Choice of impedance parameter of strawberry tissue for detection of fungal diseases

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Abstract. The application of the method of electrical impedance spectroscopy for the diagnosis of plant diseases is justified. Studies were carried out to identify the impedance parameters of strawberry leaf tissue associated with the effects of three pathogens of fungal diseases of garden strawberry, using the impedance analyzer WK 6505B. The analysis of variance of the mean values of the obtained data revealed that the most informative part of the changes in all parameters lies in the low-frequency region of the α-dispersion of bioimpedance. As the main informative parameter, it is proposed to use reactance measured at frequencies of $200 \ldots 10^4$ Hz, at which extremes of its change appear for healthy and diseased strawberry leaf blades.

1. Introduction
Strawberry is widespread in the world because of the obvious advantages compared to other berry crops. Strawberry have valuable healing properties and a bright attractive appearance. It is nutritious, has an abundant biochemical composition and has high palatability. It’s share in the global production of berries is more than 70% [1]. However, over 30 fungal, viral and bacterial diseases affect garden strawberries. Most diseases (about 80%) cause fungi [1]. The early detection of fungal diseases of infected fruits is very important both for the producers of this product and for its consumers. Currently, a great number of special methods for detecting diseases are used in diagnostics with their inherent advantages and disadvantages. Visual diagnosis of fungal diseases is the most common and does not lose its relevance, as it is often preliminary for other diagnostic technologies. The main disadvantages of the visual method can be considered unreliability due to the coincidence of external signs of plant infection and physiological disturbances caused by adverse external influences, the need for a highly qualified expert and the late detection of diseases. Most diagnostic methods for diseases of fruit crops are based on the detection of specific antigens (immunochemical methods) or nucleic acid (molecular methods). However, the known methods are invasive, require the use of complex and expensive equipment and highly qualified maintenance [2]. The use of existing methods of technical vision, for example, by counting image pixels in the space of color channels of red, green and blue (R, G, B) for the diagnosis of fungal diseases, requires the development of a complex pattern recognition system and the use of artificial intelligence systems, for example, artificial neural networks [2-4]. In addition, the effects of pathogens of viral and bacterial diseases, chemical leaf burns that occur during pest control, as well as sunburn, give identical symptoms in the color picture of leaf blade damage, which reduces the reliability of identification of a specific fungal disease by technical vision methods.
2. Problem definition

It is convenient to study biological objects by observing their reaction to a weak external effect that does not damage the tissue of a living object. The method of spectroscopy of electric impedance (Spectroscopy of an electric impedance - EIS) allows to obtain information about the processes of transport of charge carriers in any materials and allows to characterize systems which electrochemical behavior is due to several inextricably linked processes [5, 6]. When obtaining information about the complex processes of charge transfer in animal and plant tissues, the method of impedance spectroscopy is often indispensable. Electrical impedance spectroscopy is a relatively new technology in which a signal with a wide pass band and a continuous frequency band is used as an excitation source for measuring the impedance characterizing the electrical properties of the object under study, studying the structure and physicochemical characteristics of inorganic and organic materials. The EIS method allows obtaining a significant amount of information about the processes of transport of charge carriers in solid and liquid materials. It is extremely important to study charge transfer in heterogeneous systems, including phase boundaries, electrode boundaries, and microstructure elements. A methodology has been developed for determining the physiological status of plant tissues by measuring electrical impedance. For example, this methodology is widely used in studies of plants and soils [7-9], including the disease of cultivated plants [10].

The purpose of the research is to determine the possibility of detecting white, brown, and angular spots of strawberries using informative bio-impedance parameters of plant tissues of its leaves and to create a relatively simple and inexpensive non-invasive express method for the diagnosis of fungal diseases of garden crops.

3. Theory

Biological cells have components that act as electrical elements that support cell health by regulating the amount of electrical charge.

It is believed that the impedance of plants is determined by the state of physiology and pathology of plants. In many materials, especially those that are not usually considered to be conductors of electric current, the impedance varies depending on the frequency of changes in the applied voltage and is associated with the properties of the material. For a plant, this may be due to the structure of the plant cell or to the physiological properties of the plant, healthy or diseased, or to a combination of the two.

Characterization in electrical impedance spectroscopy (EIS) can comprehensively reflect changes in the internal structure and composition of organisms. EIS typically uses electrical equivalent material circuits to characterize the experimental frequency response of the impedance. The physical properties of materials can be quantified by tracking changes in the parameters of the equivalent circuit.

Active $R$ and reactance $X$ is determined by the formulas [11]:

$$R = |Z| \cos \theta$$

$$X = |Z| \sin \theta,$$

where $|Z|$ modulus of the total electrical resistance, hereinafter impedance, $\theta$ is its phase angle.

Figure 1 shows four models of equivalent circuits used in world practice for studying the functional state of plant tissues [11].

The Hayden model for plant tissues, which is shown in figure 1a, takes into account the intracellular resistance $R_i$, the extracellular resistance $R_e$, as well as the capacity and resistance of the cell membrane, $C_m$ and $R_m$. The membrane resistance $R_m$ can be neglected, since the value of $R_m$ is much larger than the values of other parameters.

Thus, the Hayden model can be expressed as a simplified Hayden model (figure 1b) [12]. This model represents the structure of a single cell and describes the exact semicircle in the hodograph of resistances. Nevertheless, tissues consisting of numerous cells can have a corresponding distribution in time and the resistance hodograph is described as a half ellipse.
To model this semi-ellipse, a constant phase element ($\varphi$) [13] is usually used instead of $C_m$ (figure 1c). Since the use of the element $\varphi$ can facilitate accurate fitting of the model to the equivalent circuit, it was used in studies [14].

The expression $\varphi$ can be described as:

$$Z_{CPE} = \frac{1}{j\omega^qT} = \frac{1}{\omega^qT} \cos \left(\frac{q\pi}{2}\right) - j \frac{1}{\omega^qT} \sin \left(\frac{q\pi}{2}\right),$$

(3)

where $j$ is the imaginary unit, $\omega$ is the angular frequency, $T$ is the coefficient $\varphi$, and $q$ is the index $\varphi$ in the range $0 \ldots 1$, which describes the distribution of the time constant in the system. After analyzing and observing impedance data, the hodograph form is an exact half-ellipse. The complex impedance of a modified model can be described as [14]:

$$Z = \frac{R_e(1+\omega^qT[2R_e+R_i] \cos \left(\frac{q\pi}{2}\right) + \omega^qT(R_e+R_i) \cos \left(\frac{q\pi}{2}+1\right)) + j \omega^qT^2R_i^2}{\omega^qT(R_e+R_i)^2 + 2\omega^qT(R_e+R_i) \cos \left(\frac{q\pi}{2}+1\right)} + j \omega^qT^2R_i^2$$

(4)

Coefficient $T$ varies depending on $q$ values. Therefore, $q$ values must be recorded for accurate analysis of capacitive components of the cell membrane. The value of $T$ can be calculated from the expression [16]:

$$C = T \omega_m q^{-1}$$

(5)

The angular relaxation frequency (the angular frequency at which the imaginary part of the impedance is minimal) remains unchanged:

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

(6)

From equations (4) and (5), we can obtain the following expression:

$$C_m = T^{1/q} (R_e + R_i)^{1-q/q}$$

(7)

In the study, it is necessary to determine the capacity of the cell membrane by substituting the values of each parameter in a modified model.

Considering that the analytical equipment used allows measurements to be carried out according to two equivalent schemes (figures 1a, 1b), it is advisable to first conduct research on these two schemes, and from the measurement results to select the most effective model and adjust (by calculation) the values of the model elements [15, 16].

4. Experimental results

The studies were carried out using a WK 6505B precision impedance analyzer (Wayne Kerr Electronics, UK). The impedance measurements were carried out on three varieties of garden strawberry: Eliani, Tanyusha and Darenka.
Diagnosis of diseases was carried out by generally accepted methods of visual analysis of symptoms and microscopic analysis of the sporulation of pathogen fungi [17, 18].

The plants were dug up in the morning on the day of measurements at the collection site of the SibFTI garden of the Siberian Scientific Center for Scientific and Technical Research of the Russian Academy of Sciences - the testing ground of the laboratory for experimental research. The plants were dug up with a lump of soil around the roots (about 3 liters in volume), watered and then transferred to the laboratory premises for measurements. Before starting physical measurements, an assessment was made of the age of the leaves selected for research (less than 5 weeks, more than 5 weeks, more than 10 weeks) and an assessment of the intensity (degree) of damage to the leaf blades, as well as the immediate tissue sites (halves of the leaf blades), where contact measuring electrodes.

The assessment of the intensity (degree) of the lesion was carried out using the assessment methodology and the visual scale for spotting [18]. The intensity of the lesion of each disease (spotting) was determined in points by the surface area of the leaf blade occupied by necrotic spots (separately for each of the 3 plates of a complex leaf of wild strawberry), expressed as a percentage (%) of its total area. Also, in a similar way, the intensity (degree) of damage to a particular half of the leaf blade (separately to the left and to the right of its central vein) was additionally evaluated, on which physical parameters were then measured.

On each leaf plate of all leaves in a selected sample of a bush of wild strawberry, measurements were made of 8 bio-impedance parameters, namely: impedance module ($Z$), phase shift ($\theta$); active ($R$) and reactance ($X$) with a serial and parallel equivalent circuit of the object of study; capacity ($C$) and loss tangent ($D$).

The bioimpedance parameters of the plant tissue of strawberry leaves were measured using sensor electrodes consisting of two non-polarizing H124SG cup electrodes with a diameter of 8 mm manufactured by COVIDIEN (USA) and superimposed on the upper side of the sheet with a constant clamping force, which is ensured by means of “clip” type clamps.

To reduce the contact resistance between the electrodes and the surface of the sheet, the electrodes were lubricated with a special electrode gel.

During the measurement, the temperature and relative humidity of the ambient air were controlled using an IVA-6.

The necessary data on the selection of samples of strawberry, its variety, the numbering of healthy and diseased leaf plates, on which the electrodes were superimposed, the degree of damage and the specific form of the fungal disease, the ambient temperature, were entered in the work journal.

2624 dependences of 8 impedance parameters on the frequency in the range from 20 to 5 MHz were obtained (active resistance $R$ and reactance $X$ with a serial and parallel circuit of the electrical model of the measurement object, the total electrical resistance module (impedance module) $Z$, phase shift $\theta$, capacitance $C$, loss tangent $D$) healthy and white, brown and angular spotted leaf blades of 3 grades.

Thus, measurements of 8 bioimpedance parameters were carried out on leaf blades of the three above varieties of garden strawberry. They were exposed to studies of the electrical properties of tissue for all 8 impedance parameters: 28 plates affected by angular spotting, 46 plates affected by white spotting and 103 plates affected by brown spotting. The total number of measurements of 8 bio-impedance parameters of healthy plates on all the studied bushes of garden strawberries was 151 units.

The dependences of the averaged bioimpedance parameters on frequency $f$ are shown in figures 2-3.

Given the large number of obtained dependences of the parameters of impedance and artifacts that affect the value of the bio-impedance parameters of garden strawberry tissue (temperature and humidity, environmental shape, thickness and area of the leaf plate, etc.), for all the obtained research results and varieties, the choice of informative parameters was carried out according to the results of two-way analysis of variance of the mean values of 7 bio-impedance parameters at 3 fixed frequencies $f$, determined by the maximum dispersion of the parameters. The phase shift parameter $\theta$ is excluded due to low information content.
Figure 2. Dependences of the mean values of impedance parameters on frequency by type of disease (sequential model circuit).

Figure 3. Dependences of the mean values of impedance parameters on frequency by type of disease (parallel model circuit).
The type of disease and the variety of the studied plant were considered as factors. To test the significance of pairwise differences in the mean values of parameters between types of diseases, the Tukey's criterion was used. The results of analysis of variance for 3 varieties are summarized in tables 1-6.

**Table 1.** Analysis of variance for electrical capacitance $C$ at a frequency $f = 118$ Hz.

| Parameters       | Df | SS     | MS     | F      | p-value |
|------------------|----|--------|--------|--------|---------|
| Pathogen         | 3  | 338542 | 112847 | 17.129 | < 0.001*** |
| Variety          | 2  | 224737 | 112368 | 17.056 | < 0.001*** |
| Pathogen × Variety | 4  | 30850  | 7713   | 1.171  | 0.328   |
| Residuals        | 101| 665398 | 6588   |        |         |

Significance level: *** 0.001; ** 0.01; * 0.05.

**Table 2.** Tukey’s test for the significance of pairwise differences between mean values of electrical capacitance $C$ at a frequency $f = 118$ Hz.

| Pairs  | Difference | p-value |
|--------|------------|---------|
| 1-0    | -126.439   | < 0.001*** |
| 2-0    | -136.757   | < 0.001*** |
| 3-0    | -114.551   | 0.004**   |

Significance level: *** 0.001; ** 0.01; * 0.05

Pathogen code: 0 – healthy; 1 – Ramularia tulasnei; 2 – Marssonina potentillae; 3 – Dendrophoma obscurans.

**Table 3.** Analysis of variance for electrical impedance $Z$ at a frequency $f = 1158$ Hz.

| Parameters       | Df | SS     | MS     | F      | p-value |
|------------------|----|--------|--------|--------|---------|
| Pathogen         | 3  | 743592 | 24786  | 17.221 | < 0.001*** |
| Variety          | 2  | 61436  | 30718  | 21.343 | < 0.001*** |
| Pathogen × Variety | 4  | 21598  | 5400   | 3.752  | 0.007**  |
| Residuals        | 104| 149684 | 1439   | 104    |         |

Significance level: *** 0.001; ** 0.01; * 0.05.

**Table 4.** Tukey’s test for the significance of pairwise differences between mean values of electrical impedance $Z$ at a frequency $f = 1158$ Hz.

| Pairs  | Difference | p-value |
|--------|------------|---------|
| 1-0    | 76.221     | < 0.001*** |
| 2-0    | 50.897     | < 0.001*** |
| 3-0    | 20.624     | 0.632   |

Significance level: *** 0.001; ** 0.01; * 0.05

Pathogen code: 0 – healthy; 1 – Ramularia tulasnei; 2 – Marssonina potentillae; 3 – Dendrophoma obscurans.

**Table 5.** Analysis of variance for electrical reactance $X$ (with a series combination) at a frequency $f = 5303$ Hz.

| Parameters       | Df | SS     | MS     | F      | p-value |
|------------------|----|--------|--------|--------|---------|
| Pathogen         | 3  | 7604   | 2535   | 18.236 | < 0.001*** |
| Variety          | 2  | 9593   | 4797   | 34.511 | < 0.001*** |
| Pathogen × Variety | 4  | 1222   | 306    | 2.199  | 0.074   |
| Residuals        | 101| 14038  | 139    |        |         |

Significance level: *** 0.001; ** 0.01; * 0.05.

**Table 6.** Tukey’s test for the significance of pairwise differences between mean values of electrical reactance $X$ (with a series combination) at a frequency $f = 5303$ Hz.
7

Pairs | Difference | \( p \)-value
---|---|---
1-0 | -19.623 | 0.002**
2-0 | -19.193 | < 0.001***
3-0 | -16.011 | 0.068

Significance level: *** 0.001; ** 0.01; * 0.05
Pathogen code: 0 – healthy; 1 – *Ramularia tulasnei*; 2 – *Marssonina potentillae*; 3 – *Dendrophoma obscurans.*

5. Results and discussion

It is known that as a result of exposure to a biostressor, the normal interaction between the individual components of the cell is disrupted, as a result of which pathological reactions arise with the formation of toxic substances, which causes a change in the polarization properties of plant tissue [10].

In the low frequency range from 20 to 100 Hz, an oscillatory process of changing the mean values of the phase shift \( \theta \), active resistance \( R \) and reactance \( X \) (with a sequential circuit of the electrical model of the object of study) is observed. This makes measurements of bioimpedance parameters in this area uninformative (figure 2).

According the data shown in tables 1 – 6, and the analysis of the nature of the dependencies presented in figures 2 and 3, it can be argued that the most informative parameter for the detection of leaf plate diseases is the reactive electrical resistance \( X \) in a sequential model of the measurement object.

When analyzing the dependences, it was found that the most informative part of the changes in all parameters lies in the low-frequency region of the \( \alpha \)-dispersion of bioimpedance: from 200 Hz to 10 kHz. With \( \alpha \)-dispersion, the polarization of whole cells occurs as a result of ion diffusion, which takes a relatively long time. In this area, the capacitive resistance of the membranes is very high, therefore, currents that envelope the cells and electrolyte solutions flowing through the surrounding cells predominate.

The analysis of experimental data showed the possibility of early diagnosis of white, brown, and angular spotting of garden strawberries based on the simultaneously obtained difference of reactive electrical resistances measured on the pathogen and healthy plates of garden strawberry leaf (see figure 4).

![Graph](image.png)

**Figure 4.** The dependence of the values of the reactive electrical resistance module \( |X| \) from the frequency \( f \) healthy (solid line) and affected (dash-dotted lines), 3 fungi pathogens (degree of damage 1 point), leaf blades of strawberry garden varieties *Tanyusha*. 08/14/2019.

6. Conclusion

To detect fungal diseases of garden strawberries, it is proposed to use reactance \( X \) as an informative parameter of bioimpedance in a serial circuit of an electrical model of an object, measured in the low-
frequency region of α-dispersion (200...10³ Hz), at which extremes of reactance change appear for healthy and affected by fungal diseases of leaf blades of strawberry garden.

A two-way (type of disease and variety) analysis of variance was carried out, which revealed the significance of the effect of the type of disease on the index of successive reactance at frequencies of 118, 1158, 5303 Hz.

Significant differences were obtained between the reactivities of healthy and fungal pathogens of white, brown, and angular spotting of leaf blades of garden strawberry varieties Eliani, Tanysusha and Darrenka with a degree of damage to these diseases equal to 1 point.

The research results will be used to create a portable device and develop a methodology for early non-invasive diagnostics of white, brown and angular spotting of wild strawberries.

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