HOSTED BY
ELSEVIER
Available at www.sciencedirect.com
INFORMATION PROCESSING IN AGRICULTURE 2 (2015) 161–168
journal homepage: www.elsevier.com/locate/inpa

Impedance analysis of *Labisia pumila* plant water status

Diyana Jamaludin [a], *Samsuzana Abd Aziz* [a,*], *Desa Ahmad* [a], *Hawa Z.E. Jaafar* [b]

[a] *Department of Biological and Agricultural Engineering, Faculty of Engineering, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[b] *Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

A R T I C L E I N F O

Article history:
Received 20 November 2014
Received in revised form
7 July 2015
Accepted 24 July 2015
Available online 28 October 2015

Keywords:
Impedance spectroscopy
Plant water status
Labisia pumila
Impedance
Reactance
Resistance

A B S T R A C T

Labisia pumila (Kacip fatimah) is a popular medicinal plant in Malaysia. The constituents of this plant have been reported to possess anticancer, antioxidant and anti-inflammatory properties. The growth and production of *L. pumila* is greatly influenced by the plant water status. Current techniques to measure plant water status are generally based on the plant soil moisture, which apparently did not indicate the real water content inside the plant. There are other techniques to measure directly on the plant such as based on leaf water potential (LWP) and relative water content (RWC). However, these techniques are destructive and time consuming. In this study, four levels of evapotranspiration replacement (ER) treatment which were 100% ER, 75% ER, 50% ER and 25% ER was applied to 30 polybags of *L. pumila* plants. The plant water status was measured using an impedance spectroscopy technique. A pair of electrocardiogram (ECG) electrode connected to an impedance analyzer board was used to measure the impedance value of the leaf samples non-invasively. Plant water status parameters such as LWP, RWC, volumetric moisture content (VMC), and leaf thickness were measured using standard methods. The results show that after 20 weeks of treatment, 25% ER had the highest impedance value ranged from 0.10 MΩ to 0.15 MΩ at the frequency of 70–100 kHz. The resistance of 100% ER at 20 weeks of treatment increased from 0.70 kΩ to 1.23 kΩ as the reactance decreased from 0.51 kΩ to 0.28 kΩ. Comparatively, the resistance of 25% ER increased from 1 kΩ to 1.10 kΩ as the reactance decreased from 0.88 kΩ to 0.83 kΩ. The polynomial regression of impedance measurements with plant water status parameters (VMC, leaf thickness, LWP and RWC) shows that LWP and RWC had the highest R^2 (0.78, 0.73). The results show that impedance measurement technique is auspicious to evaluate plant water status.

1. Introduction

The use of herbal plants and their related products in the health-food market has become important due to the increasing awareness towards maintaining health with natural products. Previous studies reported that the estimated total economic value of medicinal plants can reach up to

* Corresponding author. Tel.: +60 3 89464326; fax: +60 3 89466425.
E-mail address: samsuzana@upm.edu.my (S. Abd Aziz).
Peer review under the responsibility of China Agricultural University.
http://dx.doi.org/10.1016/j.inpa.2015.07.004

RM250,000 [1]. *Labisia pumila* or locally known in Malaysia as Kacip Fatimah is one of the popular herbs with estimated demand around 24.4 metric ton yearly [2]. The demand is due to its therapeutic effects and antioxidative activity that was reported to be higher than vitamin C and E [3]. In order to provide sufficient *L. pumila* supply and demand, environmental factors that affect the growth and cultivation of this herbal plant need to be studied. Water is one of the important factors that need to be maintained for plants to continue its growth and to perform physiological processes, such as photosynthesis and nutrient uptake. Therefore, when plants do not receive sufficient water, they are subjected to water stress. This will disrupt plant cells and the whole plant functions, which will give a negative impact on plant growth and reproduction [4]. Conversely, studies also showed that plants need a certain amount of stress to induce its growth and reproduction [5]. Thus, a precise method to determine plant water status is essential for optimum growth and cultivation of the plant.

In many studies, plant water status is determined using a range of plant and environment parameters such as soil moisture content (SMC). A general problem with the estimation of SMC arises due to the substantial heterogeneity within soils; thus, changes in bulk soil water content do not necessarily reflect plant's water demand [6]. Irrigation decision according to plant water status is more reliable than soil water status [7] such as measuring leaf water potential (LWP) and relative water content (RWC). The LWP indicates the demand for water within a plant, the resistance to water movement within the plant, and the demands for transpiration imposed by the environment. However, these measurement techniques are destructive and time consuming [8].

It is important to measure plant water status in real time for proper decision on irrigation management. Recently, electrical measurement of agricultural materials has been explored by many researchers for non-destructive and real time applications. For example, [9] showed that the impedance of biological material is related to the resistance and capacitive reactance of the tissue. Their electrical behavior is relatively resistive as tissue composition contains water, electrolytes, free ions, salts and other components [10]. This is supported by many previous works such as study on impedance properties of citrus fruits to determine citrus acidity based on pH value which showed that impedance and pH gave highest correlation at frequency of 1 MHz [11]. There was also a study on microstructure changes on heated potato tissue [10] using two stainless steel needle electrodes that gave significant result on impedance parameter at temperatures above 60 °C, indicating loss of turgor pressure and rupture of membranes cell walls. Earlier study by Stout [12] showed that resistance of intracellular stems had increased as the effect of cold acclimation. Similarly, study by [12] showed the intracellular resistance of the shoots of *Olea europea* increased during the winter resting period, whereas intracellular resistance of the leaves decreased.

Measurements of electrical properties on the leaves for plant water status indication was also explored by many researchers. Mizukami et al. [13] had used stainless steel electrodes connected to a LCR meter to measure dielectric properties of tea leaves at frequency of 10 Hz–10 MHz. It was found that impedance and capacitance value showed significant effect to measure moisture content at frequency 3 kHz. The dielectric properties of rubber leaves and oil palm leaves at 4–18 GHz frequency also had been measured by using coaxial probe and showed that moisture content can be estimated by using Debye Cole model [14]. Afzal et al. [15] did a research to estimate leaf moisture content using capacitance measurements at frequency 100 kHz and 1 MHz. The result showed that dielectric constant at 100 kHz was more accurate in determining the leaf's moisture content. These findings showed that electrical properties of plant leaves such as impedance, resistance, capacitance and dielectric constant can be used to determine plant water status.

In this study, the impedance of *L. pumila* leaves was measured non-destructively using a pair of ECG electrodes connected to an impedance analyzer at 100 Hz–100 kHz. The objectives of this study were to evaluate changes in plant water status and its electrical impedance subjected to evapotranspiration replacement (ER) treatment and to explore the potential use of impedance characteristic as plant water status indicator. The impedance measurements were compared to the standard parameters to evaluate plant water status such as LWP, RWC, leaf thickness and volumetric moisture content (VMC).

2. Materials and methods

2.1. Sample preparation and evapotranspiration replacement (ER) treatment

The experiments were performed on *L. pumila* var. pumila that were grown in 14 cm diameter polybags with soilless medium containing coco-peat, burnt paddy husk and well-composed chicken manure in 5:5:1 (v/v) ratio in growth houses at the Faculty of Agriculture Glasshouse Complex, Universiti Putra Malaysia (longitude 101°44′N and latitude 2°58′S, 68 m above sea level). The ER treatment was applied based on the previous study by [5]. In total of 30 plants were chosen for sampling of ER measurement from a total of 192 polybags. The initial weight of polybag, media and plant were determined (A kg). All polybags initially received an equal volume of water (0.6 L per 2 L of media) and water was allowed to drip for 24 h. The dripped out water from the polybag was collected to determine the saturated level of the soil (Y mL). The polybag with media and plant was weighed again after the following 24 h (B kg) and after 48 h (C kg). The amount of water held by media was calculated by Z (mL) = (600 − Y) mL. Water loss by evapotranspiration was calculated by, D (kg) = (B − C) kg. The irrigation was based on the amount of water lost by evapotranspiration, which were 25%, 50%, 75% and 100% of D. The 100% irrigation represents well watered, 75% represents moderate water stress, 50% represents high water stress and 25% represents severe water stress. The conversion of 1 kg = 1 L was applied to calculate the amount of water to irrigate based on weight loss. This method was calibrated every two or three days to ensure good readings of water requirement and loss. The ER treatment was applied for 20 weeks with three measurement stages at week 5, 10 and 20.

2.2. Impedance spectroscopy measurement

After five weeks of ER treatments, the impedance and plant parameters measurements were carried out. In this method, two electrocardiogram (ECG) electrodes were used as the test fixture. The young, fully expanded leaves (generally the second or third leaf from the tip of the stem) was selected for impedance measurement. The leaf surface was cleaned using dry tissues before attaching the ECG electrodes. The electrodes were connected to an impedance analyzer board (AD5933, Analog Device, Massachusette USA) and a personal computer (Fig. 1). The reactance, X and resistance, R of the leaves at every 200 Hz intervals within 1 kHz–100 kHz frequency range were recorded in Microsoft Excel (see Fig. 2). The impedance was calculated using Eq. (1):

$$\text{Impedance }(\Omega) = \frac{1}{\text{Gain Factor} * \text{Magnitude}} \quad (1)$$

where the magnitude of the impedance was calculated as:

$$\text{Magnitude} = \sqrt{R^2 + X^2} \quad (2)$$

and the gain factor was determined using Eq. (3) where the know impedance was set to be 200 kΩ connected between V_{OUT} and V_{IN} of the AD5933 impedance analyzer board (Fig. 3). The calibration magnitude was calculated based on R and X values of the known impedance as described in the data sheet of the board [16].

$$\text{Gain Factor} = \frac{\left(\frac{1}{\text{Known Impedance}}\right)}{\text{Calibration Magnitude}} \quad (3)$$

2.3. Leaf water potential (LWP) measurement

The LWP of the *L. pumila* leaf samples were measured using a pressure chamber (Model 600, PMS Instrument Company, Oregon USA). The same leaf used for impedance measurement was cut from the stem and placed in a chamber with the cut surface protruding through the chamber lid. Pressure was applied to the leaf and reading was taken when water first appeared at the cut surface. The unit of pressure used was bar (1 bar = 14.5 psi). The amount of pressure to make water appear at the cut surface described the amount of tension experienced by the leaf.

2.4. Relative water content (RWC) measurement

Samples of leaf disc were taken using a cork borer. In total of 18 leaf discs were taken from each leaf sample and placed on

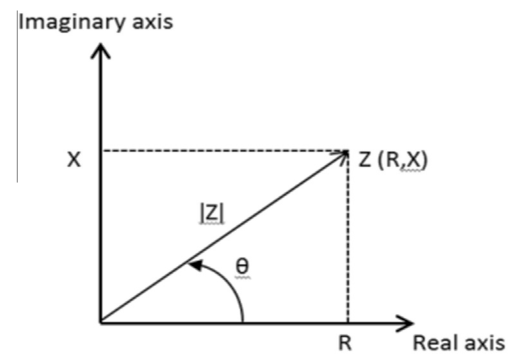

Fig. 2 – Impedance plot in a complex plane. *R* is the real or resistive component, and *X* is the imaginary or reactance component.

a pre-weighed clear container, and fresh weight (FW) was determined using digital balance (Shimadzu TX432L, Kyoto, Japan). The samples were then floated in distilled water and kept in the dark for 24 h to regain full turgor. Then, the leaf discs were removed from the vial and excess water was removed using a paper tissue, and then the turgid weight (TW) was measured. The samples were subsequently oven-fried to a constant weight at 60 °C for 48 h and the dry weight (DW) of the sample was recorded. The RWC was calculated based on Eq. (4).

$$\text{RWC }(\%) = \frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}} \times 100\% \quad (4)$$

where FW is the fresh weight, DW is the dry weight and TW is the turgid weight.

2.5. Leaf thickness measurement

A micrometer (Digimatic, Mitutoyo Corporation, IP65, Japan) was used to measure the leaf thickness. The measurement range was from 0 to 25.4 mm with the accuracy of ±0.00127 mm. The leaf thickness value shown on the display screen was recorded.

2.6. Volumetric moisture content (VMC) of planting medium

The VMC of soil generally is used as plant water status indicator for irrigation management. In this study, the VMC of the *L. pumila* planting medium was measured using a soil moisture

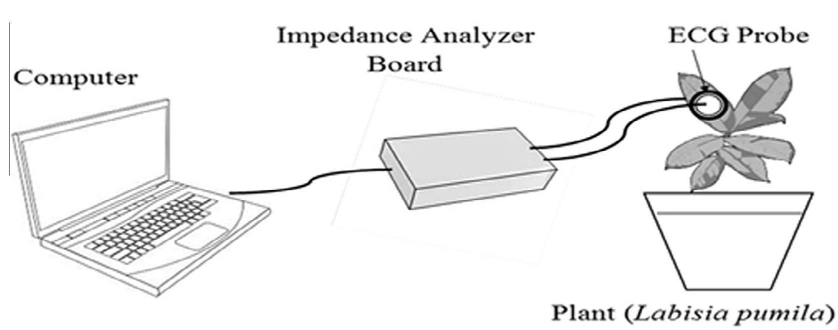

Fig. 1 – Layout of instrumentation for impedance measurement of *Labisia pumila* leaves using electrocardiogram (ECG) electrode with an impedance analyzer board connected to a computer.

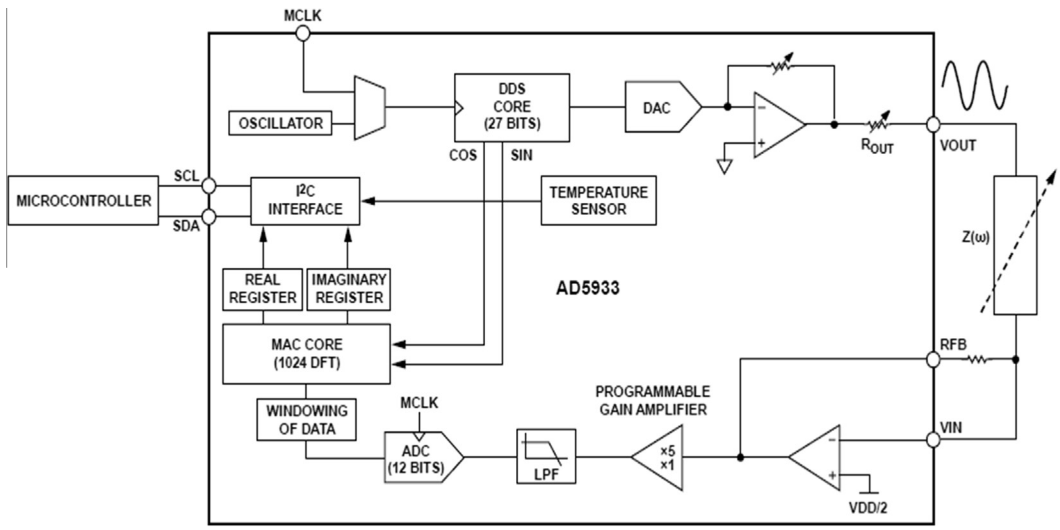

Fig. 3 – Block diagram of impedance analyzer AD5933 with for impedance measurement of *Labisia pumila* leaves. Known impedance (200 kΩ) was placed between V_{OUT} and V_{IN}.

Table 1 – *Labisia pumila* plant water status parameters at week 5, 10 and 20 of evapotranspiration replacement (ER) treatment.

	ER (%)	LWP (-bar)	RWC (%)	LT (mm)	VMC (%vol)
Week 5	100	8.67 ± 0.61[a]	86.05 ± 0.81[a]	0.280 ± 0.005[a]	33.95 ± 0.43[a]
	75	9.29 ± 0.55[ab]	85.99 ± 0.82[a]	0.263 ± 0.006[a]	31.89 ± 0.57[b]
	50	9.79 ± 0.74[ab]	83.36 ± 1.44[a]	0.246 ± 0.004[b]	31.68 ± 0.40[b]
	25	11.33 ± 0.95[b]	83.16 ± 1.67[a]	0.244 ± 0.004[b]	31.49 ± 0.41[b]
Week 10	100	5.50 ± 0.50[a]	90.25 ± 0.88[a]	0.250 ± 0.004[a]	26.07 ± 0.54[a]
	75	6.96 ± 0.60[ab]	89.53 ± 1.24[a]	0.250 ± 0.004[a]	24.42 ± 0.84[a]
	50	8.30 ± 0.82[b]	88.40 ± 1.32[a]	0.234 ± 0.004[b]	19.24 ± 0.68[b]
	25	8.18 ± 0.58[b]	87.83 ± 1.61[a]	0.235 ± 0.004[b]	17.12 ± 1.16[b]
Week 20	100	4.27 ± 0.21[a]	81.39 ± 1.33[a]	0.285 ± 0.007[a]	19.93 ± 0.98[a]
	75	6.09 ± 0.59[ab]	80.86 ± 2.61[a]	0.268 ± 0.006[ab]	14.40 ± 1.36[b]
	50	7.40 ± 0.59[bc]	80.25 ± 1.98[a]	0.262 ± 0.004[b]	9.53 ± 0.75[c]
	25	8.86 ± 0.32[c]	78.71 ± 3.44[a]	0.243 ± 0.006[c]	8.95 ± 0.70[c]

LWP is leaf water potential (-bar), RWC is relative water content (%), LT is leaf thickness (mm), and VMC is volumetric moisture content (%vol). Values represent mean ± S.E. data. Means not sharing a common letter were significantly different at $p \leqslant 0.05$, $n = 48$.

meter (IMKO, Trime FM3, Germany), which gave volumetric moisture content of the planting medium. It has three-rod probes inserted into the medium and the value of percent volume (%vol) moisture content was displayed on the LCD within 10 s. Two points were measured for each planting medium to obtain the average data.

3. Results and discussion

3.1. *Effect of evapotranspiration replacement (ER) treatment on plant water status parameters*

Analysis of variance (ANOVA) was performed to determine the effect of ER treatment on plant physiology that was associated with the plant water status (Table 1). The LWP of *L. pumila* leaves for 100% ER to 25% ER significantly increased at each measurement week with a significant difference between each treatment. The result infers that well watered plants needs less pressure to pull out water to the stem surface as the plant cells itself are in high water tension condition. This supports the study by [17], which found that LWP decreased continuously with time after imposition of water deficit on rice.

The RWC from 100% ER to 25% ER continuously decreased at each week of treatment, which shows that plants that received less amount of water replacement have lower value of RWC than well-watered plants. Although ANOVA showed no significant difference of RWC between ER treatment, at week 20, it shows that the 100% ER plants had the highest RWC (81.39%), followed by 75% ER (80.86%), 50% ER (80.25%) and 25% ER (78.71%). It proves that plants that received less amount of water replaced had less water inside the cell.

Well-watered plants have thicker leaf than plants that received less amount of water replacement. Table 1 shows that the leaf thickness decreased as the amount of water replaced decreased. There was a significant difference of leaf thickness between ER treatments, especially at week 20. This might be due to the changes in the leaf cell structure, which is influenced by turgidity of the cell wall and thus affect the leaf thickness [18].

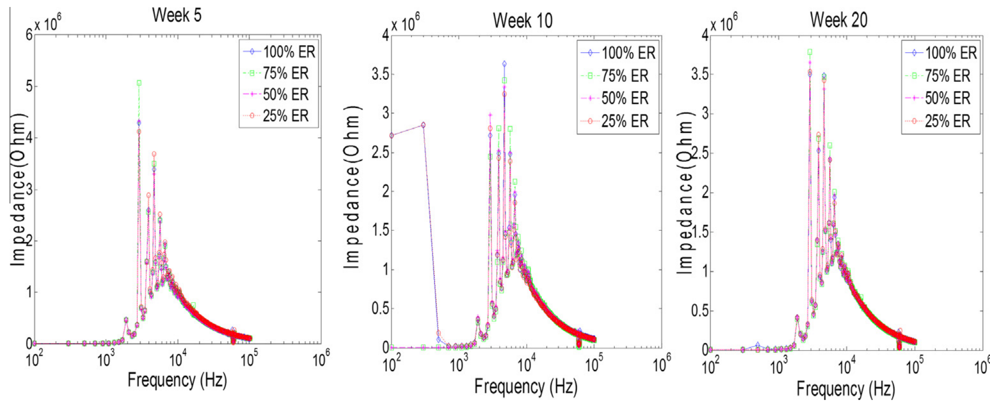

Fig. 4 – Full frequency range from 100 Hz to 100 kHz of *Labisia pumila* impedance at different evapotranspiration replacement (ER) treatments.

Generally, the VMC of *L. pumila* planting medium decreased as the irrigation decreased from 100% ER to 25% ER. This corresponds to the applied water treatment based on the amount of water loss by evapotranspiration. ER treatments apparently affected the VMC as there was a significant difference of VMC between treatments with p value $\leqslant 0.05$.

3.2. *Impedance measurement at different frequencies*

Generally, the impedance measurement of *L. pumila* leaves decreased as the frequency increased. This decrement can be seen clearly at frequency 70 kHz–100 kHz as it has more stable pattern with less noise compared to the frequency below 70 kHz (Fig. 4). This decreasing graph shows that at 70 kHz, the highest impedance value was 25% ER (0.154 MΩ), followed by 50% ER (0.147 MΩ), 75% ER (0.145 MΩ) and 100% ER (0.144 MΩ) (Fig. 5a). This proves that plants that received less water have more resistance and thus have higher impedance value compared to the plants that received more water. The same trend was observed in week 10 of treatment (Fig. 5b). However, in week 20, there was a slight difference of the trend for 100% ER, where it shows that the impedance value of 100% ER was higher than 75% ER and 50% ER (Fig. 5c). The results also show that after 20 weeks of treatment, 25% ER showed the highest impedance value range from 0.10 MΩ to 0.15 MΩ at the frequency of 70–100 kHz. This might be due to changes in the environment such as temperature and transpiration that affected the performance of plant water intake. Since the plants have been implied with ER treatment for 20 weeks, changes in the cell structure and ion concentration affect the impedance value inside the leaf. A similar decrease of impedance value was also reported in durian [19] and kiwifruit [20].

3.3. *Changes between resistance and reactance*

Instead of impedance and frequency plot, resistance and reactance components of impedance can be plotted to explore the variation of electrical impedance pattern for each ER treatment. This real and imaginary part of impedance graph is also known as Nyquist plot. For week 5 of treatment, it can be seen that the reactance was a continuously decreased as resistance increased at frequency ranged from 70 kHz to 100 kHz (Fig. 6a). The 100% ER had the lowest reactance value compared to other ER treatments. The data shows clear data lines at 100 kHz compared to 70 kHz, where some data points were mixed up. This implies that higher frequency shows better variation between ER treatments.

The pattern of the plot shows clear separation between each ER treatment when prolonged up to 20 weeks. In week 10 of treatment, the resistance values at 100 kHz, for 25% ER

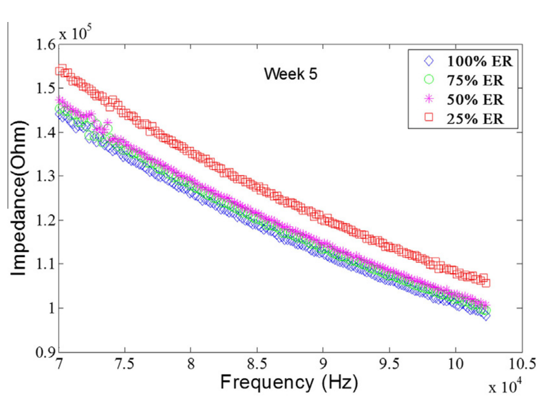

Fig. 5a – Impedance (ohm) of *Labisia pumila* leaves at different evapotranspiration replacement (ER) treatments at week 5 under treatment.

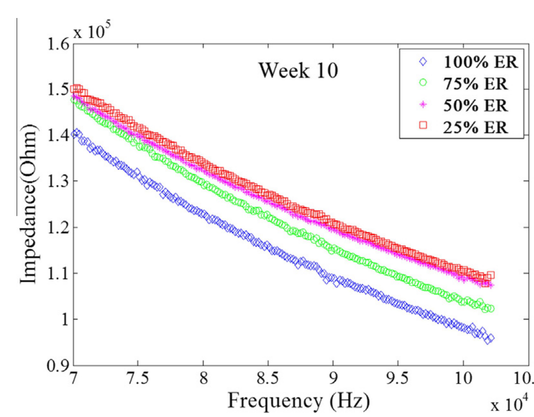

Fig. 5b – Impedance (ohm) of *Labisia pumila* leaves at different evapotranspiration replacement (ER) treatments at week 10 under treatment.

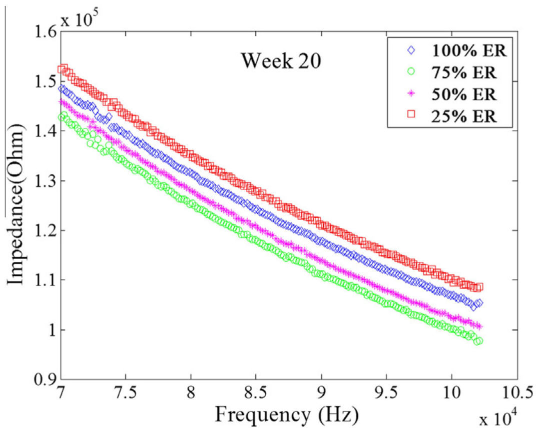

Fig. 5c – Impedance (ohm) of *Labisia pumila* leaves at different evapotranspiration replacement (ER) treatments at week 20 under treatment.

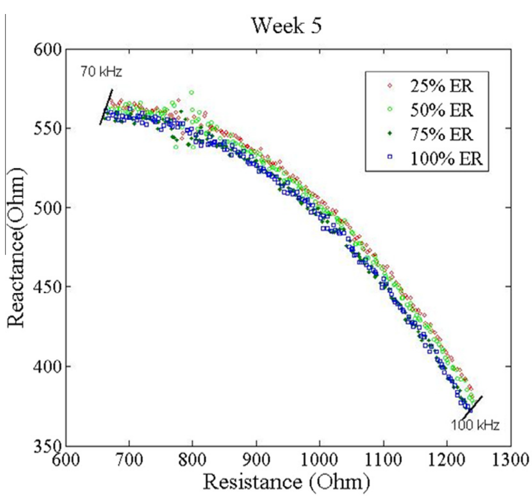

Fig. 6a – Reactance (ohm) versus resistance (ohm) of *Labisia pumila* leaves at different evapotranspiration replacement (ER) treatments at week 5.

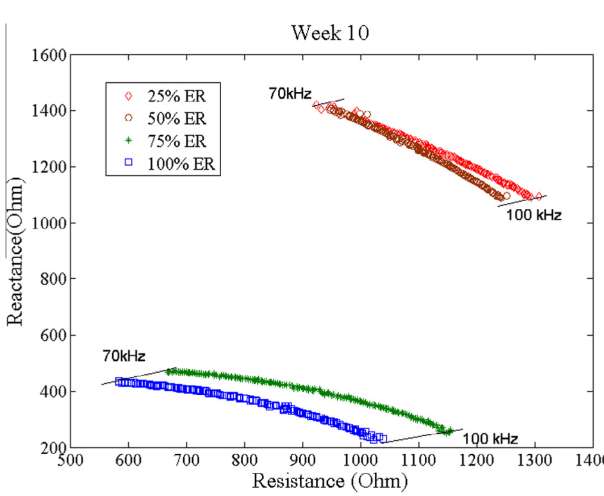

Fig. 6b – Reactance (ohm) versus resistance (ohm) of *Labisia pumila* leaves at different evapotranspiration replacement (ER) treatments at week 10.

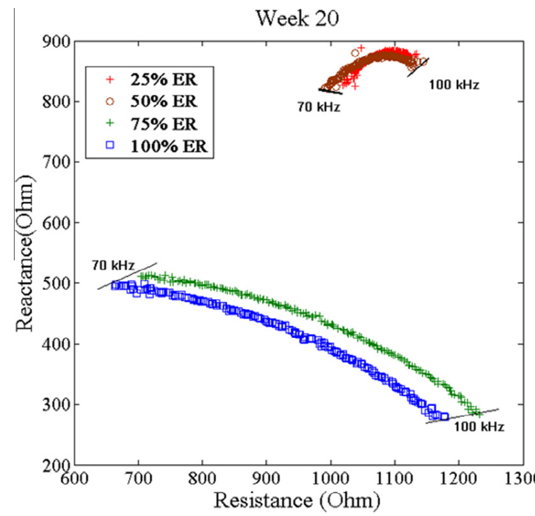

Fig. 6c – Reactance (ohm) versus resistance (ohm) of *Labisia pumila* leaves at different evapotranspiration replacement (ER) treatments at week 20.

and 50% ER were higher (>800 Ω) than resistance values for 75% ER and 100% ER (<700 Ω). Similarly, the reactance values of *L. pumila* leaves under 25% ER and 50% ER were higher (>1 kΩ) than 75%ER and 100% ER (<600 Ω) (Fig. 6b). At 20 week of treatment, plants of 50% ER had reduced resistance and reactance compared to week 10 (Fig. 6c). This implies that *L. pumila* leaves experiencing a higher degree of water stress as treatment was prolonged.

3.4. *Relationship between impedance and physiological parameters of L. pumila leaf*

The relationship between impedance and plant physiological parameters was further explored with the development of mathematical model and determination of its coefficient of determination (R^2). The frequency selected on each plant physiological parameters was based on the highest R^2 at 20 weeks of ER treatments. The R^2 for each plant physiological parameters was between 0.66 and 0.78 (Table 2). The relationship between impedance and LWP show the highest R^2 at 100 kHz. The impedance measurements increased when LWP increased in polynomial forms with $R^2 = 0.78$ (Fig 7a). The low impedance value represents high LWP, which indicates more available water inside the cell. Since water conducts electricity more easily with less resistivity, leaves with higher water content have lower impedance. Leaves could be regarded as dielectric and have stronger conductivity when they contained much water [8].

The impedance of *L. pumila* leaves was increased as the RWC decreased with $R^2 = 0.73$ at frequency of 90 kHz (Fig. 7b). Since high RWC indicates high water content, this result shows that the impedance will be much higher at a high level of leaf water stress. Zheng et al. [8] also found that RWC parameters had a very significant correlation with the electrical property of corn leaves by linear regression in different growing stages.

The regression analysis of impedance and leaf thickness shows highest $R^2 = 0.70$ at 80 kHz. Leaf thickness shows decreasing value with the increase of impedance. The

Table 2 – The coefficient of determination for regression equation used to predict plant water status parameters using impedance value at the optimum frequency.

Plant water status parameter	Frequency (kHz)	Equation	R^2
LWP (-bar)	70	$LWP = -2E - 9Z^2 - 0.0004Z + 27.87$	0.75
	80	$LWP = -1E - 9Z^2 - 0.0001Z + 6.35$	0.73
	90	$LWP = -3E - 9Z^2 - 0.0001Z - 7.19$	0.66
	100	$\mathbf{LWP = -5E - 9Z^2 - 0.0008Z + 36.13}$	**0.78**
RWC (%)	70	$RWC = -4E - 9Z^2 - 0.0006Z + 73.80$	0.70
	80	$RWC = -2E - 9Z^2 - 2E5Z + 114.74$	0.70
	90	$\mathbf{RWC = 1E - 9Z^2 - 0.0008Z + 153.97}$	**0.73**
	100	$RWC = 3E - 9Z^2 - 0.0011Z + 166.87$	0.72
LT (mm)	**70**	$\mathbf{LT = -6E - 12Z^2 + 5E - 7Z + 0.33}$	**0.70**
	80	$LT = 9E - 12Z^2 - 3E - 6Z + 0.53$	0.65
	90	$LT = 1E - 11Z^2 - 5E - 6Z + 0.65$	0.55
	100	$LT = 1E - 11Z^2 - 4E - 6Z + 0.56$	0.66
VMC (%vol)	70	$VMC = 3E - 9Z^2 - 0.001Z + 108.24$	0.69
	80	$VMC = 5E - 9Z^2 - 0.002Z + 116.59$	0.69
	90	$\mathbf{VMC = 4E - 9Z^2 - 0.001Z + 117.47}$	**0.70**
	100	$VMC = 6E - 9Z^2 - 0.001Z + 113.45$	0.67

LWP is leaf water potential (-bar), RWC is relative water content (%), LT is leaf thickness (mm), VMC is volumetric moisture content (%vol). Z is impedance (ohm). The equation is based on the 20 week of ER treatment. The bold frequency is the highest R^2 for particular parameter.

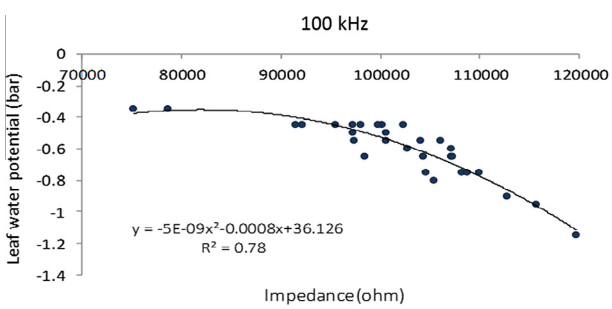

Fig. 7a – Relationship between leaf water potential (LWP) with impedance at frequency 100 kHz.

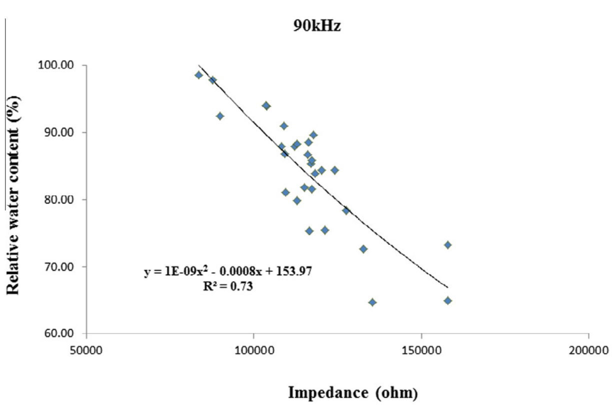

Fig. 7b – Relationship between relative water content (RWC) with impedance at frequency 90 kHz.

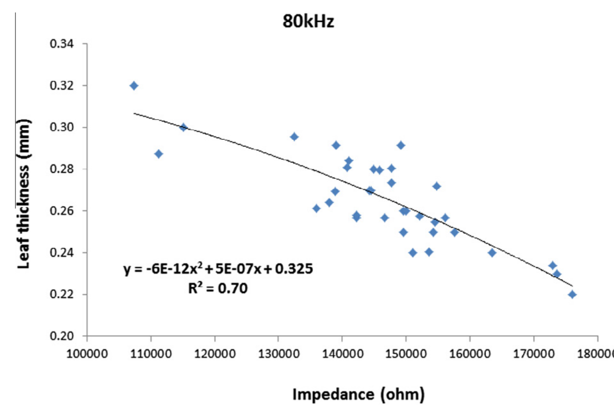

Fig. 7c – Relationship between leaf thickness (LT) with impedance at frequency 80 kHz.

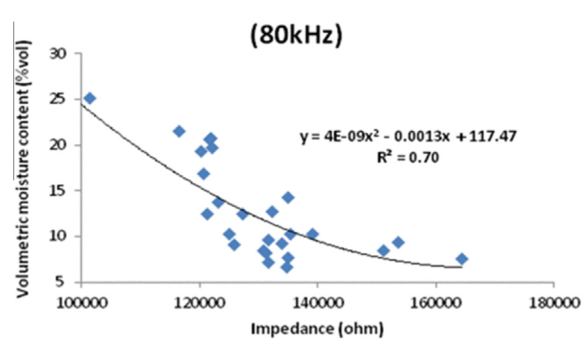

Fig. 7d – Relationship between volumetric moisture content (VMC) with impedance at frequency 80 kHz.

polynomial curves show that impedance value was changed with leaf thickness (Fig. 7c). Previous results on ER treatments show that plants receiving less amount of water replacement have a thinner leaf, which conclude that plants received less amount of water replacement have a higher value of impedance compared to well-watered plants. The relationship between impedance and VMC was analyzed because VMC generally has been used as plant water status indicator for irrigation management. The relationship between VMC and impedance showed highest $R^2 = 0.70$ at 80 kHz (Fig. 7d). The result was comparatively lower than the R^2 of impedance and LWP or RWC. This might be due to the fact that impedance, LWP and RWC were measured on the leaf surface rather than planting medium, which reflects that different medium of measurement affected the result.

4. Conclusion

The ECG probe and an impedance analyzer were used to evaluate the impedance property of *L. pumila* leaves. Performance experiments were conducted to explore the relationship between leaf impedance characteristic and water status at different ER treatments. The following conclusions are obtained from the study:

(1) ER treatments gave a significant effect on plant physiology of *L. pumila*. It shows that plants received sufficient amount of water replaced (100% ER) had the highest LWP, RWC, leaf thickness and VMC, compared to other treatments (75% ER, 50% ER, and 25% ER).
(2) In week 5 of treatment at the frequency of 70 kHz, the highest impedance value was recorded for 25% ER (0.154 MΩ), followed by 50% ER (0.147 MΩ), 75% ER (0.145 MΩ) and 100% ER (0.144 MΩ). This proves that plants that received less water had more resistance and thus had higher impedance value compared to plants that received more water inside the leaf.
(3) Impedance measurement also detected changes in tissue resistance and reactance at different treatments, which indicates that the impedance method has a good potential to be used as a plant water status indicator. The resistance value of plants in 25% ER and 50% ER were higher than in 100% ER and 75% ER. The well-watered plants (100% ER) had the lowest reactance value compared to other ER treatments.
(4) The R^2 of polynomial regression of plant water status parameters (LWP, RWC, leaf thickness, and VMC) with impedance shows that LWP and RWC had the highest R^2 (0.78, 0.73). Reflecting leaf water status by measuring impedance property is feasible and practical.

From these results, it shows that impedance measurement is auspicious to be used in the determination of *L. pumila* plant water status. This method offers in-situ and simple measurement technique for plant water status measurement. Future investigations on the leaves physiological and biological behaviors as plant water status changes and their relationship with impedance measurements need to be extended for further development of a plant water status indicator.

Acknowledgements

The authors gratefully acknowledge the financial support from the Ministry of Education Malaysia and Universiti Putra Malaysia with the UPM Research Grant Scheme (Project Code: GP-IPS/2013/9399814). The authors would also like to acknowledge Mrs. Nadirah Roslan who was involved in data collection process.

REFERENCES

[1] Mohamad Stefarzi M, Mansor P. Status penggunaan herba oleh pengilang tempatan dan potensi penanaman di Malaysia. Seminar on Medicinal and Aromatic Plants. Kuala Lumpur: FRIM; 2000.
[2] Norini H, Azmi MIM. Economic valuation of medicinal plants in Ayer Hitam Forest Reserve (AHFR), Puchong, Selangor Darul Ehsan. Pertanika J Trop Agric Sci 2008;30(2):117–29.
[3] Jamia AJ, Ibrahim J, Khairana H, Juriyati J. Perkembangan penyelidikan dan pembangunan Kacip fatimah. 2004 p. 13–9
[4] Bray EA. Life science. Plant response to water-deficit stress. John Wiley & Sons, Ltd.; 2001. p. 1–5.
[5] Jaafar HZE, Ibrahim MH, Mohamad Fakri NF. Impact of soil field water capacity on secondary metabolites, phenylalanine ammonia-lyase (PAL), maliondialdehyde (MDA) and photosynthetic responses of Malaysian Kacip fatimah (*Labisia pumila* Benth). Molecules 2012;17(6):7305–22.
[6] Jones HG. Irrigation scheduling: advantages and pitfalls of plant-based methods. J Exp Bot 2004;55(407):427–2436.
[7] Turner NC. Plant water relations and irrigation management. Agric Water Manage 1990;17(1–3):59–73.
[8] Zheng L, Wang Z, Sun H, Zhang M, Li M. Real-time evaluation of corn leaf water content based on the electrical property of leaf. Comput Electron Agric 2014 (in press).
[9] Harker FRDJ. Electrical impedance studies of nectarines during cool storage and fruit ripening. Postharvest Biol Technol 1994;4:125–34.
[10] Fuentesa A, Vázquez-Gutiérreza JL, Pérez-Gagob MB, Vonasekc E, Nitinc N, Diane MB. Application of nondestructive impedance spectroscopy to determination of the effect of temperature on potato microstructure and texture. J Food Eng 2014;133:16–22.
[11] Juansah J, Budiastra IW, Dahlan K. The prospect of electrical impedance spectroscopy as non-destructive evaluation of citrus fruit acidity. Int J Emerging Technol Adv Eng 2012;2 (11):58–64.
[12] Stout DG. Effect of cold acclimation on bulk tissue electrical impedance. I. Measurements with birdsfoot trefoil at subfreezing temperatures. Plant Physiol 1988;86:275–82.
[13] Mizukami Y, Sawai Y, Yamaguchi Y. Moisture content measurement of tea leaves by electrical impedance and capacitance. Biosyst Eng 2006;93(3):293–9.
[14] Chuah HT, Lee KY, Lau TW. Dielectric constants of rubber and oil palm leaf samples at X-band. IEEE Trans Geosci Remote Sens 1995.
[15] Afzal A, Mousavi SF, Khademi M. Estimation of leaf moisture content by measuring the capacitance. J Agric Sci Technol 2010;12:339–46.
[16] Analog Device. AD5933 data sheet 1 MSPS, 12-bit impedance converter, network analyzer. 2013. p. 40.
[17] Jongdee B. Designing a national breeding program for developing drought-tolerant rainfed lowland varieties: the Thailand experience, in breeding rice for crought-prone environments. Los Banos; 2003. p. 64–9.
[18] Sancho-Knapik D, Alvarez-Arenas TG, Pequero-Pina JJ, Fernandez V, Gil-Pelegrin E. Relationship between ultrasonic properties and structural changes in the mesophyll during leaf dehydration. J Exp Bot 2011;62:3637–45.
[19] Kuson P, Terdwongworakul A. Minimally-destructive evaluation of durian maturity based on electrical impedance measurement. J Food Eng 2013;116(1):50–6.
[20] Bauchot AD, Harkera FR, Michael Arnold W. The use of electrical impedance spectroscopy to assess the physiological condition of kiwifruit. Postharvest Biol Technol 2000;18(1):9–18.