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Frost hardiness accuracy assessment by electrical impedance spectroscopy in *Catalpa* spp. during frost hardening

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ABSTRACT

Three species of *Catalpa* spp. were used to measure soluble sugar content and membrane permeability as well as electrical impedance spectroscopy (EIS) of stems during frost hardening. The relations between parameters of the EIS and corresponding soluble sugar content and membrane permeability were analyzed respectively. The comparatively satisfying correlated EIS parameters were selected for estimating frost hardiness of stems. Also the conventional conductivity method was applied to estimate frost hardiness of stem in three species of *Catalpa* spp.. The most outstanding parameter of the EIS was chosen by comparison of these two estimation frost hardiness methods, for the purpose of testing the accuracy of frost hardiness assessed by means of the EIS method. Those study results reveal: 1) During frost hardening, soluble sugar content and membrane permeability increased; 2) Shapes of impedance spectra were changed in different species of *Catalpa* spp. stem; 3) The EIS parameters of specific low-frequency resistance r_1 and specific extracellular resistance r_e had significant positive correlations with soluble sugar content as well as membrane permeability. Among them r_1 rank on the top for frost hardiness of stem with estimation accuracy of 81.83%. In conclusion, the EIS data can be used to assess frost hardiness of stem in three species of *Catalpa* spp. with greater reliability.

KEYWORDS

Catalpa spp.; Frost hardiness; Accuracy; Electrical impedance spectroscopy; Soluble sugar; Membrane permeability.

INTRODUCTION

Catalpa spp. is a precious high-quality timber species and famous ornamental tree species because of the excellent material quality and beautiful tree form[1,2]. It is cultivated in subtropical regions of hemisphere with warm temperature. *Catalpa* spp. growth and breeding are limited by low temperature mainly during winter, sometimes in early spring and later fall. These low temperatures are common in northern hemisphere. In order to select the premium frost hardy resource of *Catalpa* spp., it is necessary to establish fast and objective methods to evaluate frost hardiness (FH).

There are several physiological, biochemical and biophysical changes in cells and tissues during frost hardening, as well as the changes of FH of the plants[3-5]. The current FH assessment method is time-consuming which requires expensive equipment, and a considerable amount of material. The EIS is considered as a new fast and effective technique for studying the structure of organic and inorganic material, which can get the changed information of physicochemical properties of cells without destroying it. Recently the EIS has been developed in many fields[6-8]. The studies showed that the EIS parameters can not only assess the FH of plant effectively, but also correlate significantly with FH[9,10]. In the end of frost hardening, however, the FH is underestimated by the EIS than by the other methods[11,12]. Most studies focus on the correlation between the EIS parameters and the FH assessed by electrolyte leakage (EL). However, data is still scarce in the accuracy of FH.

The aim of the present research was to study the accuracy of FH assessed by means of the EIS method. We select the better correlated EIS parameters through the verification of accuracy, which can complement and improve the EIS method to assess FH of plant.

MATERIALS AND METHODS

Plant material

The current-year shoots were sampled in a one-year provenance field trial with three *Catalpa* spp. at Agricultural University of Hebei in Hebei province (N 38°50′, E 115°26′, elevation 25 m, China). Those three *Catalpa* spp. were *Catalpa fargesii* f. *duclorxii* of Yunnan (N 98°51′, E 25°01′, elevation 1640 m, China), *Catalpa fargesii* of Gansu (N 107°88′, E 36°03′, elevation 1026 m, China) and *Catalpa ovata* of Henan (N 114°02′, E 32°83′, elevation 88 m, China). In each of the three provenances, 10 grafting plants were selected with the same treatment of container and soil. Measurements of the current-year shoots were carried out from 20 October, 2009 to 20 January, 2010. Samples from 10 grafting plants of each provenance were taken at one-month intervals. A total of four measurements were used.

Impedance analysis of non-frost-exposed shoots

The electrical impedance spectra of the 8 shoots from each provenance at each sampling date were measured in laboratory immediately after sampling. A 15-mm section was cut from the middle of the shoot samples. The impedance spectra were measured as described by Repo et al[9]. The sample placed in direct contact with the electrode paste and the Ag/AgCl electrodes (RC1, WPI Ltd, Sarasota, USA) was set in contact with the paste. The impedance spectrum was measured at 42 frequencies between 80 Hz and 1 MHz (HP 4284A LCR meter, Agilent Technologies, USA). The input voltage level of the sine signal was 100 mV (r.m.s.).

The parameters of the distributed circuit model (single-DCE) were estimated by means of a complex non-linear least squares (CNLS) fitting program, which uses Cole-Cole model. The parameters were fetched as described by Zhang et al.[8], which included specific high-frequency resistance r, specific low-frequency resistance r_l, specific extracellular resistance r_e, specific intracellular resistance r_i, relaxation time τ and distribution coefficient of relaxation time ψ.

Determination of memerane permeability

A 10-mm section was cut from the middle of the shoot samples. Electrolyte leakage (EL) was measured as described by Zhang et al.[8]. The samples were shaken at room temperature for 24 h before the first conductivity measurement (C_1). The conductivity measurement of control (deionized water only) was C_{01}. The samples were killed at 100 ℃ for 20 min and shaken for another 24 h before the second conductivity measurement (C_2), and the conductivity measurement of control is C_{02}. The relative EL was defined as equation 1:

$$EL=\frac{C_1-C_{01}}{C_2-C_{02}}\cdot 100\% \qquad \textbf{(1)}$$

Determination of soluble sugar concentration

Soluble sugar concentration was determined with anthrone colorimetric assay. Determination of each treatment was repeated four times[13].

Measurement of FH

Controlled freezing tests

The shoots of four freezing tests were rinsed three times by tap water and three times by deionized water to remove surface pollutants. Every plastic bag was put into six branches of sample with a little deionized water in order to avoid excessive super cooling. One sample bag of each provenance was exposed in each of seven temperatures (TABLE 1). The freezing temperatures included temperatures that killed the samples and temperatures that caused no damage. The rate of cooling was 6 ℃· h^{-1}. The samples were kept at the target temperature for 4 h and then moved into 4 ℃ to thaw gradually for 24 h. Immediately after the freezing test, the degree of frost damage in the shoots was quantified by the EIS and the EL method, respectively.

TABLE 1 : The temperatures used for determining FH in four controlled freezing tests

Date	Temperature /℃						
20 Oct.	4	-3	-6	-10	-15	-20	-30
20 Nov.	4	-6	-13	-20	-25	-35	-45
20 Dec.	4	-8	-14	-22	-30	-38	-46
20 Jan.	4	-8	-16	-24	-34	-46	-72

FH assessed by EIS and EL

EIS: After freezing test, eight shoots were used in each provenance and each freezing temperature. To obtain the frost hardiness, the EIS parameter was modeled by a logistic sigmoid function (in equation 2) with respect to the treatment temperature:

$$y = \frac{A}{1+e^{B(C-x)}} + D \quad (2)$$

where y is one of EIS parameters, x is treatment temperature, B is slope at inflection point C, C is frost hardiness and A and D determine the asymptotes of the function.

EL: After freezing treatment, four shoots from each freezing temperature for each provenance were selected for the EL test. To obtain the frost hardiness, the relative conductivity was modeled by a logistic sigmoid function (in equation 2) with respect to the treatment temperature: where y and x refer to the EL and the exposure temperature, respectively, A and D define asymptotes of the function, and B is the slope at the inflection point C.

RESULTS

Soluble sugar concentration and membrane permeability of stems

The soluble sugar concentration of stems of *Catalpa* spp. was increasing during frost hardening except *Catalpa fargesii* f. *duclorxii* in Jan., and differed significantly among three species of *Catalpa* spp. between Jan. and Oct. ($P<0.05$) (Figure 1A). Sugar concentration of *Catalpa fargesii* differed significantly from *Catalpa ovata* in Oct. and from *Catalpa fargesii* f. *duclorxii* in Jan. There were no significant difference between three species of *Catalpa* spp. in Nov. and Dec. ($P>0.05$).

The membrane permeability of stems of three species *of Catalpa* spp. followed a similar pattern as soluble sugar concentration of stems except that significant difference was found in Jan. ($P<0.05$) (Figure 1B). The membrane permeability of *Catalpa fargesii* f. *duclorxii* differed significantly from *Catalpa ovata* and *Catalpa fargesii* in Jan., and there were no significant difference between three species *of Catalpa* spp. in Oct., Nov. and Dec..

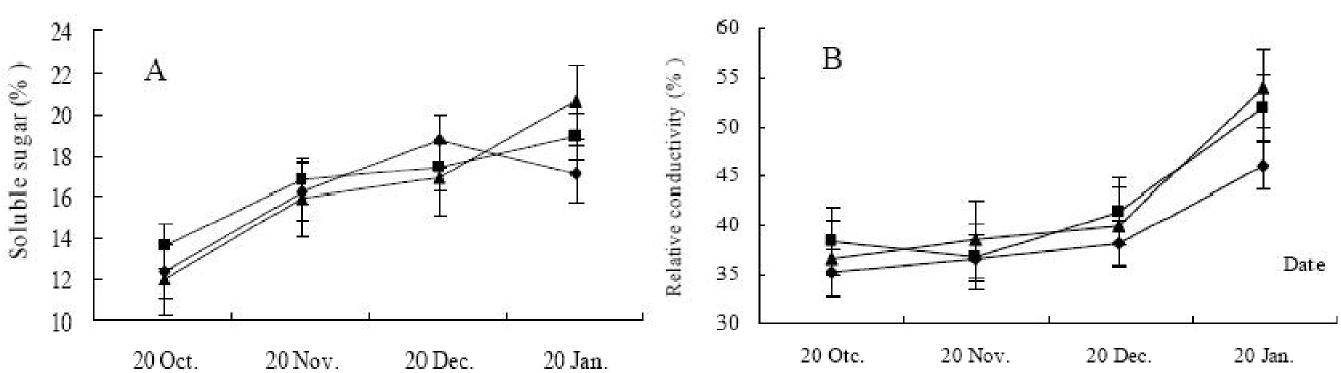

Figure 1 : The variation of soluble sugar (A) and membrane permeability (B) of different *Catalpa* spp. stems during frost hardening; ◆ *Catalpa fargesii* f. *duclorxii* ▲ *Catalpa fargesii* ■ *Catalpa ovata*

EIS and EIS parameter analysis of stems

Shapes of EIS of stem were changed in different species of *Catalpa* spp. and different period during the frost hardening, which could demonstrate changing in tissue structure and physiological and biochemical change in cells. The spectra of three species of *Catalpa* spp. were clearly characterized by single arc (Figure 2). The arc of *Catalpa fargesii* f. *duclorxii* was smaller than that of *Catalpa fargesii* and *Catalpa ovata* during frost hardening (Figure 2A-D), however, the arc of *Catalpa fargesii* was bigger than that of *Catalpa ovata* in Nov. and Dec. (Figure 2B and C) and the arc of *Catalpa ovata* was bigger than that of *Catalpa fargesii* in Oct. and Jan. (Figure 2A and D). With frost hardening the arc of *Catalpa fargesii* f. *duclorxii* became bigger in early stage and then smaller later, the maximum value of top arc was -10.24 kΩ in Oct. and the minimum value of top arc was -18.55 kΩ in Jan.. During frost hardening, the arc of *Catalpa fargesii* became bigger, the maximum value of top arc was -25.91 kΩ in Oct. and the minimum value of top arc was -106.08 kΩ in Jan., while the arc of *Catalpa ovata* became smaller in early stage and then bigger later, the maximum value of top arc was -29.80 kΩ in Nov. and the minimum value of top arc was -124.76 kΩ in Jan..

All of the parameters of *Catalpa ovata* increased during the frost hardening except distribution coefficient of relaxation time ψ. It had a sharp rise in Jan. (Figure 3A-E). In Jan. the value of *Catalpa ovata* was higher than that of *Catalpa fargesii* and *Catalpa ovata* in r, r_i, τ, and ψ, but it was lower than *Catalpa fargesii* in r_l and r_e (Figure 3B and C). EIS parameters values in *Catalpa fargesii* and *Catalpa ovata* were higher than those of *Catalpa fargesii* f. *duclorxii* in all parameters (Figure 3A-F), and differed significantly in r_l, r_e and τ in Jan. (P<0.05). The trend of ψ was similar for three species of *Catalpa* spp., which was higher for parameter values in Nov. and Dec. and lower in Oct. and Jan..

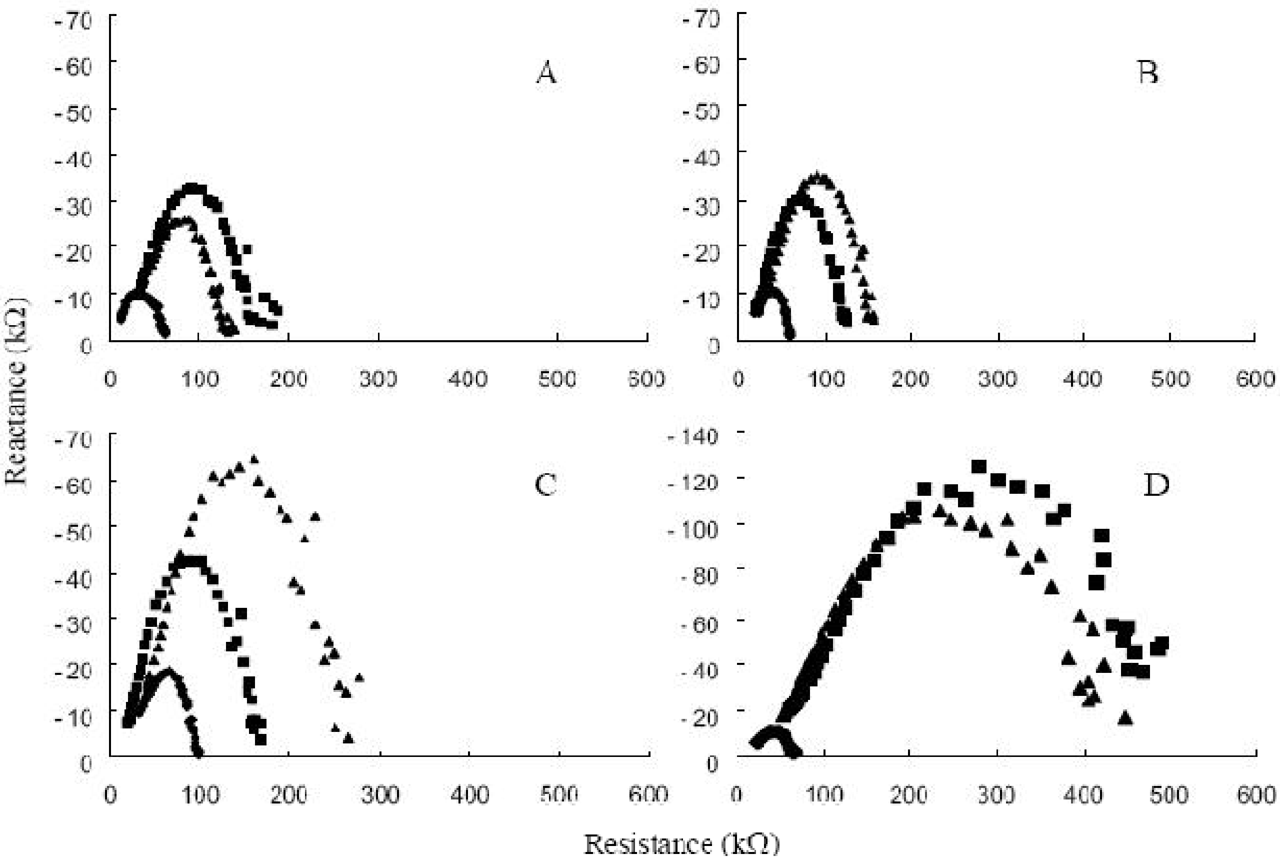

Figure 2 : Impedance spectra of stems of different *Catalpa* spp. during frost hardening; A: The date of 20 Otc., B: The date of 20 Nev., C: The date of 20 Dec., D: The date of 20 Jan.); The spectra were the pooled data of each month and composed of 42 different frequencies ranging from 80 Hz to 1 MHz (from right to left, respectively); ◆ *Catalpa fargesii* f. *duclorxii* ▲*Catalpa fargesii* ■ *Catalpa ovata*

Correlation between EIS parameter and soluble sugar concentration as well as membrane permeability

Some EIS parameters and soluble sugar concentration as well as membrane permeability are correlated. The parameters of r_1 and r_e had significant positive correlations with soluble sugar as well as membrane permeability. The coefficient of determination (R^2) between r_1 and soluble sugar concentration as well as membrane permeability was 0.621 and 0.823, respectively. The coefficient of determination (R^2) between r_e and soluble sugar concentration as well as membrane permeability was 0.624 and 0.828, respectively (TABLE 2).

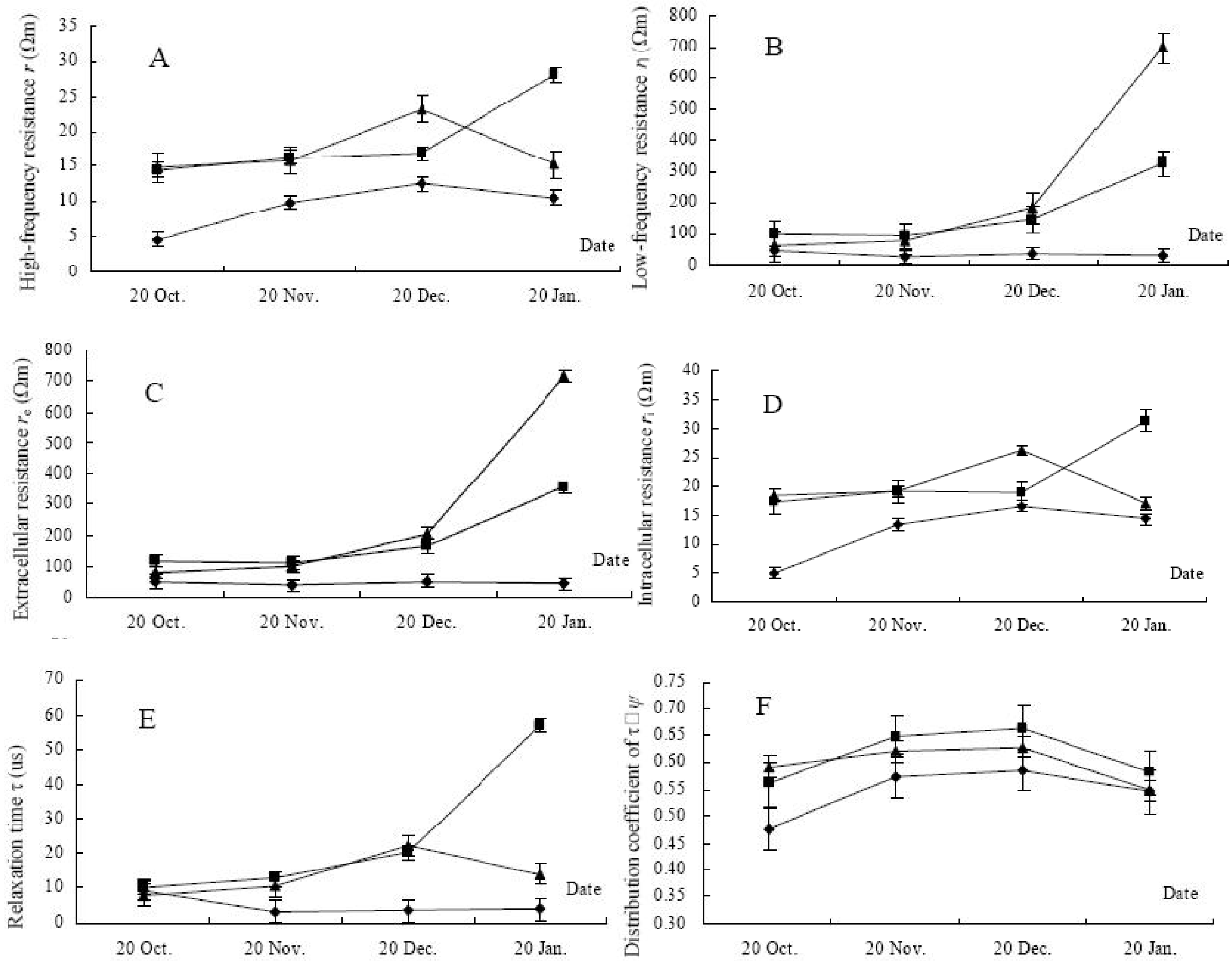

Figure 3 : EIS parameters of stems in different *Catalpa* spp. during frost hardening; ◆ *Catalpa fargesii* f. *duclorxii* ▲ *Catalpa fargesii* ■ *Catalpa ovata*

TABLE 2 : Correlation between EIS parameters and soluble sugar concentration as well as membrane permeability in stems of *Catalpa* spp. during frost hardening

EIS parameters	Soluble sugar concentration	Membrane permeability
Specific high-frequency resistance r	0.435	0.484
Specific low-frequency resistance r_l	0.621*	0.823*
Specific extracellular resistance r_e	0.626*	0.828*
Specific intracellular resistance r_i	0.439	0.454
Relaxation time τ	0.349	0.566
Distribution coefficient of relaxation time ψ	0.264	-0.105

*** means that correlation is significant at the 0.05 level, and for stem, n=36.**

FH assessed by EIS parameters

The sugar concentration and membrane permeability were reliable physical signs to assess the FH of plants. So it is more reliable for EIS parameters to assess FH of *Catalpa* spp. as a result of significant correlation between EIS parameters of r_1, r_e and soluble sugar concentration as well as membrane permeability. The FH of each *Catalpa* spp. assessed by r_1 were similar to that assessed by r_e during frost hardening except Jan. (TABLE 3). The FH of each *Catalpa* spp. was significant different between two methods in Jan. (P<0.05).

TABLE 3 : Comparison of FH in stems of different *Catalpa* spp. assessed by EIS parameters

Date	*Catalpa fargesii*		*Catalpa ovata*		*Catalpa fargesii* f. *duclorxii*	
	r_l FH/℃	r_e FH/℃	r_l FH/℃	r_e FH/℃	r_l FH/℃	r_e FH/℃
20 Oct.	-10.368a	-10.359a	-11.383a	-12.051a	-9.848a	-9.806a
20 Nov.	-19.781a	-19.715a	-22.104a	-22.078a	-31.655a	-34.306a
20 Dec.	-22.173a	-22.426a	-38.006a	-38.393a	-21.331a	-21.637a
20 Jan.	-24.728a	-12.247b	-28.298a	-16.217b	-27.451a	-29.776b

Accuracy test of FH assessed by EIS

The FH of three species of *Catalpa* spp. assessed by r_1 and r_e was tested by EL. The results showed that FH results of EL correlated well with FH results assessed by r_1 and r_e. The coefficient of determination (R^2) of three species *Catalpa* spp. was over 0.5, and root mean square error (RMSE) and relative error (RE) were lower. The parameter r_1 was the best to assess the FH with R^2 = 0.80, RMSE = 3.815, RE = 18.17%, and the assessed accuracy was 81.83% (TABLE 4).

TABLE 4 : The fit evaluation indicators of the FH measured values by the relative conductivity (x) and EIS parameters (y) during frost hardening

EIS parameters	Regression equation	R^2	RMSE	RE (%)
Specific low-frequency resistance r_l	y = 0.9413 x - 0.479	0.80	3.815	18.17
Specific extracellular resistance r_e	y = 0.8473 x - 1.1443	0.55	6.629	26.64

DISCUSSION

EIS can provide information for basic physicochemical properties of cells, tissues and organs[14]. In the present study, during frost hardening the sugar concentration and membrane permeability are increased. The EIS and EIS parameters have corresponding changes, which can be an indication of changes in physicochemical properties of cells. This is in accordance with previous studies[9]. The membrane permeability is increased because of low temperature, which leads to increase of electrolyte exosmose and concentrations of intercellular substance. The *Catalpa* spp. would increase the sugar concentration through osmoregulation to enhance the concentration of cell sap and the capacity of water in order to improve the FH, thus the impedance of tissues and organs has changed. Electric potential difference can be kept by effective transport system and alternative infiltration characteristics would create when current through the cell membrane. Thus, the EIS characteristics can show the change of impedance of extracellular resistance and intracellular resistance because of change of the concentration of cell sap and membrane permeability.

The soluble sugar concentration had significant positive correlations with FH[15]. The identification of FH by membrane permeability accorded with that of field performance[16,17], so the soluble sugar concentration and membrane permeability are often used as identifying signal of FH of plants. In agreement with Ryyppö et al.[12], the soluble sugar concentration and membrane permeability are closely related with FH, and those of stems of *Catalpa* spp. are significant positive correlation with r_1 as well as r_e. The changes of EIS parameters reflect changes of the soluble sugar concentration and membrane permeability and can also assess the FH of *Catalpa* spp.. These studies show the r_1 is the best EIS parameter for assessment of FH and have the better accuracy compared to other parameters. The FH assessed by r_1 is reliable to be used in *Catalpa* spp..

In conclusion, EIS is a new approach to assess the FH of plants and to filter the plant resource with high FH. The study confirmed the rule of change of the soluble sugar concentration as well as membrane permeability of three species of *Catalpa* spp. and the best EIS parameter on assessment of FH. All of these studies will improve the application of EIS technology on assessing FH.

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