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Measurement of Fresh Tea Leaf Growth Using Electrical Impedance Spectroscopy

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Abstract: Electrical Impedance Spectroscopy (EIS) was applied to identify EIS parameters for the growth assessment of fresh tea leaves. We compared the EIS parameters of a distributed model with the dry matter content, which is a commonly applied parameter for growth assessment. As growth progresses, EIS parameters such as the relaxation time, resistances (except intercellular resistance) and dry matter content increased. According to the results of multiple linear regression analysis of the response surface model using fifty samples, the relaxation time displayed a good correlation with the dry matter content; however, the other parameters had no significant correlation. We concluded that the relaxation time would be useful for the growth assessment of tea leaves.

Key words: Growth, tea leaf, impedance spectroscopy, relaxation time, dry matter content

INTRODUCTION

The quality of green tea in terms of its shape, colour, aroma and taste of the leaf as well as the colour of the liquor is strongly influenced by manufacturing conditions and the maturity of the fresh tea shoots[1], which include the bud, leaves and internodes (stem). As growth progresses, the tea leaves become rigid and heavy and the dry matter content increases on maturation[1,2]. Several methods are used to assess the growth of the tea shoots e.g. the weight of one hundred tea shoots, bulk density (kg m^{-3}) and dry matter content[1-3]. These methods provide information regarding the various properties of fresh tea shoots. However, all have limitations and time-consuming measurements. An easy and rapid method for assessing the growth of fresh tea shoots is still lacking in green tea manufacture.

Electrical Impedance Spectroscopy (EIS) has been widely used to assess the in vivo conditions of animal and plant tissue because it is a rapid and easy method of measurement[1]. In this method, Alternating Current (AC) causes polarization and relaxation in the sample leading to changes in the amplitude and phase of the applied AC signals. Based on these changes, the impedance of the sample, which comprises a real and an imaginary part in a complex plane, can be determined. When the real and imaginary parts are measured at different frequencies, an impedance spectrum is obtained. In biological samples, the proportion of current passing through the apoplastic and symplastic spaces in a tissue depends on the AC frequency. At a low frequency, AC current passes through the apoplast. The conductivity of cell membranes increases as the frequency of the current increases. At sufficiently high frequencies, the apoplast and symplast form a parallel circuit that facilitates calculation of the symplastic resistance. Hence, information regarding different tissue features e.g. intra- and extracellular fluids and a specific interface may be revealed by EIS[4-7]. In many reports, electrical impedance was used to measure the growth of roots[8,9]; fruit ripening[10-12] and plant tissue during cold accumulation[13-15] and following freeze-thaw treatment[16,17], exposure to ozone and carbon dioxide[18] and planting in the saline condition[19]. The EIS spectrum was also measured during green tea manufacture processes e.g., steaming[3], primary drying [20] and rolling[21]. However, the EIS spectra of tea shoots demonstrating vigorous growth has not been investigated.

Since a tea shoot has more leaves than internodes and buds, we focused on the growth of fresh tea leaves in this study. We attempted to asses growth by investigating the changes in the dry matter content and equivalent circuit EIS parameters. If there is a relationship between growth (dry matter content) and the EIS parameters, then it could be used for growth assessment. Our discovery will provide valuable information for green tea production.

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MATERIALS AND METHODS

Plant material: Tea bushes (*Camellia sinensis* L.) (twenty-seven years old) were planted in an experimental field at the National Institute of Vegetable and Tea Science (34°48'N, 138°08'E, 202 m.a.s.l.) in Japan. A vigorously growing shoot from a bush being plucked produced a minimum of 4 leaves before it temporarily ceased growth and one leaf emerges at 5-6 day intervals. To clarify the difference among EIS parameters during growth, we used the 'Yabukita' variety, which is widespread in Japan; its growth is divided into four stages until shoot emergence. Fig. 1 illustrates the numbering of the leaves on the shoot. The first stage commenced on April 25, when a bud and one leaf (first leaf) emerged. The second stage commenced on May 3 (two leaves), the third on May 9 (three leaves) and the last on May 15 (four leaves). At each stage, thirty tea shoots were collected from the tea bushes. The tea shoots were placed in plastic bags immediately after sampling and transported to the laboratory.

To clarify the relationship between the EIS parameters and dry matter content for growth assessment, tea shoots belonging to the Meiryoku, Saemidori and Okumidori varieties were plucked between May 4 and May 8 from different divisions in the experimental field. The leaves (n = 40) from each shoot were examined.

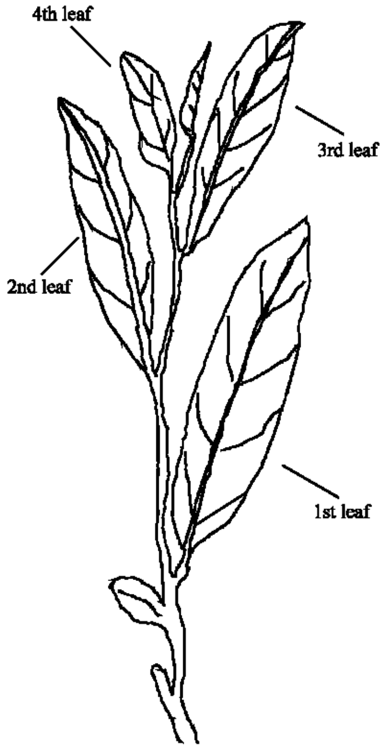

Fig. 1: Numbering of the tea leaves in a new shoot

Determination of the dry matter content in the leaf: For measuring the dry matter content of a tea leaf at each position (Fig. 1), four leaves present at the same position in different shoots were subjected to microwave heating for 12 min at 400 W. These measurements confirmed that the dry matter content in the tea leaves as determined by microwave heating was similar to that determined by the standard method, which involves heating in an oven at 105°C for 24 h. The determination was repeated in triplicate and then the average and standard deviation (s.d) were calculated.

Electrical impedance measurement of the leaf: A rectangular section (5×10 mm) dissected from the central position of each leaf was used for EIS analysis. The EIS spectra were measured using two Ag/AgCl electrodes (homebuilt) connected to an impedance meter (3532-80, Hioki, Tokyo, Japan). The Ag/AgCl electrodes were kept in contact with the samples using a conductive paste (of the type commonly used for electrocardiograms) to maintain minimum electrode/tissue interface polarization. Further, the device was calibrated by using OPEN/SHORT circuit correction to eliminate the polarization impedance of the electrode-paste surface. The real and imaginary

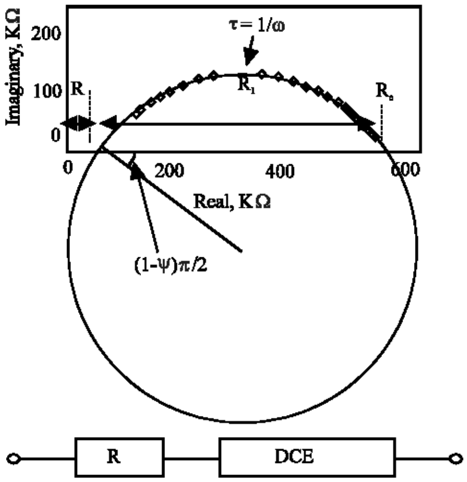

Fig. 2: Determination of the distributed model parameters of an Impedance spectrum of tea leaf (◇) (schematic). Real part of impedance on the x-axis and imaginary part of impedance on the y-axis. Frequency increases from right (0.5 kHz) to left (500 kHz). Resistances (R, R_e and R_1) of the equivalent model are obtained according to the intersections of the circles with the x-axis. The centre of the circles is below the x-axis i.e., depressed centre defined by the parameter ψ, which is the distribution coefficient of the relaxation time. Relaxation time τ is obtained from the apex of the circles

values of impedance were then measured within a frequency range of 0.5 to 500 kHz at 25 frequency points. The input voltage of the signal was 50 mV (rms). Each measurement from different shoots was repeated a minimum of five times and then the average and s.d. were calculated.

Modelling of impedance in the leaf: To utilize the data obtained by EIS analysis, the plant tissue being tested must be represented by an equivalent electrical circuit. Impedance analysis in plants is performed using mainly two types of equivalent circuits i.e., lumped[3-7, 9, 10-12, 16, 20, 21] and distributed models[8, 13-15, 17-19]. In this study, the values obtained for the real and imaginary parts of the impedance by EIS were fitted to the models described by the distributed circuit in Fig. 2. The model used was a single distributed circuit element (DCE) in series with a resistor. The impedance of the single arc (ZARC) is defined as follows[8, 13-15, 17-19]:

$$Z_{ZARC} = \frac{R_1}{1 + (i \cdot \tau \cdot \omega)^{\psi}} \tag{1}$$

where, Z is impedance; R_1, resistance; ω, angular frequency; i, imaginary unit; τ, relaxation time and ψ, the distribution coefficient of the relaxation time. By increasing the ψ value, the impedance spectrum approaches a symmetric arc (attained when ψ = 1).

A low frequency current may not cross the cell membranes; instead it flow into the apoplastic space. Therefore, the extracellular resistance (R_e) is calculated when ω is 0, as shown in Fig. 2. At high frequencies, the current may cross the cell membranes and accordingly, it flows into both the apoplastic and symplastic spaces. Intracellular resistance (R_i) is calculated as follows:

$$R_i = R\left[1 + \frac{R}{R_1}\right] \tag{2}$$

The resistance parameters were normalized with respect to the cross-sectional area and length of the sample. The specific resistances are obtained using the following Eq:

$$r = \frac{R \cdot A}{l} \tag{3}$$

where, R is the estimated resistance; A, the cross-sectional area of the sample and l, the length of the sample. τ and ψ do not need to be normalized.

Individual parameters of the equivalent circuits were estimated by using the method of non-linear least squares curve-fitting program using Microsoft Excel.

RESULTS AND DISCUSSION

To clarify the differences among the EIS spectra during growth, the real and imaginary parts of the impedance of the first leaf at each stage are illustrated in Fig. 3. The impedance spectrum increases as growth progresses. At high frequencies (left in Fig. 3), the real part of the impedance at each stage appeared to be identical. Besides, at low frequencies, the real part of the impedance increased with growth (right in Fig. 3). Fig. 4 shows the changes in the EIS parameters and dry matter content in the leaf with growth. The estimated relative standard deviation (RSD = (s.d./average)×100) of r_e, r_1 and r_i remained below 23%. Additionally, the RSD values of ψand τ/ remained below 9%. This might be because normalization of ψand τ/ is not required. The parameters of r_e, r_1 and τ increased with growth, whereas r_i was greatest at the beginning of growth and then tapered off. ι remained comparatively constant; it ranged from 0.55 to 0.64 during growth. In this experiment, the RSD values of r_e and r_1 remained higher than that of τ. It took less than 1 h to perform the measurements after plucking. During the measurement, we confirmed that the EIS parameters did not change within the range of 23% RSD. Therefore, τ appeared to predict the growth of the leaves rather than the parameters r_e and r_1. The dry matter content in the leaf increased as growth progressed. During growth, the dry matter content in the first leaf increased from 19.8 to 27.5% i.e., the water content decreased from 80.2 to 72.5%. We observed that the water content did not change within the range of 3% RSD during the measurements.

To clarify the relationship between the EIS parameters and dry matter content in the leaf, individual data (n = 50)

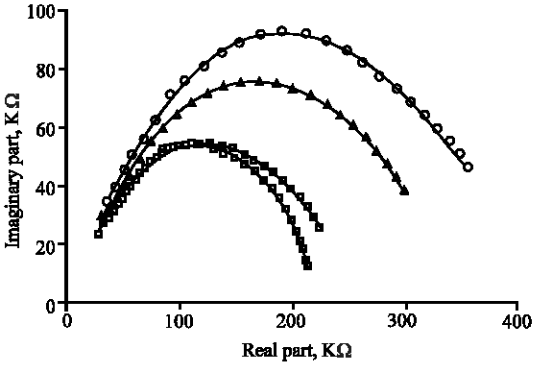

Fig. 3: Typical impedance spectra of the first leaf during growth. Symbols: ♦ =/April 25, /= May 3, /= May 9 and/= May 15. Frequency increases from right (0.5 kHz) to left (500 kHz)

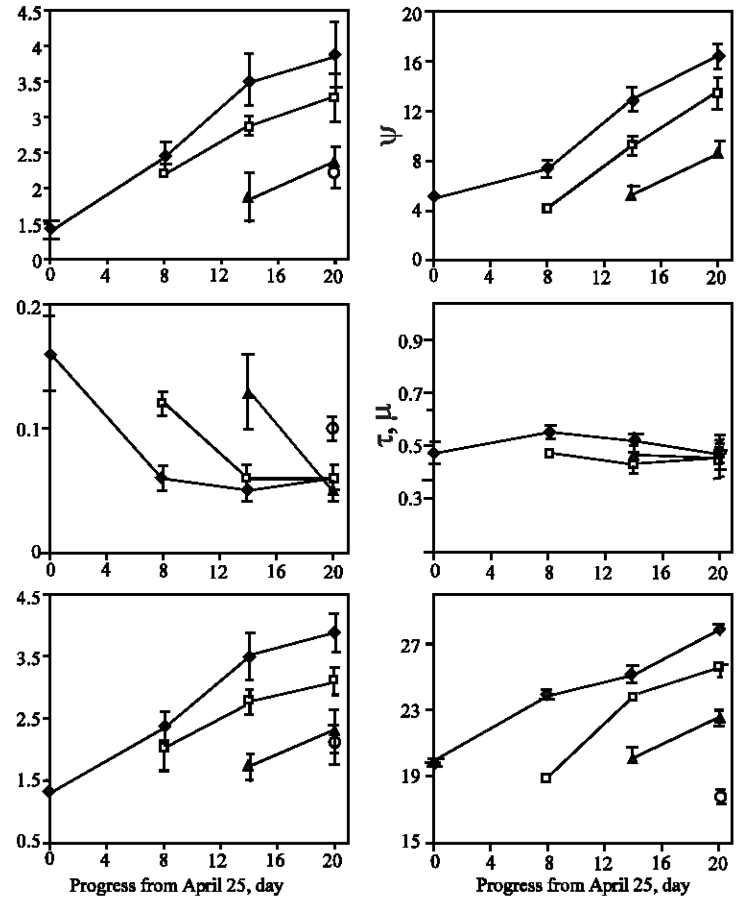

Fig. 4: Changes in the parameters for a single-DCE model of the tea leaf by impedance analysis and dry matter content in the leaf. Symbols: ♦ =/1st leaf,/= 2nd leaf,/= 3rd leaf and/= 4th leaf. The parameters r_e, r_i and r_l are resistances, ι is the distribution coefficient of the relaxation time (τ). The progress from April 25 is shown on the x-axis. Each point is the mean of 5 measurements and the bars indicate standard deviation

included the Yabukita, Meiryoku, Saemidori and Okumidori varieties that were used for Multiple Linear Regression (MLR) analysis of the response surface model by using Microsoft Excel. The variance ratio and probability (P) value were calculated using this model. The MLR results provide extensive information. In particular, the parameter with a higher variance ratio is considered to be more significant than that with a lower variance ratio. Table 1 listed the variance ratio and P value obtained by using the model and the coefficient of determination R^2 of the linear regression model, which was obtained by comparing the EIS parameters and dry matter content in the leaf. τ displayed a good correlation ($R^2 = 0.79$) with the dry matter content in the leaf. Further, τ with a variance ratio of 16.53 ($p<0.001$) had the highest value among the parameters investigated in this experiment. The other EIS parameters had no significant correlation with the dry matter content in the model. It was clarified that τ had a good correlation with the dry matter

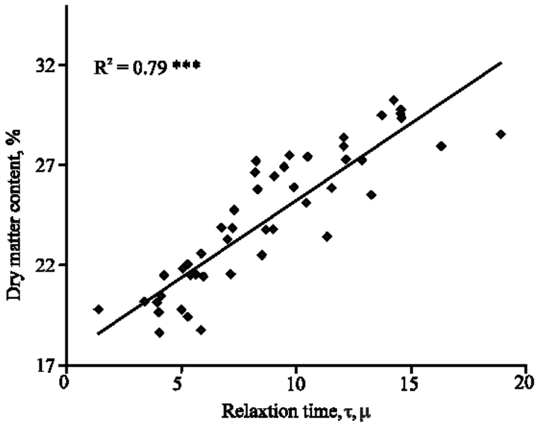

Fig. 5: Relationship between the relaxation time (τ) and dry matter content in the leaf (n = 50). R^2 is the coefficient of the determination and *** indicates $p<0.001$

content Fig. 5. Since the dry matter content in the leaf is used to assess the growth, τ would be useful for growth assessment.

τ increased as growth progressed and it had a good correlation with the dry matter content in the leaf. In mathematical terms, τ is obtained from the apex of the frequency arc of the impedance spectrum (Fig. 2). Using the normalized values for r_1 and capacitance (c) we can calculate the relaxation time τ as follows[18]:

$$\tau = r_1^{\frac{1}{\psi}} \cdot c \tag{4}$$

The change in ion mobility in a cellular compartment is represented by r_1 and at an interface in the tissue, it is represented by c. The c remained constant ranging from 0.30 to 0.54n F cm^{-1}, as calculated from the results in Fig. 4. τ increased with the increase in r_1 and they were linearly correlated (data were not shown). Further, ψ remained comparatively constant Fig. 4. Therefore, from Eq. 4, it was clear that the change in τ was due to r_1 during growth. Our results showed that both r_e and r_1 increased with growth Fig. 4. The increase in r_1 is due to a decrease in the charge-carrying ion content inside for outside the cell[17,18]. In addition, r_e is dependent on the extracellular air space; in tissues with ample air space r_e would be high and in wet layers between cells it would be low[6]. r_e of *Brassicea oleracea* leaves also tended to be higher at the later developmental stages[7]. Hence, the change in r_1, which contributed to τ, would be dependent on the condition of the extracellular space and the change in the charge-carrying ion content inside for outside the cell during growth.

According to the MLR results, τ had a good correlation with the dry matter content, but r_e and r_1 were not significant. Although this might suggest that the values of the resistances and capacitance were dependent on the varieties, age of the tea bush and the cultivation methods, interestingly, the τ was due to the dry matter content, i.e., growth. The changes in the EIS parameters would be associated with the concomitant changes in cellular properties e.g., cell wall thickness and rigidity, the size of the apoplastic space, membrane fluidity and symplastic compartmentalization[8,9,13,15,17-19]. In this experiment, these changes may partly explain the increase in the EIS parameter of τ, which occurred with the increase in dry matter content i.e., growth.

CONCLUSION

In conclusion, this was first time that EIS was applied to identify parameters to predict the growth of fresh tea leaves. As growth progressed, τ increased due to the increase in r_1. The change in r_1 was due to the condition of the extracellular space and the change in the charge-carrying ion content inside for outside the cell during growth. The result of MLR analysis of the response surface model reveals that τ of the electrical impedance arc of tea leaves would be useful for understanding their growth.

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