Early diagnosis and monitoring of nitrogen nutrition stress in tomato leaves using electrical impedance spectroscopy

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Abstract: Nitrogen (N) is a life element for crop growth. In tomato growth and development, N stress often occurs and degrades crop yield and quality. Superfluous N can noticeably increase the nitrate content, which can be degraded into strong carcinogenic substance- nitrite. An accurate and timely monitoring and diagnosis of nutrition during crop growth is premise to realize a precise nutrient management. Crop N monitoring methods have been developed to improve N fertilizer management, and most of them are based on leaf or canopy optical property measurements. Although many optical/spectral plant N sensors have already commercialized for production use, low accuracy for phosphorus (P) and potassium (K) detection and diagnosis remains an important drawback of these methods. To explore the potential of N diagnosis by electrical impedance and perform study for nutrition status of plant NPK meanwhile by the electrical impedance, it is necessary that evaluate the N nutrition level by leaf impedance spectroscopy. Electrical impedance was applied to determine the physiological and nutritional status of plant tissues, but few studies related to plant N contents have been reported. The objective of this study was to evaluate the N nutrition level by leaf impedance spectroscopy and realize the early diagnosis and monitoring of N nutrition stress in tomato. Five sets of tomato plant samples with different N contents were cultivated in a Venlo greenhouse. N content of leaves was determined, and electrical impedance data were recorded in a frequency range of 1 Hz to 1 MHz. The obtained impedance data were analyzed using an equivalent circuit model for cellular tissues. The variation of equivalent parameters along with N content was analyzed, and the sensitive impedance spectroscopy characteristics of N nutrition level were extracted. Furthermore, the effect of moisture content on impedance measurement was discussed and the prediction model for N content was developed. Results showed that electrical impedance can be conveniently applied to early diagnosis and monitoring for tomato N nutrition stress.

Keywords: electrical impedance spectroscopy, nitrogen stress, tomato (*Solanum lycopersicum*) leaves, nitrogen nutrition, diagnosis, monitoring, nondestructive detection

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1 Introduction

Nitrogen (N) is the material basis of plant life and occupies primacy in life activities of plants, which plays an important role in tomato growth. N deficiency decreases crop quality and yield. Superfluous N can noticeably increase the nitrate content. Although the nitrate in vegetable is harmless to vegetable itself, nitrate is degraded into the nitrite ion in human body, which is a kind of strong carcinogenic substance when vegetables are eaten. Furthermore, excessive N fertilizer leads to environmental pollution and increases production cost. Therefore, an early, timely and accurate monitoring of the N nutrition level in tomato is considerably important to realize an accurate nutrient management, save loss as

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well as to guide irrigation scheduling and fertilization. Moreover, it can also save fertilizer and reduce environment pollution. This type of monitoring is the most suitable method to provide nutrition according to the requirements of plant growth. However, nutrition diagnosis is a premise for accurate variable fertilization. Although traditional chemical testing methods for N nutrition with high detection accuracy, they are time consuming and cannot realize the real-time and rapid detection of crop nutrient. In recent years, crop N monitoring methods have been developed to improve N fertilizer management, and most of them are based on spectral and image technology. Although many optical/spectral plant N sensors have already commercialized for production use[1], low accuracy for phosphorus (P) and potassium (K) detection and diagnosis remains an important drawback of these methods. Our previous studies have demonstrated the potential and feasibility for early, rapid and non-destructive for PK detection and diagnosis using leaf electrical impedance. Image technology can be well characterized the leaf appearance change causing by nutrition stress, it can be hardly applied to the detection and diagnosis of nutrition level until apparent symptoms appear. Namely, the early diagnosis for nutrition stress could not been achieved by image technology. Therefore, an early-stage, rapid, non-destructive method for N nutrient diagnosis and detection is necessary to achieve a rapid early-stage, rapid, non-destructive monitoring and diagnosis.

Nutrition deficiency can often disturb growth and metabolic balance in plants, thereby causing variations of crop internal structure. Morphological changes are not clearly evident until internal structural changes develop to a certain extent. When symptoms appear, crops have already been subjected to nutrient deficiency. Even by fertilizing at this time, the loss is not avoided. An accurate and early detection of plant nutrition before the emergence of visible symptoms is important to guide fertilization and save production.

The electrical properties of plant tissues are also altered by their physical structure, chemical processes, or a combination of both[2]. Compared with reflectance spectroscopy and image technology, impedance spectroscopy measurement method has the potential to obtain earlier information about biology and realize the early-stage and rapid diagnosis of crop nutrition level. Comprehensive qualitative and quantitative analyses on the inner components of composition and microstructure of the object can be conducted[3]. In addition, the physical and chemical changes in biological tissue can be accurately described by electrical impedance spectroscopy (EIS) parameters. When the variation in the internal structure of crops can be monitored using EIS measurement methods, the early detection and diagnosis of N nutrition levels can be achieved before the plants present evident symptoms.

EIS has been applied to crop nutrition stress monitoring[4-6], early detection and monitoring of plant disease[7], quality and injury evaluation of fruit[8-10], vegetable[6], water stress[11], salt stress[12], root growth[13,14], low-temperature stress of tea leaves[15], detection of avian influenza virus[16] and early detection of pressure ulcers[17]. Nevertheless, few studies have studied on nutrient level detection in plants. The electrical impedance measurements for phosphorous- and potassium-deficient *Trifolium subterraneum* plants were studied[18]. Total potassium content in maize leaf was monitored based on the electrical impedance measurement of leaf tissue juice; results demonstrated that impedance value could be a predictor for maize leaf potassium[6]. A plant-based sensing method for nutrition stress monitoring was also investigated, and results indicated that the relation between nutrition index and stress caused by lack of mineral nutrients is a monotonic function[4]. The electrical impedance response of lettuce (*Lactuca sativa* L.) was studied at different N statuses, and a high and positive correlation was found between plant N content and frequency values at minimum phase angle[6]. However, the sensitive frequencies and impedance spectrum characteristics about N were not provided in their studies and thus quantitative analysis for N contents could not been realized. Therefore, the N nutrition level in tomato based on leaf impedance spectroscopy was evaluated in this study to quantitative analysis for N contents in leaves and early diagnosis and

detection of N nutrition level. The N uptake of tomato is throughout the whole growth period and increases sharply after fruiting of the first inflorescence, which accounts for more than 85% of the total N uptake during the entire growth stages[19]. Fruit period of the first inflorescence is the critical point for N nutrition monitoring. To realize an early and rapid monitoring of the N nutrition level of tomato and thus avoid crop losses, tomato leaf samples from fruiting of the first inflorescence were selected as research objects in this study.

As mentioned above, electrical impedance has been widely used to determine the physiological status of plant tissues and agricultural product quality due to the simplicity and effectiveness of the method. However, limited research on plant nutrition status detection has been conducted, and few studies have focused on analyzing the effect of plant N status on electrical impedance. These research results provided a theoretical basis and some successful cases regarding the detection and diagnosis of tomato N nutrition levels by using EIS. A method of implementing the early-stage, rapid and non-destructive detection of N by leaf impedance spectrum has yet to be established. The objective of this article is to analyze how the electrical impedance response of tomatoes is influenced by their N nutrition status and explore the feasibility of early diagnosis and monitoring of N nutrition stress using EIS.

2 Materials and Methods

Experiments were carried out in the Venlo greenhouse and the laboratory of the Agricultural Engineering Institute of Jiangsu University in China, which was under controlled conditions such as photoperiod of 16/8 h (light/darkness), at a daily air temperature and relative humidity ranging from 21°C to 25°C and between 70% and 85%, respectively, and light radiations of 800 μmol/(m^2·s). Experiments were performed from April to August.

2.1 Samples preparation

The cultivar Hybrid "908" (Long March Seed Co., Ltd., Shanghai, China) of tomato (*Solanum lycopersicum*) is widely planted in China and has large fruits. Tomato seedlings were planted in a Venlo greenhouse, cultivated in perlite, and watered with nutrient solution. The nutrient solution followed the Yamazaki formula[20], which is the preparation formula for tomato standard nutrient solution. Same-sized seedlings were selected. The samples with different N nutrition levels were cultivated using nutrient solution with five different N amounts. The N contents were 25%, 50%, 75%, 100% and 125% of the Yamazaki formula, which were marked as N0, N1, N2, N3 and N4, respectively (Table 1). The standard Yamazaki solution for tomato growth was obtained using thirteen kinds of chemical reagents. The reagents and corresponding contents used in the standard Yamazaki solution were same as those of N3, as shown in Table 1. N0, N1 and N2 were obtained by discounting about 75%, 50% and 25% of $NH_4H_2PO_4$ and KNO_3 quotients from the standard Yamazaki solution, and the usage amount of other chemical reagents in N0, N1, N2 and N3 was the same, except for $NH_4H_2PO_4$, KNO_3, NaH_2PO_4 and KCl. To ensure the K and P contents in nutrient solution for all treatments were same, NaH_2PO4 and KCl were added proportionally in N0, N1 and N2. N4 was obtained by adding $(NH_4)_2CO_3$ with corresponding quotients (4 mg/L) on the basis of the standard Yamazaki solution. Their specific sources are given in Table 1.

Table 1 Chemical composition of nutrient solutions with various N concentrations based on Yamazaki solution for tomato plants unit: mg/L

	N4	N3	N2	N1	N0
$Ca(NO_3)_2·4H_2O$	354	354	266	177	89
KNO_3	404	404	303	202	101
$NH_4H_2PO_4$	77	77	58	39	19
$MgSO_4·7H_2O$	246	246	246	246	246
NaH_2PO_4	0	0	20	40	60
$(NH_4)_2CO_3$	4	0	0	0	0
KCl	0	0	75	149	224
Na_2Fe-EDTA	25	25	25	25	25
H_3BO_3	2.13	2.13	2.13	2.13	2.13
$MnSO_4·4H_2O$	2.86	2.86	2.86	2.86	2.86
$ZnSO_4·7H_2O$	0.22	0.22	0.22	0.22	0.22
$CuSO_4·5H_2O$	0.08	0.08	0.08	0.08	0.08
$(NH_4)_6Mo_7O_2·4H_2O$	0.02	0.02	0.02	0.02	0.02

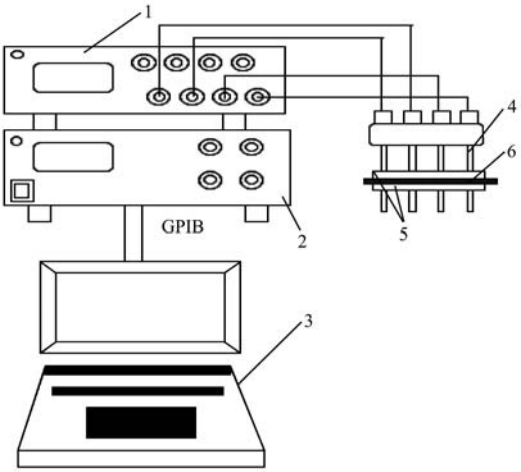
Tomato seedlings were watered with the standard solution at early stages and via nutrient solution with five different N contents after 3-4 true leaves appeared.

Seedlings were classified as five groups according to different treatments: N0, N1, N2, N3 and N4. Each treatment had seven replicates and total 35 samples were acquired. From each treatment, four samples were randomly chosen as calibration samples and the rest as test samples, thus a total of 20 and 15 samples were applied to build and test the model, respectively.

The online impedance measurements of disease-free leaves from five different treatments were conducted, and each sample was replicated three times. The 7th tomato leaf (from top to bottom) was selected, and the middle part of each blade away from the main vein was chosen as the test area. The tested area was quickly cleaned using a tissue prior to impedance measurement. The average value of the three replications was used in the analysis of results. The leaves from different treatments were picked and placed in polythene seal bags with labels immediately after electrical impedance measurement. The plucked leaves were then sent to the laboratory, and N content was determined by continuous flow analytical system (Auto Analyzer 3, Seal Analytical Instruments Co., Ltd, and England).

2.2 Electrical impedance measurement

The combination of 1294 impedance interface and 1260A Impedance/Gain-Phase Analyzer (Solartron Analytical, England, UK) was used for impedance measurements. The schematic for the measurement system is shown in Figure 1, and Figure 2 presents the experimental device.



1. 1294 Impedance interface 2. 1260A Impedance/Gain-Phase Analyzer
3. PC 4. Electrode 5. Leaf 6. Insulation board with holes

Figure 1 Schematic of the measurement system for tomato leaves via four electrodes

Figure 2 Experimental device for electrical impedance measurement of tomato leaf

For impedance measurements, 100 mV of generator voltage and scanned 91 spot frequencies (logarithmic frequency intervals) between 1 Hz and 1 MHz were used. Four-terminal configuration was used in this study. The detailed measurement method followed [19] and not repeated herein.

EIS is based on the measurement of current that passes through the samples. Electric current is strongly linked to the contact area between the sample and electrode. Leaves were sampled for on-line electrical impedance measurement, which were located at the seventh level from the top of the stem.

To obtain high reproducibility in the measurements, the contact interface between the sample and electrode should generally be the same. The corresponding measures can be found in [27] and not repeated here. Moreover, to avoid contamination among samples, electrodes were washed with deionized water and wiped dry with a filter paper after each measurement.

2.3 Determination of the total N content in leaf

Immediately after electrical impedance measurement, fresh tomato leaves were collected, and the N mass fraction of tomato leaves was determined via Kjeldahl method[21]. A continuous flow analytical system (Auto Analyzer 3, Seal Analytical Instruments Co., Ltd, England) was also used. The N content of tomato leaf samples from five treatments is shown in Figure 3. The conditions for tomato growth, except for the N concentrations, were the same. Therefore, the difference of N content in samples was solely attributed to N element.

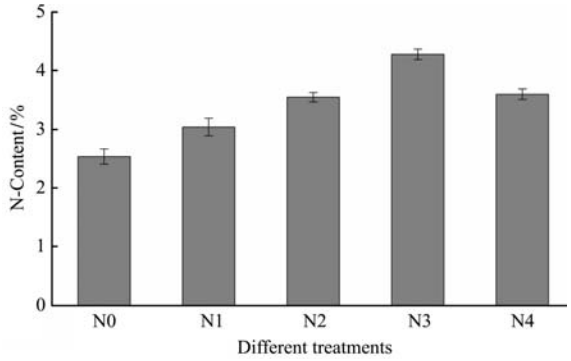


Figure 3 N content averages (n=5) related to the N treatment. Each data point is the mean± SE of three replicates per plant. Values are significantly different at the p=0.05 level (Duncan's multiple –range test)

2.4 Models and curve fitting

The variation in parameters of the equivalent circuit can characterise the physical properties of the materials. Therefore, the equivalent circuit for leaves are provided and analysis for variation in corresponding parameters of the equivalent circuit is conducted.

By analysis and observation for impedance data, the shape of Cole–Cole plot is exactly right semi-ellipse with its centre below the X-axis. In order to model this semiellipse, a constant phase element (CPE) was used[22]. Since the use of a CPE can make it easy to fit the model to the equivalent circuit accurately, a CPE has been used in many studies[23-25]. The expression of CPE can be described as:

$$Z_{CPE} = \frac{1}{j\omega^q T} = \frac{1}{\omega^q T}\cos(\frac{q\pi}{2}) - j\frac{1}{\omega^q T}\sin(\frac{q\pi}{2}) \quad (1)$$

where, j is the imaginary unit; ω is the angular frequency; T is a CPE coefficient, and q is a CPE exponent in the range of 0-1 that describes the time constant distribution in the system. Upon the analysis and observation for impedance data, the shape of Cole-Cole plot is exactly right semiellipse. Hence, the modified model (Figure 4) was applied for the tomato tissue samples in this study.

The complex impedance of the modified model can be described as[26]:

$$Z = \frac{R_e\{1+\omega^q T[(2R_i+R_e)\cdot\cos(q\pi/2)+\omega^q T R_i(R_e+R_i)]\}}{\omega^q T(R_e+R_i)^2+2\omega^q T(R_e+R_i)\cdot\cos(q\pi/2)+1} - j\cdot\frac{\omega^q T R_e^{\ 2}\cdot\sin(q\pi/2)}{\omega^q T(R_e+R_i)^2+2\omega^q T(R_e+R_i)\cdot\cos(q\pi/2)+1} \quad (2)$$

where, R_i is intracellular resistance and R_e is extracellular resistance. The equivalent parameters were estimated with complex nonlinear least squares (CNLS) curve-fitting. In the CNLS fitting, the sum of squares of the real and imaginary residuals was minimized.

The unit of T is different with the values of q. Hence, values of q must be fixed for the accurate analysis of the capacitive components of the cell membrane. T can be expressed apparent C by using Equation (3)[26]. Note that there is an assumption that the relaxation angular frequency (the angular frequency at which the imaginary part of the impedance Z is minimum) keeps the same.

$$C = T\omega_m^{\ q-1} \quad (3)$$

where, ω_m is the relaxation angular frequency. ω_m of the modified model can be calculated from the following equation[26]:

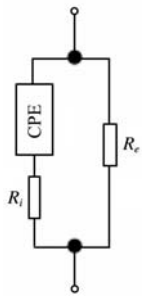
$$\omega_m = \frac{1}{T(R_e+R_i)^{-q}} \quad (4)$$

From Equations (2) and (3), the following expression can be obtained,

$$C_m = T\frac{1}{q}(R_e+R_i)^{\frac{1-q}{q}} \quad (5)$$

In this study, C_m was defined as the cell membrane capacitance and calculated by substituting the values of each parameter in the modified model into Equation (5).

The calculation for resistance (R), reactance (X), and estimation for equivalent parameters (R_e, R_i), followed the [27]. Z-view software was used to perform curve fitting, and data analysis was conducted using Matlab 7.1.



Note: CPE: constant phase element; R_i: intracellular resistance; R_e: extracellular resistance.

Figure 4 Modified equivalent circuit model for biological tissues

2.5 Statistical analysis

A one-way analysis of variance (ANOVA) and principal component analysis (PCA) were performed using the Matlab 7.1 program. Mean separation

procedures were carried out by Duncan's test with the least significant difference (LSD) (p<0.05).

3 Results

3.1 Behaviour of the impedance characteristics for different N nutrition levels

As the frequency increased, the impedance of the samples dropped obviously (Figure 5). The impedance modulus of different treatments showed the obvious difference. Lower N content was associated with higher impedance modulus. The variations in the Cole–Cole plot of the leaf samples with different N contents were shown in Figure 6. The right side of each semi-ellipse described a low-frequency region, and the left-side was a high-frequency area. Each curve demonstrated a semi-elliptical arc, with its centre below the X-axis. The curves could be characterised by the modified model as shown in Figure 4. The measurement values from different treatments were close in the high-frequency region. At low-frequency region, the difference among measurement values from different treatments was apparent.

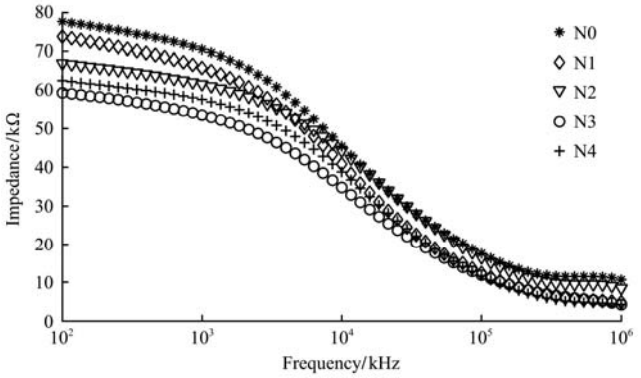


Figure 5 Variation in the impedance spectra of tomato leaf for different N treatments plotted against frequency

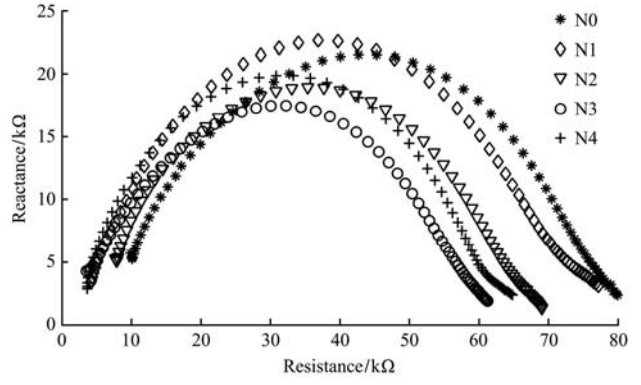


Figure 6 Changes in the Cole–Cole plot of the tomato leaf for different N treatments

3.2 Validity of equivalent circuit models and analysis of N content on the effects of electric parameters

To quantify the impedance characteristics in Figures 5 and 6, the parameters of equivalent circuit were calculated. To test the validity of the built equivalent circuit model, the comparison between predicted and measured values was carried out when N content is 2.54% (Figure 7). The established model implied high accuracy for R and X, and the corresponding R_R^2, R_X^2, and standard deviation (SD) were 0.917, 0.925 and 43 Ω, respectively. These results signified that the developed model could describe test samples. The detailed calculation process of R_R^2, R_X^2 and SD could be seen in [27] and not repeated here.

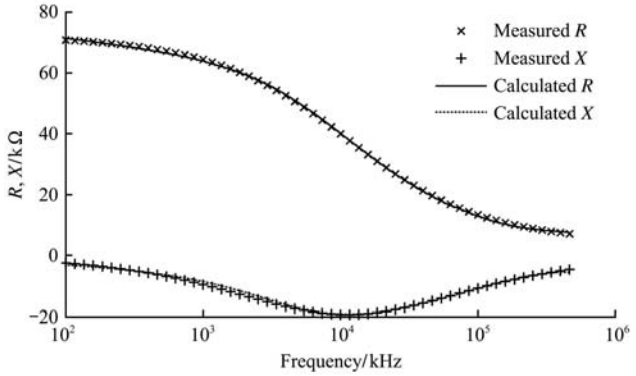


Figure 7 Approximation results of the model and experimental results of tomato leaf at N content of 2.54%

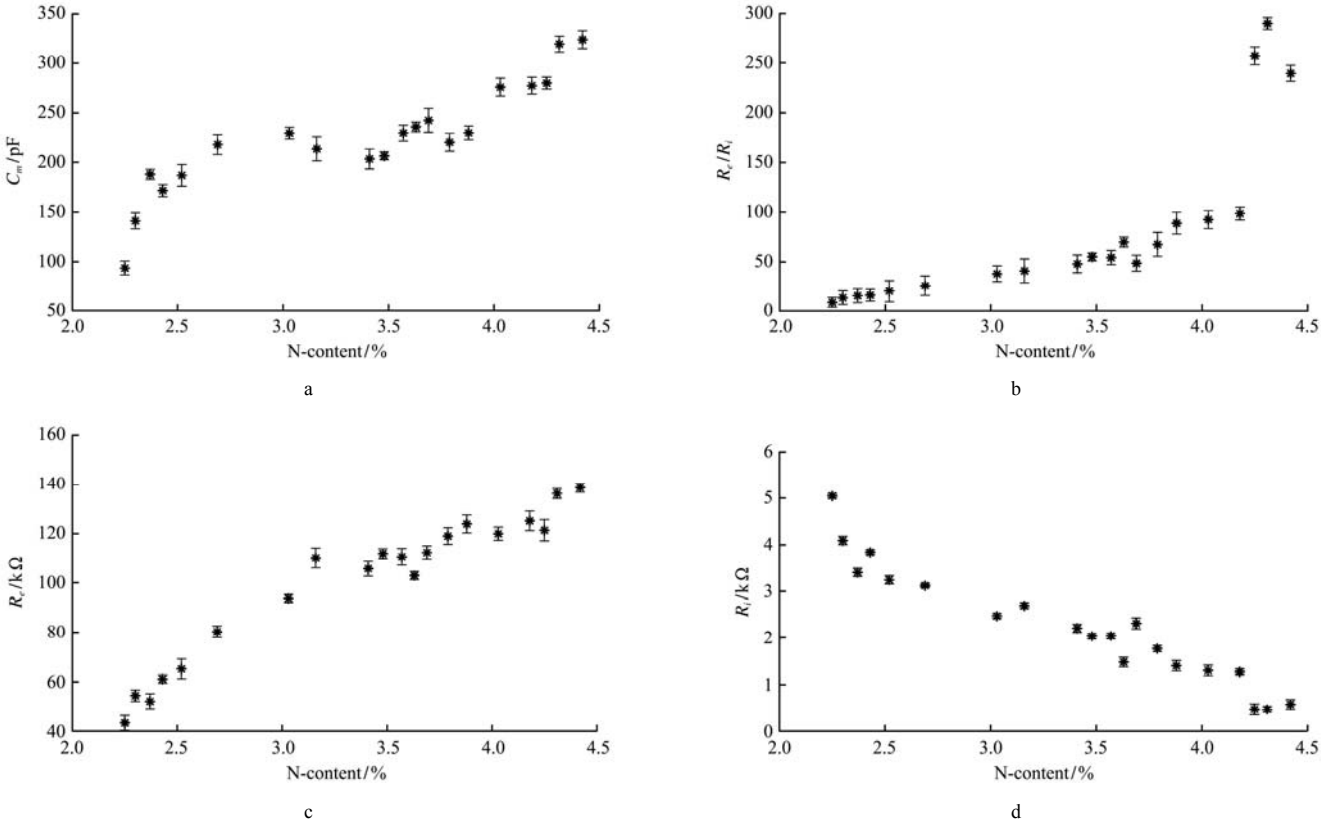
The phase angles of C_m were altered flexibly by introducing the constant phase element CPE into the model. The modified model rightly represented the impedance characteristics of the inhomogeneous tissues of samples. This result was also verified by Yasumasa, et al.[26] The above analysis demonstrated the correctness of the modified model.

The modified model (Figure 4) was also used to analyse the impedance spectra of other samples (except for sample with N content 2.54%) with different N contents. The experimental data also indicated a good fit with the approximations. The R_R^2 values for each sample were 0.909-0.957, and the R_X^2 values were 0.921-0.969. The ratio of the standard deviation and average values (coefficient of variation) was smaller than 0.869 for measured R, 0.942 for calculated R, 0.981 for measured X, and 0.990 for calculated X.

To clarify the influencing mechanism of N deficiency on impedance at the cellular level, the relationship between the N content of tomato leaves and electrical parameters were studied (Figure 8). C_m became small and R_e decreased sharply when N content was less than 2.69 % (Figures 8a and 8c).

R_i and R_e kept virtually constant when N content was higher than 3.48 % (Figure 8). At N content below 3.48%, the capacitance of the cell membrane dropped as the N content deceased obviously. At N content less than 2.69%, this decline in membrane capacitance was noticeable.

In this study, the early monitoring and diagnosis could be achieved by the proposed method 4-6 d in advance before apparent symptoms of N stress were visually observed, this showed that the feasibility and effectiveness of the proposed method in realizing early monitoring and diagnosis.



Note: C_m: capacitance of cell membrane, R_i: intracellular resistance, and R_E: extracellular resistance. Each data point is the mean± SE of three replicates per plant. Values are significantly different at the p=0.05 level (Duncan's multiple -range test).

Figure 8 Relationship between N content and the equivalent circuit parameters

3.3 PCA for tomato leaf impedance

The cumulative contribution rate of PC1 and PC2 exceeded 96% (Figure 9), thus reflecting the original information of multidimensional data indicators. Thus, the 91 impedance values could be represented by these two principal components, and the covariance between these two principal components was equal to zero. Figure 9 showed that samples under different N treatments were distributed in five different regions.

3.4 Stepwise regression model based on tomato leaf impedance and N mass fraction

About 70%-90% water were available in plants. The large content of moisture and metal ions inside tomato leaves allowed the leaves to act as good electrical conductors. Impedance was influenced by the moisture content[26-28]. The logarithmic functions of impedance and capacitance could be used to estimate the moisture content at 3.98 kHz[29]. To improve the diagnostic accuracy of N nutrition status, in this study, the moisture content in leaves was predicted by using the logarithmic functions of impedance and capacitance at 3.98 kHz.

The positive correlation between leaf impedance values and N mass fractions was apparent (Figure 10). Significant correlation was present when the frequency was higher than 4.642 Hz. The correlation coefficient exceeded 0.85 in the frequency ranges from 215.2 Hz and

398.1 kHz. These frequency bands with good correlation showed a good quantitative relationship between leaf impedance value and N content. Therefore, to estimate and predict the N content in tomato leaves, a stepwise regression model was developed for 20 leaf samples. The modulus of impedance at 49 frequency points between 215.2 Hz and 398.1 kHz was chose as independent variable, x and N contents were used as dependent variable, y. The experimental samples were divided into two groups for the development of the stepwise regression model. The first group of 20 samples was used to build the regression model. The second group included 15 samples and was used to validate the regression model. Data analysis was conducted by SPSS software. The regression model of N content was described as:

$$y = 9.133\ln(x_{2.51\ \mathrm{kHz}}) - 8.794\ln(x_{13.59\ \mathrm{kHz}}) + 0.102\ln(x_{3.98\ \mathrm{kHz}} / C_{3.98\ \mathrm{kHz}}) + 0.209 \quad (6)$$

where, y is the N mass fraction,%; $x_{2.51\ \mathrm{kHz}}$, $x_{3.98\ \mathrm{kHz}}$ and $x_{13.59\ \mathrm{kHz}}$ are the impedance modulus values (Ω) at 2.51 kHz, 3.98 kHz and 13.59 kHz, respectively; $C_{3.98\ \mathrm{kHz}}$ is the capacitance values (F) at 3.98 kHz.

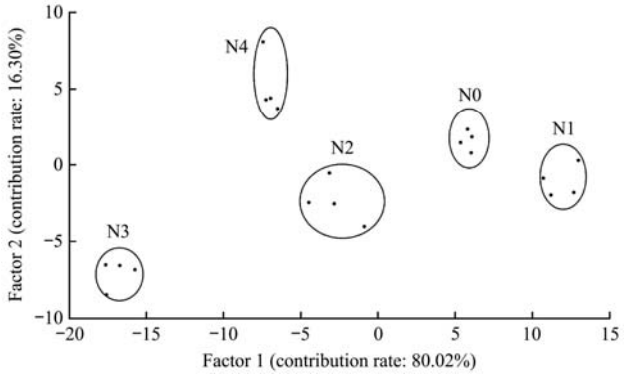


Figure 9 Score plot obtained from PC1 and PC2 for different treatments

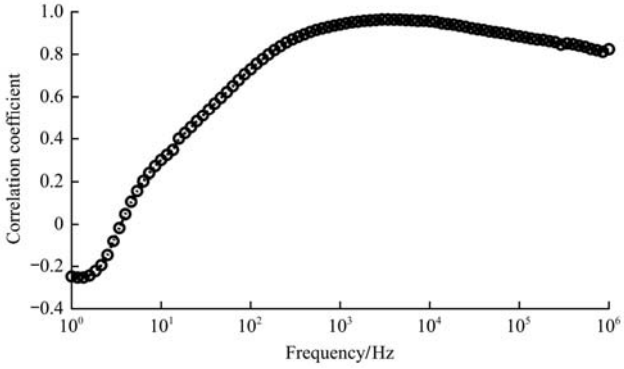


Figure 10 Correlation curves of tomato leaf impedance value and N content

Good coefficient of determination (R^2=0.8635) and root mean square error (*RMSE*=0.1729) were acquired during the calibration process. Verification of the built model before using it for the prediction of the new sample is necessary. Therefore, model validation was conducted by using 15 test samples, and satisfactory prediction result was obtained (R^2=0.8374 and *RMSE* = 0.6871). The relationship between measured and predicted values indicated the high accuracy of the established model (Figure 11). The above analysis showed that the extraction of N sensitive frequencies (2.51 kHz and 13.59 kHz) was reasonable. The determination of sensitive frequencies laid a good foundation for the design of N nutrition level impedance analyzer in the future.

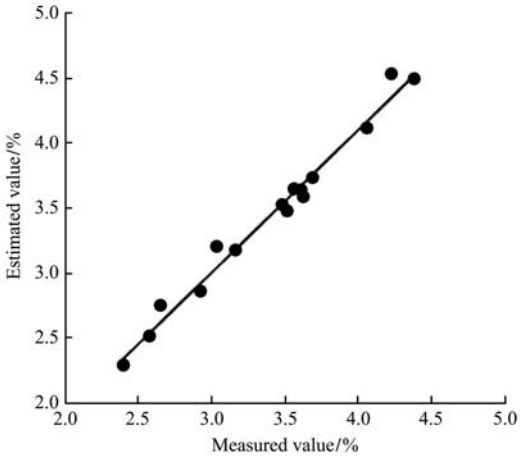


Figure 11 Estimated vs. measured values of N levels in tomato leaves and the 1:1 line

4 Discussion

In the presented work, the impedance modulus of different N content showed the obvious difference. Lower N content was associated with higher impedance modulus and vice versa (Figure 5). This phenomenon can be explained as follows: nitrogen in leaves or plants exists in the form of protein, nucleic acid NH_4^+ (Ammonium ions), NO_3^- (Nitrate ions), adenosine triphosphate (ATP) and so on [30,31]. The conductance of samples is a function of the ion content of the samples[32]. Ion content in leaf samples with low N content is low. Therefore, high impedance modulus is correlated with low N content.

Results in this study showed the impedance of the samples decreased obviously as the frequency increased

(Figure 5). This result agrees with previous studies[5,6,26]. These differences could be explained as follows: In the low-frequency zone, due to the high electrical capacity of cell membranes, the electrical current flowed only through extracellular fluid, which has relatively high resistance. However, in the high-frequency area, the impedance decreases greatly because the current can flow through intracellular fluid, which has relatively low resistance. This phenomenon, which causes by cell structures in biological tissue, is called β dispersion[26].

The measurement values from different treatments were close in the high-frequency area while the apparent difference in low-frequency region was virtually observed (Figure 6). This phenomenon can be interpreted as follows: electrical current in low frequency regions only flows through the extracellular fluid, which has a relatively high resistance. The impedance mainly depends on extracellular resistance due to the high electric capacitance of cell membranes. However, according to the basic ion potential theory[33], the cell membrane is a semipermeable membrane with ion channels. Channel proteins, which inlay the cell membrane and provide a particular gateway for charged ions or molecules, are the communication gateways between intracellular tissues and extracellular fluid. N in leaves or plants exists in the form of NH_4^+ (Ammonium ions), NO_3^-, protein and nucleic acid, which cannot freely transport between intracellular and extracellular fluid via channel proteins. Thus the impedance of samples mainly rests with the NH_4^+ and NO_3^- concentration in extracellular fluid. In addition, the impedance also depends on the integrity of cell membrane. The N contents in samples from distinct treatments are different. Thereby, the noticeable difference among impedance measurements from different treatments appears in the low-frequency region.

At high frequencies, the capacitance of the cell membrane is negligible because the values are largely less than those of other parameters. Impedance mainly depends on extracellular and intracellular resistances. Although N stress has effect on capacitance to a certain extent, this effect can be ignored at high frequencies, and thus measurement values from different treatments are close.

Results in this study suggest C_m became small and R_e decreased sharply when N content was less than 2.69% (Figures 8a and 8c). These findings show that the cell membrane is seriously injured, and the evident variation is resulted from N deficiency. In normal cells, intracellular fluid with a relatively high electrolyte and low extracellular fluid concentrations is separated by the cell membrane[26]. The current results could be interpreted by the cell membrane of the sample being damaged by N deficiency, such that intracellular fluid can leak from the inside of the cells to the outside. Thus, R_e decreased and R_i increased, which caused R_e/R_i decline (Figure 8b). The R_e and R_i values were close to each other as the aggravation of N deficiency. This observation provides support for our suggested explanation.

R_e/R_i can reflect the extent of damage to the cell membrane from another point of view. In a normal cell, R_e is greater than R_i because extracellular fluid has a relatively low electrolyte concentration. The cell membrane is probably damaged as the nutrition stress aggravates. Thus, intracellular fluid leaks to the intracellular fluid via the microholes in the cell membrane. With further leakage, the electrolyte concentration in the intracellular fluid gradually approach and yet still higher than the electrolyte concentration in the intracellular fluid. Therefore, the R_e/R_i values for samples without N stress were higher than 1 and gradually became close to 1 as the N deficiency. The initial R_e/R_i value was approximately 289 and decreased with further N deficiency. The above findings show that N deficiency influences the cell membrane, and R_e/R_i could be an index of cell health.

Nitrogen is the constituent of nucleic acids and phospholipids of cell membrane and the main composition of the protein. With the increase of N, the vitamin and soluble sugar contents in vegetables drop significantly[34]. The protein synthesis, cell division and growth are hampered and chlorophyll content is reduced when N deficiency in plant occurs. Therefore, this phenomenon is perhaps that the decline in cell membrane capacitance occurs at low N, and cell membrane is

damaged in N content less than 2.69% (Figure 8).

PCA analysis showed samples under different N treatments were distributed in five different regions (Figure 9). This phenomenon implies that the impedance measurement method may be conducive to the qualitative diagnosis of N nutrition status.

Higher correlation between leaf N content and impedance modulus values at two characteristic frequencies 2.51 kHz and 13.59 kHz is found. However, a low correlation between total-N in lettuce leaves and electrical impedance magnitude at characteristic frequencies is demonstrated[6]. This disagreement of two results is perhaps caused by the differences in experimental conditions and physiological feature between tomato and lettuce, the detailed reason can be interpreted by scientific research in the future.

Age of plant organ or plant tissue have a significant influence on the magnitude of phase angle of the impedance[26]. It should be mentioned that the measurement data could be influenced by age-dependent characteristic changes in plant tissue which seems difficult to consider and avoid during leaf sampling. In this study, to reduce this effect, only the tomato leaf samples from fruiting of the first inflorescence were selected as research objects. The electrical impedance measurement are also influenced by the conditions where plants are cultivated such as water and nutrient availability, temperature and moisture content, each of which has to be considered when measurements are conducted, especially when intact plants will be used for EIS experiments.

The advantages of EIS technique is that it can be used to measure what's happening to plant tissue without having to destroy the plants[12]. The measurements are easy, quick and repeatable. However, there is a major problem with the interpretation of electrical impedance method, because a few different equivalent circuit models can have identical impedances[35]. The impedance spectrum of tomato leaves was composed of one arc, similarly to Scot pine and Norway spruce[36]. These results obviously suggested the heterogeneity of the tissues studied, which will make the biological interpretation of impedance models difficult.

5 Conclusions

The impedance characteristic of tomato leaves with different N contents was investigated. The mechanism of how the electrical impedance response of tomatoes was influenced by N nutrition status was evaluated, and the feasibility of detecting N deficiency using EIS was explored. Results showed that EIS could be accurately discriminated tomato leaves with different N contents at fruiting of the first inflorescence stage. The electrical impedance spectroscopy could be used to early-stage diagnosis, warning and monitoring for N stress in tomato. This study also indicated that the electrical impedance spectroscopy might be a suitable method for assessing N nutrient status of plants without damaging effects on tissues. This method could also be extended to evaluate other nutrient status meanwhile such as P and K in tomato leaves as well as other plants such as cucumber, lettuce and maize. This research provided some theoretical basis and practical technology for precision management of crop nutrition and had important significance for food traceability and quality safety.

In future researches, the effect of other factors, such as complicate interactions among N, P and K, disease, levels of other nutrients, and physiological variables, on electrical impedance measurements should be assessed. The EIS feature extraction and method for different nutrition from other different growth stage and a web-based monitoring and early warning system for tomato nutrition should be developed with EIS and Internet of things (IoT).

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