

Assessing electrical impedance spectroscopy as a non-invasive method in plant tissue



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ABSTRACT

Electrical impedance spectroscopy (EIS) has provided a non-invasive methodology of characterizing the electrical properties (resistance, capacitance) of many systems, including biological tissues. It elaborates on the structural-functional studies of physiological events, biochemical cross talk's, plant soil-root interactions, root size, leaf water relations, and fruit quality characteristics. In EIS measures, the plasma membrane acts as an electrical insulator that controls the movement of ions and electrolytes across the cells. An electric current under an applied voltage on the tissue flows through the cell walls and from cell to cell and in the fluids within the conducting elements giving rise to electrical impedance. The magnitude of the impedance and its phase angle can be measured at multiple frequencies using a precise impedance analyzer. The fundamental concepts of electrical impedance spectroscopy are re-examined and a brief review is given of the role that EIS has played in the development of our understanding of cellular and synthetic membranes, cell biophysics and ionic systems in biological tissues. Inside the tissue, ions are the main current carriers that demonstrate the total impedance. The symplastic and apoplastic resistance form a parallel impedance circuitry at a given frequency. A description is given of a new computer-controlled, four-terminal digital impedance spectrometer and measurements were carried out in the range 1Hz-1MHz with 10 points per decade and a 100mV applied voltage at 10-day period at various aluminum (Al) concentrations (0, 15, 30, 45 and 60 mg/l), in the desert shrub, *Calotropis Procera*. We explained the impedance dispersions in terms of Nyquist graphs that demonstrate the complex impedance of *C. Procera* stem via arcs of different peaks. Nyquist graphs of biological tissues are composed of one or two arcs in the complex plane, depending on the sample under study and the range of frequencies used. The parameters of the best fitting circuit are estimated using an optimization technique. To analyze and interpret experimental data, an equivalent circuit model should be used. The spectra and changes that occur as a result of perturbations to the system can be readily assessed and interpreted. This paper reviews EIS theory, instrumentation, summarizes its application, model validation and data assimilation in the model plant *Calotropis Procera* under aluminum stress.

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1. Introduction

Electrical Impedance Spectroscopy (EIS) is a non-destructive method, are gaining significant attention among the scientific community due to their global application at field and laboratory scale and capacity to provide pertinent information on plant health, biochemistry and plant physiology via measuring the electrical impedance. EIS technique has been developed to measure the electrochemical status (resistance and capacitance) of plant tissues. EIS has been used in medical sciences (analysis and responses given by different human body tissues, body mass index calculation, and nutritional and hydration status evaluation. Several workers demonstrated EIS application in plant stress physiology, e.g. evaluating electrophysiological status of potato tubers and carrot roots (Zhang and Willison, 1991), impedance response of Scots pine needles (Zhang and Willison, 1992), detection of damaged tissue in bruised apples (Jackson and Harker, 2000), fruit biochemical properties, pH, sugar content, ripening (Liu, 2006), willow root system (Cao et al., 2010), water status in tomato (He et al., 2011), phosphorus and potassium deficiencies detection in *Trifolium subterraneum* (Greenham et al., 1982). However, there are few studies that have used EIS

for evaluating heavy metal stress in plants. EIS measurements are non-invasive, fast and less sensitive to environmental variables and can monitor a variety of physiological and biochemical processes at a cellular level. Since the applications of the EIS technique on intracellular biochemical responses have not been fully researched, therefore, the present article will evaluate aluminum stress on electrical impedance characteristics in a desert shrub, *Calotropis procera* and propose new approaches and equivalent circuit models for determination of plant stress physiology. If the process of plant growth and development can be monitored in a predictable manner, then the extracellular and intracellular properties can be demonstrated using this technology. Data assimilation, selection of the suitable equivalent electrical model and using appropriate impedance software to obtain the best electrical data fitting is performed in order to exploit and highlight the entire process of electrical impedance spectroscopy.

Here, we discuss and summarize the status of different biological tissues under abiotic/biotic stress that measured through typical impedance-based techniques.

2. Plant biotic and abiotic/biotic stress and electrical impedance spectroscopy

2.1. Plant phenotypic plasticity

Cao et al. (2010) used electrical impedance spectroscopy (EIS) for hydroponically raised willows (*Salix schwerinii*) to

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estimate the root system size and area. The lumped model parameters were correlated with the contact area of the roots and/or stems raised in the hydroponic solution. Dietrich et al. (2012) found that the capacitance of barley (*Hordeum vulgare*) appeared to be determined, not by the mass of their root system, but by the cross-sectional area of roots at the solution surface. They observed that capacitance was not linearly related to the mass of roots in solution when root systems were partly submerged and (ii) that excising the root below the solution surface had a negligible effect on the capacitance measured. Several researchers have reported the correlations between EIS and root mass (Tsukahara et al., 2009). A form of single frequency (128 Hz) measurement, the earth impedance method, was introduced to estimate the absorbing root surface area in the field. The impedance was related to the basal area over a large range of stem diameters, which was further assumed to be related to the absorbing root surface area. Based on the single-frequency measurements, equivalent models were formulated using resistors in the case of willow cuttings with their root system raised in hydroponic systems (Cao et al., 2010). In that particular study, it was found that the resistance decreased in relation to an increase in the contact surface area of roots using the solution. The single-frequency alternating current was used to assess the capacitance or resistance of the root system since those attributes were assumed to provide a measurement of the active root surface area.

2.2. Plant water relations

Electrical impedance parameters for water uptake in *Schefflera arboricola* were used to measure the electromagnetic properties. Results showed that the capacitance of leaf and stem was periodically decreased by 51 and 0.8 PF/hr during night and increased by 62.3 and 18 PF/hr during the day respectively and its resistance increased by 3.6 K Ω /hr at night and decreased by 92.3 K Ω /hr during the day (Sinha and Tabib-Azar, 2015). Greenham (1966) observed that electrical impedance in the crown tissue of alfalfa was decreased during freezing. In earlier, the impedance analytical approaches were used to study the physiological status of the plant cell (Zhang and Willison, 1992). de León et al. (2010) exhibited that scheduled irrigation based on the trunk shrinkage signal intensity was not always possible due to the temporal changes in the reference values that occurred as trees aged.

2.3. Evaluation of metal and salt toxicity through impedance

Jócsák et al. (2010) used an electrical impedance technique to study the effect of flood and cadmium stress on Pea roots. They concluded that EIS can be used for detecting structural changes in plants caused by environmental stresses. Moreover, sudden flooding encouraged the growth of vacuoles, which act as an electrical conductor in the cell and decreased resistance and capacitance values.

Tavakkoli et al. (2012) worked on the electro-physical response of barley to salinity under laboratory and field conditions. The results showed a significant difference in physiological response because field conditions were more stressful as compared to the laboratory due to the soil heterogeneity and the occurrence of different abiotic stresses. Other physical factors such as the availability of water and nutrients, air and soil temperatures and moisture content could also account for the observed differences. Tavakkoli et al. (2012) stated a negative correlation was observed between the resistance and the hydroponic culture condition ($R = -0.9$), while it was positive between the latter and the capacitance ($R = 0.96$). The opposite correlations were observed between sand culture conditions and both electrical impedance parameters. Under controlled greenhouse conditions, the electrical resistance was positively correlated with the treatment of plant with 100 mM

NaCl ($R = 0.85$), while its correlation with 400 mM NaCl treatment was negative ($R = -0.53$). The differences in the electrical impedance between hydroponic (liquid) and sand (solid) culture conditions could be explained by the influence of osmotic pressure which is higher in the liquid solution. Sand-grown plants were likely to have more time to adapt to the salt concentration than plants in hydroponic systems.

2.4. Environmental stresses and impedance measurements

Electrical impedance can be used to measure different environmental stress. Protocols for such evaluations have been developed. Similarly, several protocols for estimating the resultant injury such as differential thermal analysis (Mancuso, 2000), visible injury, chlorophyll fluorescence (Havaux, 1987), electrolyte leakage (Steponkus, 1984) and electrical resistance (Mancuso 2000) are currently used.

Many explorations have been directed in the field towards impedance measurements and environmental stress (Repo et al. 1997; Mancuso and Azzarello, 2002). Repo et al. (2000) revealed the relationship between frost hardiness (FH) of stems and needles with equivalent circuit EIS parameters. Repo et al. (1994) demonstrated that the distributed circuit element (DCE) model was used to calculate the extracellular resistance according to the impedance spectra. However, the intracellular resistance in plants has been increased with the frost hardening due to the increased concentration of the intracellular sap and impaired intracellular ions mobility. The equivalent circuit parameter of impedance analysis is a useful tool for studying the extracellular and intracellular resistance as well as plant physiology (Repo et al., 2004).

Mancuso et al. (2004) worked on stem impedance analysis and electrolyte leakage of leaves and shoots of ten cultivars of olive to determine heat injury. They found a significant decrease in Z ratio, together with an increase in electrolyte leakage, in all genotypes after heat treatment. In addition, Jócsák et al. (2010) revealed that resistance (R_s) of roots of hydroponically grown seedlings decreased continuously to one-fifth of the starting value over the period of our experiment. A recent study of Bazihizina et al. (2015) showed the work on intra and extracellular resistance of plants against the different concentrations of Ni^{2+} . They reported an increase in intracellular resistance at higher Ni^{2+} concentrations (1000 and 3000 μ M).

2.5. Plant water relation

Numerous researches have demonstrated the plant water relation by using electrical impedance and proved that changes occurred rapidly at the root level, through rapid systemic long-distance signals; mediate stomatal behavior. On the contrary, several studies have shown that heavy metal caused blockage of water transport from roots to the above parts of the plant (Kholodova et al., 2011). Similarly, many researcher have studied that surplus amount of Ni^{2+} impedes photosynthesis through various mechanisms, e.g., the disruption of the photosynthetic electron transport (Velikova et al., 2011), negative effects on the water-splitting site of PSII (Velikova et al., 2011) and the inhibition of photosynthetic pigment biosynthesis (Küpper et al., 1996). A summary of the application of impedance spectroscopy (EIS) in various biological systems under different abiotic and biotic stresses was discussed in Table 1.

3. Electrical impedance theory

As a general phenomenon, during the EIS measurements, an electrical voltage is applied to plant tissue. Electrical impedance determines how an applied alternating-current flow generated by an external electrical field is "impeded" by biological tissues. This caused an immediate change in the cell that showed the ionic polarization and relaxation that occurs after removing

the electric field, according to the time constant of the polarized system. The change in relaxation time with frequency from one value to another is called dispersion and is due to ionic movements. The principal mechanism of dispersion is the accumulation of charges on both sides of a dielectric (i.e. cell

membranes) (Fig. 1a). At low frequency, the current flows in the apoplasmic space of the tissues where ions are the main current carriers, which determine the total impedance. At higher frequencies, the current can flow inside the cell organelles (Fig. 1b).

Table 1
The double dispersion Cole-Cole model parameters (standard deviation in parenthesis) for stems of Calotropis Procera in the four experimental set-ups of aluminum (0, 15, 30, and 45 ppm) concentration.

	Al (ppm)	R ₁ (MΩ)	C ₁ (μF)	α ₁	τ ₁	R ₂ (MΩ)	C ₂ (nF)	α ₂	τ ₂ (10 ⁵)
Stem	0	1.00 (2.03)	1.25 (0.10)	0.686 (0.01)	0.720 (0.02)	0.037 (0.0)	0.284 (1.02)	0.591 (0.02)	1.06 (0.01)
	15	1.00 (1.20)	1.25 (0.4)	0.633 (0.03)	0.705 (0.07)	0.038 (0.001)	0.309 (0.99)	0.572 (0.09)	1.32 (0.17)
	30	8.04 (1.00)	1.11 (0.10)	0.594 (0.05)	0.025 (0.02)	0.059 (0.002)	0.413 (1.10)	0.545 (0.03)	0.609 (1.03)
	45	10.00 (1.52)	1.88 (0.22)	0.588 (0.012)	0.006 (0.0001)	0.566 (0.01)	9.45 (1.04)	0.649 (0.04)	1.08 (0.25)

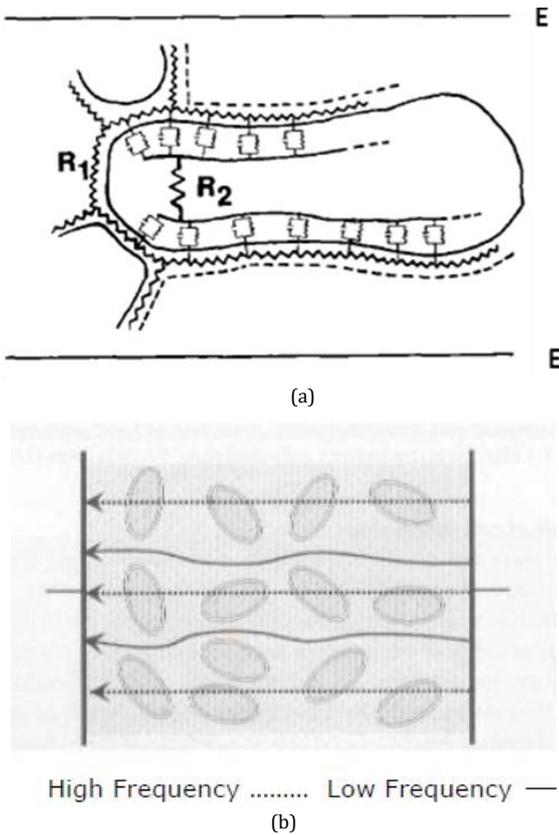


Fig. 1. (a) An illustration of Scot pine mesophyll cell and its equivalent electrical circuit. Extracellular resistance (R₁), Extracellular resistance (R₂) and electrodes (E). (b) The path of low and high-frequency current in plant tissue. High frequency has been demonstrated through dashed lines while the low frequency was represented by a solid line.

number, constituted of two components in the following equations:

$$Z(\omega) = Z_r(\omega) + jZ_i(\omega),$$

where,

$$Z_r(\omega) = |Z| \cos \theta$$

is the real component,

$$Z_i(\omega) = |Z| \sin \theta$$

is the imaginary component or reactance, and

$$\theta = \tan^{-1} \frac{Z_i}{Z_r}$$

is again the impedance phase angle. The phase angle can vary from 0 to 90°. When the angle is 0 the circuit is purely resistive and at 90° it is purely capacitive, while at 45° the circuit has an equal amount of capacitive reactance and resistance and known as the Warburg impedance. Finally, the relation between impedance and its individual component (resistance and reactance) can be represented as a vector whose magnitude is:

$$|Z(\omega)| = \sqrt{Z_r^2 + Z_i^2}$$

The real and imaginary parts of Z describe the resistance and reactance, respectively. If the real part is plotted on the X-axis and the imaginary part on the Y-axis, an impedance spectrum, using the frequency as the parametric variable.

3.2. Data collection and processing

Once the raw data from the experiment has been obtained, it is important to extract characteristic parameters to analyze the system properties. Typically, the spectrum (Nyquist plot) of plant tissues is composed of one or two arcs in the complex plane, depending on the sample under study and the range of frequencies used. However, it is imperative to mention here that there are several recent attempts to propose cheap and portable impedance measurement devices such as those reported previously. However, all “direct” impedance measurement techniques suffer from a major drawback which is the necessity of having data post-processing using a suitable software optimization (data fitting) technique. The parameters of the best fitting circuit are then estimated using an optimization technique. The freely available EIS Spectrum Analyzer software is an example of software that can do this.

3.3. Selection of an equivalent circuit

To analyze and interpret experimental data, it is best to have an equivalent circuit model that provides a representation of the

3.1. Methodology, instrumentation and measurement procedure

EIS studies the impedance response of an object under test by measuring its electrical impedance (Z) and its phase angle (θ) at several frequency points (ω_i: i = 1, 2, 3, ...) from the voltage-current data at the object surface. In EIS, a constant amplitude sinusoidal voltage or current signal is injected to the object surface at different frequencies and the boundary current or voltage are measured at each frequency to estimate the impedance using an array of electrodes attached to the object surface. A general set-up for EIS measurements is shown in Fig. 2.

If the injected signal is a voltage, the setup is known as a potentiostat setup. Here, impedance Z is the quotient of the applied voltage v(t) and the resultant current i(t):

$$Z(t) = \frac{v(t)}{i(t)} = |Z| \angle \theta$$

where θ is the phase difference between the voltage and the current. In Cartesian coordinates, impedance becomes a complex

electrical properties and represents a realistic picture of the impedance data. The choice depends on the characteristic of the system under study and on the intuition of the researcher. There can be potential problems caused by the fact that equivalent circuits are rarely unique and several circuit models can have identical or very similar impedances. The impedance spectra can be modeled by an equivalent circuit through well-known double-dispersion Cole model (Cole and Cole, 1941):

$$Z_{tot} = R_{\infty} + \frac{R_1}{1 + s^{\alpha_1} R_1 C_1} + \frac{R_2}{1 + s^{\alpha_2} R_2 C_2}$$

This model is composed of:

- R_{∞} is the very high-frequency resistance, usually very small in value.
- R_1 , C_1 , and α_1 are the parameters for the first dispersion while R_2 , C_2 , and α_2 are the parameters for the second dispersion.

It is important to note that $\alpha_{1,2}$ are unit-less and are known as the dispersion coefficients which measure the distribution of relaxation times in the material and hence its closeness to ideal capacitive behavior ($\alpha = 1$). It is also important to note that $C_{1,2}$ are pseudo capacitances.

However, there are few studies that have used EIS for evaluating heavy metal stress in plants. EIS measurements are non-invasive, fast and less sensitive to environmental variables and can monitor a variety of physiological and biochemical processes at a cellular level. If the process of plant growth and development can be monitored in a predictable manner, then the extracellular and intracellular properties can be demonstrated using this technology. Data assimilation, selection of the suitable equivalent electrical model and using appropriate impedance software to obtain the best electrical data fitting is performed in order to exploit and highlight the entire process of electrical impedance spectroscopy.

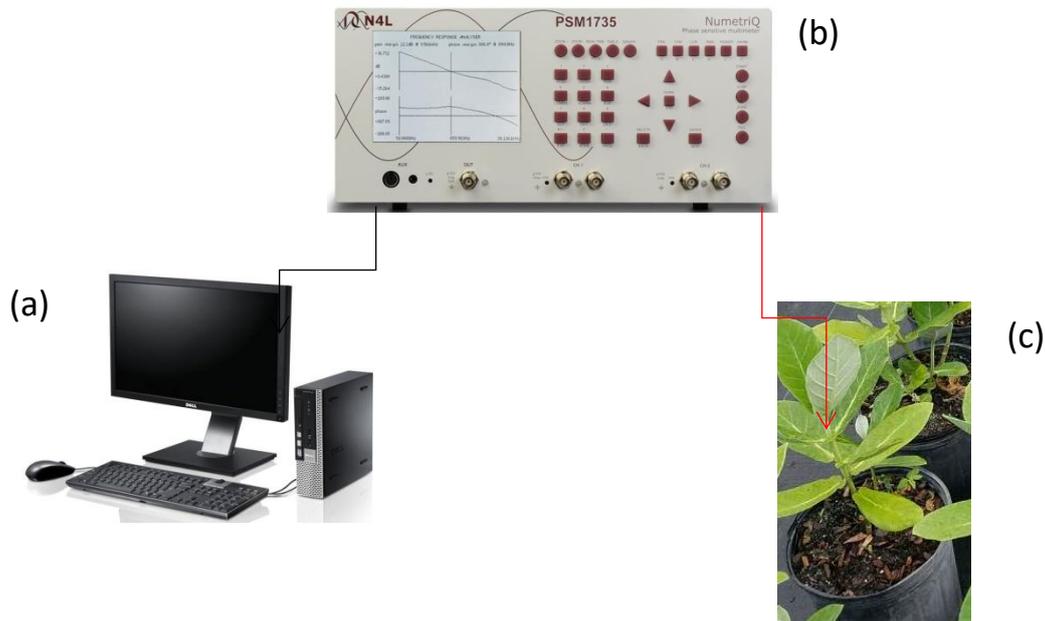


Fig. 2. An illustration to show Electrical Impedance measurements setup (a) Desktop Computer, (b) Impedance Analyzer, (c) *Calotropis Procera* plants

4. Application

EIS evaluation was carried using a precision PSM1350 Impedance analyzer (PSM 1735, Newtons4th Ltd., Leicester, UK) in the range 1Hz-1MHz with 10 points per decade and a 100mV applied voltage at a 10-day period at various aluminum (Al) concentrations (0, 15, 30, 45 and 60 mg/l). As a model plant species, the *Calotropis Procera* stem surface was cleaned using dry tissues and two copper electrodes were inserted in the plant organ at a uniform distance. The positions were kept the same throughout the measurements and the electrodes were connected with coaxial cables to the impedance analyzer. Impedance data were collected using a standard RS232 interface with the impedance analyzer operating in potentiostatic mode; i.e. the device applies a sinusoidal voltage signal and measures the induced current at each frequency.

5. Results and discussion

Nyquist graphs demonstrated the complex impedance of *C. procera* stem via arcs of different peaks following a 10-days Al treatment (0, 15, 30, 45 and 60 mg/l) (Fig. 3). For each Al conc, a complete arc was obtained at the higher frequencies (> 2 kHz to 1MHz), but a semicircle was detected at lower frequencies (<1 kHz to 1Hz). At higher frequencies, the arcs were totally overlapped, but those of lower frequencies were separated. Generally, the impedance (imaginary and real) values were

higher in control stem as compared with Al treated 45 and 60 mg/l. At the lowest frequency (1 Hz), the impedance values of stem after 10-days were higher in control than those treated with different Al conc.

5.1. EIS model

According to the measured impedance spectra and prior knowledge of the physicochemical properties and the equivalent circuit models for different components in the experimental setup, a double-dispersion Cole model was formulated and applied for these measurements. To validate the Nyquist plot curves with the double dispersion Cole-Cole model, the data from the stem following 10-day period was computed at Al concentrations (0, 15, 30 and 45 mg/l; Table 1). The model clearly differentiates the responses of extracellular (R_1 and C_1) and intracellular (R_2 and C_2) components. In general, the extracellular resistance (R_1) was higher in the stems treated with different Al conc as compared to control. The highest R_1 in plant stem was noticed at higher Al conc (45 mg/l) that was 10-folds greater than the control plant stem. In the stem, C_1 sharply decreased from 1.25 μF (control) to 1.11 (30 mg/l Al) but again increased to 1.88 μF following Al treatment at 45 mg/l. Sinha and Tabib-Azar (2015) found that the leaf capacitance of *Schefflera arboricola* periodically decreased by 51 pF/hr during the night and increased by 62.3 pF/hr during the day.

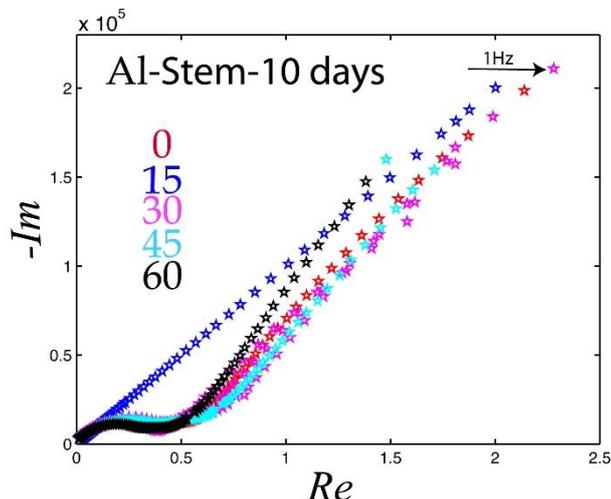


Fig. 3. Electrical impedance spectra (EIS) of the stem of *C. procerca* plants cultivated hydroponically under laboratory conditions. The term “Re” refers to the real part (Z') and “-Im” to the imaginary part of impedance (Z''). In each curve, the frequency increased from the right (1 Hz) to left (1 MHz). The values are computed following a 10-days treatment with aluminum at different concentrations (0, 15, 30, 45 and 60 mg/l).

While stem capacitance decreased by 0.8 pF/hr at night and increased by 18 pF/hr during the day. However, stem showed different behavior at the intracellular level, where R_2 gradually increased from 0.037 M Ω (control) to 0.56 M Ω after treatment with 45 mg/l Al. The intracellular resistance (R_2) of control (0.037 M Ω) plant stem was approximately equivalent to that of treated plant stem (0.038 M Ω) at 15 mg/l Al (Table 1). The intracellular capacitance (C_2) was less in control stem as compared to the treated stem at all Al concentrations. Interestingly, Al treatment resulted in an increase of C_2 at each Al treatment level. The C_2 of the 45 mg/l treated stem was almost 9-folds greater than that of the control stem. J \acute{o} c \acute{s} ak et al. (2010) evaluated the effects of flood and cadmium on Pea roots through EIS and found an increase in symplasmic resistance (R_s), and membrane capacitance (C_m) following cadmium treatment. They concluded that increasing tendency was as a consequence of the enhanced membrane rigidity, thickened cell walls and growth inhibition due to Cd. Mancuso and Azzarello (2002) found a significant decrease in impedance ratio, together with an increase in electrolyte leakage in olive genotypes after heat treatment.

6. Conclusion

EIS is a non-invasive technique that has been developed to study extracellular and intracellular resistance and capacitance in plant tissues. This article outlines the application of EIS to study electrochemical crosstalk's with the cell during plant stress physiology experiments. EIS can be used to screen the appropriate frequencies for detection, and then, single or certain frequencies are chosen for latter high-speed analyses. EIS has been used to estimate plant health, nutrient status, viruses interference, fruit damages, structural cellular variation during fruit ripening, freeze or chill damages, sensitivity to salinity, and tree root growth. EIS can fully recognize cellular behavior in response to various stimulations with high sensitivity. In the future, the integration of EIS will improve the quality and reproducibility of measured data and enhance the ability for high-throughput screening, which may bring evolution to screening techniques, such as drug candidates screening and gene function screening, etc.

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