biophysical changes related to plant N supply and water status. The DBS-PSO approach provided stable and reproducible parameter estimation. Compared with the ZFIT procedure commonly used in plant physiological research, PSO resulted in lower parameter variability, which directly increased the reliability of biological interpretation and enabled BIS parameters to be consistently used as diagnostic variables in statistical models, such as MANOVA, ROC analysis, and correlation analysis.

My results confirmed that individual BIS parameters carry different and partly complementary physiological information. Leaf extracellular resistance (R_1) responded sensitively to changes in apoplastic ion composition and showed a close relationship with both apoplastic and leaf NO_3^- content in the present experimental system. In the low-frequency range, R_1 particularly reflected ion-dynamic changes related to N supply. In parallel, vacuole fluid resistance (R_4) showed a close relationship with leaf NO_3^- content and may therefore provide information on the ion content of the vacuole and its changes. Cell membrane capacitance (C_m) can be interpreted as an indicator of membrane integrity and dielectric status, making it suitable for the sensitive description of stress-related changes in membrane status, including the effects of water supply.

The effects of N and water stress measured by bioimpedance partly overlap, which justifies the use of multiparametric and multivariate evaluation. At the

same time, the results also indicate that the two stress responses can be separated to some extent by using appropriate parameter combinations. The practical implication is that BIS diagnostics should not be based on a single indicator, but at least on the combined interpretation of R_1 , R_4 , and C_m as this parameter combination proved to be the most informative.

The strongest predictors for distinguishing early N deficiency were genotype-dependent, indicating that genotype-specific calibration is required for the development of BIS-based N diagnostics.

BIS can be used particularly effectively for diagnostic purposes in the low and medium N range, whereas at higher N levels (N3–N4), smaller bioimpedance-based differences may occur due to feedback-regulated NO_3^- homeostasis. Accordingly, the practical application of the method, including sampling strategy, threshold values, and decision rules, should be optimized for the detection of early deficiency.

Based on my results, the following practical and methodological recommendations were formulated:

1. For the diagnostic interpretation of leaf N status, the combined use of R_1 resistance, R_4 resistance, and C_m capacitance is primarily recommended. Instead of evaluating individual BIS parameters in isolation, it is advisable to rely on combined parameter patterns, as these carry the greatest physiological information.
2. Since the values and diagnostic sensitivity of BIS parameters differ among cultivars and genotypes, genotype- and objective-specific calibration of the measurement protocol and decision thresholds is required.

3. Based on the reduced variance and greater interpretative stability observed during parameter estimation, PSO-based fitting is recommended for extracting DBS model parameters, especially when the aim is to develop diagnostics based on physiologically meaningful BIS parameters.

Overall, the presented results suggest that BIS, when combined with appropriate modeling and calibration, may be a promising tool for the non-destructive, cellular-level investigation of changes related to leaf N supply and water status.

5. New scientific findings

1. The PSO algorithm was shown to produce significantly lower variability in the parameters of the Double-Shell cell model than the ZFIT algorithm, which is widely used in plant physiological research. This improved the reliability of biological interpretation.
2. It was demonstrated for the first time that, under the examined conditions, extracellular resistance (R_1) is the most sensitive indicator of apoplastic NO_3^- supply. R_1 showed a strong negative correlation with both leaf and apoplastic NO_3^- content and separated the impedance curves of N-deficient and optimally N-supplied leaves in the low-frequency range of 1–4000 Hz.
3. It was demonstrated for the first time that, under otherwise optimal nutrient supply, vacuole resistance (R_4) is closely related to the NO_3^- content of leaf tissue during changes in NO_3^- level. Significantly higher R_4 values were observed when plant NO_3^- concentration was lower.
4. It was demonstrated for the first time that, under N-deficient conditions, cell membrane capacitance (C_m) shows a close quantitative relationship with the membrane stability index (MSI). This indicates that N deficiency is associated with a measurable reduction in membrane charge storage capacity. Tonoplast capacitance (C_t) showed lower sensitivity; its values partly followed changes in leaf NO_3^- content, but its diagnostic performance was weaker than that of C_m .
5. In the examined experimental system, vacuole resistance (R_4) showed a strong negative relationship with leaf NO_3^- content and proved to be the most sensitive bioimpedance parameter for indicating differences among the studied N sources, including NO_3^- , NH_4NO_3 , and wool

pellet as an organic N source. In contrast, extracellular resistance (R_1) and cell membrane capacitance (C_m) responded not only to changes in N supply but also to the water status of leaf tissue.

6. Publications related to the thesis topic

First-author journal articles with impact factor

1. Kovács F, Juhos K, Odry Á, et al (2025) Early detection of nitrogen status in cucumber plants using bioimpedance spectroscopy. *Smart Agric Technol* 12:101610. <https://doi.org/10.1016/J.ATECH.2025.101610>, **Q1: Impact Factor: 5.7**
2. Kovács F, Juhos K, Vizvári Z, et al (2025) Bioimpedance Analysis of Cucumber Plants Exposed to Different Nitrogen Doses Under Greenhouse Conditions. *Sensors* 25:. <https://doi.org/10.3390/s25082486>, **Q1: Impact Factor: 3.5**
3. Kovács F, Odry Á, Vizvári Z, et al (2025) Electrical bioimpedance spectroscopy as a tool for non-destructive monitoring nitrogen supply in two lettuce genotypes. *Sci Hortic (Amsterdam)* 349:114229. <https://doi.org/10.1016/j.scienta.2025.114229>, **D1: Impact Factor: 4.2**
4. Kovács F, Odry Á, Vizvári Z, et al (2025) A novel approach to water stress assessment in plants: New bioimpedance method with PSO-optimized Cole-Cole impedance modeling. *Comput Electron Agric* 234:110167. <https://doi.org/10.1016/j.compag.2025.110167>, **D1: Impact Factor: 8.9**
5. Kovács, F., Odry, Á., Vizvári, Z. *et al.* Advancing nitrogen diagnostics in plants through bioimpedance spectroscopy: current evidence and future perspectives—a review. *Planta* 263, 62 (2026). <https://doi.org/10.1007/s00425-026-04934-y>, **Q1, Impact Factor: 3.8**
6. Kovács, F., Odry, Á., Vizvári, Z. *et al.* (2026) Bioimpedance spectroscopy highlights bioelectrical mechanisms of nitrogen and water interactions in cucumber leaves. *Sci Hortic (Amsterdam)*. 114810. *HORTI* 114810. <https://doi.org/10.1016/j.scienta.2026.114810> **D1: Impact Factor: 4.2**

First-author conference publications

1. Kovács F, Odry Á, Vizvári Z, Gyalai IM, Szarvas A, Odry P, Juhos K (2025) Short-term nitrate uptake and its impact on the bioelectrical impedance spectrum of lettuce leaves. In: Gyalai IM, Czóbel Sz (szerk.) 22nd Wellmann International Scientific Conference: Book of Abstracts. University of Szeged Faculty of Agriculture, Hódmezővásárhely, pp. 78–78.
2. Kovács F, Odry Á, Vizvári Z, Chen H, Gyalai I, Kabalan S, Papdi E, Odry P, Juhos K (2024) Measuring the nitrogen supply capacity using electrical bioimpedance spectroscopy. In: Gyalai IM, Czóbel Sz (szerk.) 21st Wellmann International Scientific Conference: Book of Abstracts. University of Szeged Faculty of Agriculture, Hódmezővásárhely, pp. 117–117.
3. Kovács F, Papdi E, Veres A, Szegő A, Juhos K (2024) Gyapjúhulladék alkalmazása a kertészetben és a talajerő-gazdálkodásban. In: Buró B, Molnár M (szerk.) XIX. Kárpát-medencei Környezettudományi Konferencia. Absztrakt füzet = 19th Carpathian Basin Environmental Science Conference. Book of Abstracts. HUN-REN Atommagkutató Intézet, Debrecen, p. 9.
4. Kovács F, Papdi E, Veres A, Juhos K (2024) Effect of wool pellet on soil biological activity and biomass production depending on soil organic matter content. In: Fodor M, Bodor-Pesti P, Deák T (szerk.) A 2023. évi Lippay János – Ormos Imre – Vas Károly Tudományos Ülésszak tanulmányai. Magyar Agrár- és Élettudományi Egyetem, Budai Campus, Budapest, pp. 194–200.

5. Kovács F, Papdi E, Veres A, Juhos K (2024) Effect of sheep wool pellet on nitrogen uptake by plants depending on the organic matter content and biological activity of soil. In: 100 Years of Soil Science – Past Achievements and Future Challenges: Centennial Celebration and Congress of the International Union of Soil Sciences: Abstract Book. International Union of Soil Sciences, Florence, p. 137915.
6. Kovács F, Papdi E, Kotroczó Zs, Veres A, Juhos K (2024) Effect of wool pellet on nitrogen utilization and soil biological activity depending on the organic matter content of soil. In: Program of the 9th International Symposium on Soil Organic Matter. p. 13.
7. Kovács F, Papdi E, Juhos K (2023) Alternative approaches to horticultural nitrogen fertilisation in sandy soils. In: Beloev H, Popov G (szerk.) International Scientific Conference “Conserving Soils and Water” Program. p. 13, Paper 68.
8. Kovács F, Papdi E, Veres A, Juhos K (2023) Innovative strategies for nitrogen fertilization in sandy horticultural soils. Budapest, 151 p.

7. References

- Azzarello, E., Masi, E., Mancuso, S., 2012. Electrochemical impedance spectroscopy. *Plant Electrophysiol. Methods Cell Electrophysiol.* 205–223. https://doi.org/10.1007/978-3-642-29119-7_9
- Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Anal. Biochem.* 239, 70–76. <https://doi.org/10.1006/abio.1996.0292>
- Cookson, S.J., Williams, L.E., Miller, A.J., 2005. Light-dark changes in cytosolic nitrate pools depend on nitrate reductase activity in *Arabidopsis* leaf cells. *Plant Physiol.* 138, 1097–1105. <https://doi.org/10.1104/pp.105.062349>
- Crawford, N.M., Glass, A.D.M., 1998. Molecular and physiological aspects of nitrate uptake in plants. *Trends Plant Sci.* 3, 389–395. [https://doi.org/10.1016/S1360-1385\(98\)01311-9](https://doi.org/10.1016/S1360-1385(98)01311-9)
- Czékus, Z., Milodanovic, D., Koprivanacz, P., Bela, K., López-Climent, M.F., Gómez-Cadenas, A., Poór, P., 2023. The role of salicylic acid on glutathione metabolism under endoplasmic reticulum stress in tomato. *Plant Physiol. Biochem.* 205. <https://doi.org/10.1016/j.plaphy.2023.108192>
- Ficzek, G., Mehmeti, S., Bujdosó, G., Magyar, Á., Simon, G., 2025. Assessing the Post-Brief-Storage Fruit Quality and Sensory Characteristics of Old, Local Apple Cultivars from the Carpathian Basin. *Plants* 14. <https://doi.org/10.3390/plants14132005>
- Gao, Y., Shao, G., Cui, J., Lu, J., Tian, L., Song, E., Zeng, Z., 2023. Effects of Drought Hardening and Saline Water Irrigation on the Growth, Yield, and Quality of Tomato. *Agronomy* 13. <https://doi.org/10.3390/agronomy13092351>

- Hesari, N., Szegő, A., Mirmazloum, I., Pónya, Z., Kiss-Bába, E., Kolozs, H., Gyöngyik, M., Vasas, D., Papp, I., 2023. High-Nitrate-Supply-Induced Transcriptional Upregulation of Ascorbic Acid Biosynthetic and Recycling Pathways in Cucumber. *Plants* 12, 1292. <https://doi.org/10.3390/plants12061292>
- Huang, W., Yang, S., Pu, R., Yang, S., 2014. Estimation of nitrogen vertical distribution by Bi-Directional canopy reflectance in winter wheat, in: *Sensors (Switzerland)*. IOP Publishing, pp. 20347–20359. <https://doi.org/10.3390/s141120347>
- Jamaludin, D., Abd Aziz, S., Ahmad, D., Jaafar, H.Z.E., 2015. Impedance analysis of *Labisia pumila* plant water status. *Inf. Process. Agric.* 2, 161–168. <https://doi.org/10.1016/j.inpa.2015.07.004>
- Jinyang, L., Meiqing, L., Hanping, M., Wenjing, Z., 2016. Diagnosis of potassium nutrition level in *Solanum lycopersicum* based on electrical impedance. *Biosyst. Eng.* 147, 130–138. <https://doi.org/10.1016/j.biosystemseng.2016.04.005>
- Kabalan, S., Kovács, F., Papdi, E., Tóth, E., Juhos, K., Biró, B., 2024. Residues of Symbiont Cover Crops Improving Corn Growth and Soil-Dependent Health Parameters. *Agric.* 14. <https://doi.org/10.3390/agriculture14091601>
- Karim, E., Beáta, G., Zsuzsanna, P., 2025. Essential oils under stress: How drought and salinity shape the physiological and biochemical profile of *Thymus ×citriodorus*. *Ind. Crops Prod.* 233, 121368. <https://doi.org/10.1016/j.indcrop.2025.121368>
- Khanna-Chopra, R., Selote, D.S., 2007. Acclimation to drought stress generates oxidative stress tolerance in drought-resistant than -susceptible wheat cultivar under field conditions. *Environ. Exp. Bot.* 60, 276–283.

<https://doi.org/10.1016/j.envexpbot.2006.11.004>

- Levine, T.R., Hullett, C.R., 2002. Eta Squared, Partial Eta Squared, and Misreporting of Effect Size in Communication Research. *Hum. Commun. Res.* 28, 612–625. <https://doi.org/10.1093/hcr/28.4.612>
- Li, J. zhi, Li, B., Guan, Q., Gao, J. ming, 2022. Signal molecules controlling nitrate uptake by roots. *J. Plant Interact.* 17, 301–308. <https://doi.org/10.1080/17429145.2022.2037763>
- Li, M.Q., Li, J.Y., Wei, X.H., Zhu, W.J., 2017. Early diagnosis and monitoring of nitrogen nutrition stress in tomato leaves using electrical impedance spectroscopy. *Int. J. Agric. Biol. Eng.* 10, 194–205. <https://doi.org/10.3965/j.ijabe.20171003.3188>
- Li, S., Liu, X., Yin, L., Wang, S., Deng, X., 2024. Alteration in lipid metabolism is involved in nitrogen deficiency response in wheat seedlings. *Plant Physiol. Biochem.* 214. <https://doi.org/10.1016/j.plaphy.2024.108883>
- Liu, X., Wang, S., Deng, X., Zhang, Z., Yin, L., 2020. Comprehensive evaluation of physiological traits under nitrogen stress and participation of linolenic acid in nitrogen-deficiency response in wheat seedlings. *BMC Plant Biol.* 20, 1–16. <https://doi.org/10.1186/s12870-020-02717-5>
- López-Villanueva, J.A., Rodríguez Bolívar, S., 2022. Constant Phase Element in the Time Domain: The Problem of Initialization. *Energies* 15. <https://doi.org/10.3390/en15030792>
- Mackinney, G., 1941. Absorption of Light By Chlorophyll Solutions. *J. Biol. Chem.* 140, 315–322. [https://doi.org/10.1016/s0021-9258\(18\)51320-x](https://doi.org/10.1016/s0021-9258(18)51320-x)
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010a. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive

- agriculture. Ann. Bot. 105, 1141–1157.
<https://doi.org/10.1093/aob/mcq028>
- Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., Suzuki, A., 2010b. Nitrogen uptake, assimilation and remobilization in plants: challenges for sustainable and productive agriculture. Ann. Bot. 105, 1141–1157.
<https://doi.org/10.1093/AOB/MCQ028>
- Meiqing, L., Jinyang, L., Hanping, M., Yanyou, W., 2016. Diagnosis and detection of phosphorus nutrition level for *Solanum lycopersicum* based on electrical impedance spectroscopy. Biosyst. Eng. 143, 108–118.
<https://doi.org/10.1016/j.biosystemseng.2016.01.005>
- Monteiro, M.I.C., Ferreira, F.N., De Oliveira, N.M.M., Ávila, A.K., 2003. Simplified version of the sodium salicylate method for analysis of nitrate in drinking waters. Anal. Chim. Acta 477, 125–129.
[https://doi.org/10.1016/S0003-2670\(02\)01395-8](https://doi.org/10.1016/S0003-2670(02)01395-8)
- Narasimhan, R., Wang, G., Li, M., Roth, M., Welti, R., Phytochemistry, X.W.-, 2013, undefined, n.d. Differential changes in galactolipid and phospholipid species in soybean leaves and roots under nitrogen deficiency and after nodulation. Elsevier.
- Nelson, D.W., Sommers, L.E., 1980. Total Nitrogen Analysis of Soil and Plant Tissues. J. AOAC Int. 63, 770–778.
<https://doi.org/10.1093/jaoac/63.4.770>
- Nikolic, M., Cesco, S., Monte, R., Tomasi, N., Gottardi, S., Zamboni, A., Pinton, R., Varanini, Z., 2012. Nitrate transport in cucumber leaves is an inducible process involving an increase in plasma membrane H⁺-ATPase activity and abundance. BMC Plant Biol. 12.
<https://doi.org/10.1186/1471-2229-12-66>

- Nouaze, J.C., Kim, Jae Hyung, Jeon, G.R., Kim, Jae Ho, 2022. Monitoring of Indoor Farming of Lettuce Leaves for 16 Hours Using Electrical Impedance Spectroscopy (EIS) and Double-Shell Model (DSM). *Sensors* 22, 9671. <https://doi.org/10.3390/s22249671>
- Odry, A., Vizvari, Z., Gyorfi, N., Kovacs, L., Eigner, G., Klincsik, M., Sari, Z., Odry, P., Toth, A., 2021. Application of Heuristic Optimization in Bioimpedance Spectroscopy Evaluation, in: SACI 2021 - IEEE 15th International Symposium on Applied Computational Intelligence and Informatics, Proceedings. IEEE, pp. 105–112. <https://doi.org/10.1109/SACI51354.2021.9465617>
- Prasad, A., Roy, M., 2020. Bioimpedance analysis of vascular tissue and fluid flow in human and plant body: A review. *Biosyst. Eng.* 197, 170–187. <https://doi.org/10.1016/j.biosystemseng.2020.06.006>
- Serrano-Finetti, E., Castillo, E., Alejos, S., León Hilario, L.M., 2023. Toward noninvasive monitoring of plant leaf water content by electrical impedance spectroscopy. *Comput. Electron. Agric.* 210. <https://doi.org/10.1016/j.compag.2023.107907>
- Singleton, V.L., 1981. Naturally occurring food toxicants: Phenolic substances of plant origin common in foods. *Adv. Food Res.* 27, 149–242. [https://doi.org/10.1016/S0065-2628\(08\)60299-2](https://doi.org/10.1016/S0065-2628(08)60299-2)
- Vizvari, Z., Gyorfi, N., Odry, A., Sari, Z., Klincsik, M., Gergics, M., Kovacs, L., Kovacs, A., Pal, J., Karadi, Z., Odry, P., Toth, A., 2020. Physical validation of a residual impedance rejection method during ultra-low frequency bio-impedance spectral measurements. *Sensors (Switzerland)* 20, 1–20. <https://doi.org/10.3390/s20174686>
- Xu, G., Fan, X., Miller, A.J., 2012. Plant nitrogen assimilation and use efficiency. *Annu. Rev. Plant Biol.* 63, 153–182.

<https://doi.org/10.1146/annurev-arplant-042811-105532>

- Yaseen, A.A., Takacs-Hajos, M., 2022. Evaluation of moringa (*Moringa oleifera* Lam.) leaf extract on bioactive compounds of lettuce (*Lactuca sativa* L.) grown under glasshouse environment. *J. King Saud Univ. - Sci.* 34. <https://doi.org/10.1016/j.jksus.2022.101916>
- Youden, W.J., 1950. Index for rating diagnostic tests. *Cancer* 3, 32–35. [https://doi.org/10.1002/1097-0142\(1950\)3:1<32::AID-CNCR2820030106>3.0.CO;2-3](https://doi.org/10.1002/1097-0142(1950)3:1<32::AID-CNCR2820030106>3.0.CO;2-3)
- Zhang, B., Dong, Z., Sui, X., Gao, J., Feng, L., 2024. Detection of water content in tomato stems by electrical impedance spectroscopy: Preliminary study. *Comput. Electron. Agric.* 219. <https://doi.org/10.1016/j.compag.2024.108755>
- Zhao, L.S., Li, K., Wang, Q.M., Song, X.Y., Su, H.N., Xie, B. Bin, Zhang, X.Y., Huang, F., Chen, X.L., Zhou, B.C., Zhang, Y.Z., 2017. Nitrogen Starvation Impacts the Photosynthetic Performance of *Porphyridium cruentum* as Revealed by Chlorophyll a Fluorescence. *Sci. Rep.* 7. <https://doi.org/10.1038/s41598-017-08428-6>