Article

Rapid and Efficient Determination of Relative Water Contents of Crop Leaves Using Electrical Impedance Spectroscopy in Vegetative Growth Stage

Rinku Basak ^{1,*}, Khan A. Wahid ¹ , Anh Dinh ¹, Raju Soolanayakanahally ² , Reza Fotouhi ³ and Aryan S. Mehr ¹

¹ Department of Electrical and Computer Engineering, University of Saskatchewan, Saskatoon, SK S7N 5A9, Canada; khan.wahid@usask.ca (K.A.W.); anh.dinh@usask.ca (A.D.); aryan.saadat@usask.ca (A.S.M.)

² Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, SK S7N 0X2, Canada; raju.soolanayakanahally@canada.ca

³ Department of Mechanical Engineering, University of Saskatchewan, Saskatoon, SK S7N 5A9, Canada; reza.fotouhi@usask.ca

* Correspondence: rib595@mail.usask.ca; Tel.: +1-306-203-6124

Received: 1 April 2020; Accepted: 27 May 2020; Published: 29 May 2020

Abstract: Crop water stress is a deficiency in plants in water supply when the transpiration rate becomes higher than the water absorption capacity. The stress may be detected by a reduction in soil water content, or by the change in physiological properties of the crop. The leaf water content (LWC) is commonly used to assess the water status of plants, which is one of the indicators of crop water stress. In this work, the leaf relative water contents of four different crops: canola, wheat, soybeans, and corn—all in vegetative growth stage—were determined by a noninvasive tool called, electrical impedance spectroscopy (EIS). Using a frequency range of 5–15 kHz, a strong correlation between leaf water contents and leaf impedances was obtained using multiple linear regression. The trained dataset was validated by analysis of variance tests. Regression results were obtained using the least square method. The optimized regression model coefficients for different crops were proposed by selecting features using the wrapper backward elimination method. Multi-collinearity among the features was considered and individual T-tests were made in the feature selection. A maximum correlation coefficient (R) of 0.99 was obtained for canola compared to the other crops; the corresponding coefficient of determination (R^2) of 0.98, an adjusted R^2 of 0.93, and root mean square error (rmse) of 0.30% were obtained for 36 features. Therefore, the results show that the proposed technique using EIS can be used to develop a low-cost and effective tool for determining the leaf water contents rapidly and efficiently in multiple crops.

Keywords: electrical impedance spectroscopy; correlation coefficient; coefficient of determination; relative water content; analysis of variance

1. Introduction

Water is essential for crop production. Water stress reduces the efficiency of photosynthesis and limits crop productivity [1,2]. It occurs when the water demand exceeds the available moisture during a certain period. Since plant growth and productivity are adversely affected by water stress, it is important to accurately determine the water status in plants to make timely irrigation decisions [1–3]. Water status of plant can be indicated by different tissues (such as root, stem, and leaf or the whole canopy). Compared with the other plant tissues, leaf analysis is the most important tool for evaluating nutrient and water status of a plant, which aids in fertilization and irrigation [4–7]. Therefore, the leaf water content (LWC) is an important indicator of plant water status.

Some previous studies determined water stress using an environment parameter, like soil moisture content (SMC). However, it is determined that irrigation decision based on plant water status (like relative water content (RWC) or leaf water potential (LWP)) is more reliable than SMC [7]. Photosynthesis decreases with the reduction in the RWC and LWP [2]. Leaf RWC reflects the balance between water supply to the leaf tissue and transpiration rate [5–7]. 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, determining these measurements is destructive and time consuming; therefore, measuring plant water status in real time is critical for irrigation scheduling.

Several techniques, like visible or near-infrared spectroscopy, use of pressure bomb, and use of terahertz quantum cascade lasers have been applied in the past years for predicting LWC [7–10]. Applications of hyperspectral sensors and stimulated light output of the lasers give reflectance spectra of the leaves in the visible or near infrared wavelengths, which helps determine the LWC non-destructively [7,8]. Recently, electrical measurement of agricultural materials has been explored by several researchers for non-destructive and real-time applications [11–14]. These findings showed that electrical properties of leaves, such as impedance, resistance, capacitance, and dielectric constant can be used to determine the plant water status [12,15,16].

Plant electrical properties can be represented by a simplified electrical model consisting of a parallel circuit of capacitor and a resistor. In this sense, impedance spectroscopy allows the analysis of material properties through the application of alternate electric signals (voltage or current) of different frequencies, and the measuring of the corresponding electrical output signals (voltage or current). At high frequencies, the current flows through the capacitive component, and thus decreases the overall impedance. As a result, impedance spectroscopy technique had been used in agriculture to determine the physical and physiological aspects in the plant [17–19].

Several past studies have reported methodologies using electrical impedance measurements to determine the physiological status of biological tissues [20–22]. The impedance measurement by electrical impedance spectroscopy (EIS) is a fast, non-destructive, non-invasive, easily implemented, and inexpensive compared to the other available methods [11,23–25]. Although the computation is complex and model dependent, EIS works in a wide frequency range, and is less sensitive to environmental variables than other non-invasive methods. The EIS method has been used to determine nitrogen status [12,16,26], water status or moisture content [11,14], root biomass or root growth [13,19], phosphorus and potassium status [20], plant tissue differentiation [17], leaf growth [23], citrus fruit acidity (pH measurement) [15], and soil moisture content [18]. In a recent work [26], leaf nitrogen concentration in multiple crops were determined using EIS, which was proposed as an attractive alternative to optical spectroscopy. Multiple regression analysis was employed to perform the statistical analysis [27–29]. In this paper, we proposed that EIS collect in situ data directly on the leaf for the determination of water status in multiple crops. The measurement was done by varying the signal frequency, and the correlation between leaf impedances and leaf water contents was obtained for multiple crops using a multiple regression analysis. Therefore, the main objective of this work was to obtain the correlations between leaf impedances and relative water contents of four crops—canola, wheat, soybeans, and corn—all in vegetative growth stage and under varying water stress conditions.

2. Materials and Methods

2.1. Experimental Setup

The experiment was carried out in a greenhouse at the Agriculture and Agri-Food Canada (AAFC), Saskatoon, Saskatchewan, Canada. The EIS measurement was done by an evaluation board (EVAL-AD5933EBZ from Analog Devices Inc.), which is a high precision impedance converter system with a master clock of 16.77 MHz and the operating frequency of 5–100 kHz. The AC signal injected into the sample was generated by a built-in function generator of the board. The board combines

a frequency generator with a 12-bit, 1 mega sample per second (MSPS) analog-to-digital converter (ADC), and an internal temperature sensor. The frequency generator allows an external complex impedance to be excited with a known frequency.

The experimental setup of the data acquisition system was connected to a graphical user interface (GUI) as shown in Figure 1. For impedance spectroscopy measurements, a $2V_{p-p}$ generator voltage was used. A pair of electrocardiogram (ECG) electrodes connected to the evaluation board was used to measure the impedance of the leaf samples non-invasively. A separation of 3 cm between the two electrodes were maintained for all the measurements. The measurement at particular frequencies with on-board implementation is possible using EIS. Hence, the test duration is short, though the method is time-consuming for a large number of crops.

Figure 1. Impedance measurement of soybeans leaves using the electrical impedance spectroscopy (EIS) data acquisition system.

A portable impedance converter network analyzer was used in EIS for measuring the leaf impedances of canola, wheat, soybeans, and corn by varying the frequency in a range of 5–15 kHz with 100 Hz intervals. A total of 186 samples (n) were selected: 48 each for canola and wheat, and 45 each for soybeans and corn. The selected crops are chosen based on the applications and extensive use. A low frequency range was considered because of obtained high impedance profiles for the selected crops in different experiments.

The seeds were sown on 8 February 2019 in the greenhouse environment which was maintained temperature of 23–30 degrees Celsius during daytime and 18–22 degrees Celsius at night; the environment was controlled at 45%–55% relative humidity. The experiment was conducted on a total of 64 plants. Individually, 16 plants/pots of a commercial canola, wheat, soybeans, and corn were chosen. After sowing the seeds, all pots were watered with 200 mL in the first three weeks till 4 March 2019. In the next two weeks, until the measurement of every 4 plants/pots, each crop was watered with 50 mL, 100 mL, 150 mL, and 200 mL, respectively, in every 24 h. The different water volumes were selected to observe the variations in leaf impedance profiles of the crops in various water stress. The plants were fertilized with 15-30-15 N-P-K (15% nitrogen, 30% phosphorus, and 15%

potassium) fertilizer at 4 gram/liter rate. The leaf impedance measurements were performed using EIS on vegetative tissue during 14–15 March 2019 (i.e., 5 weeks after sowing).

The impedance (Z) of the leaf samples was measured at 100 Hz intervals within the selected frequency range by using a calibration of 7.5 k Ω resistance [26]. The impedance magnitude was controlled by the calibrated gain factor and the corresponding frequency as well. The impedance at each frequency point is considered as a feature (k). Therefore, a total of 101 features were selected initially for each crop considering frequencies of $f_1, f_2, \dots, f_{101}$, respectively. For each sample of the crops the measured dataset consisted 101 impedance values and the total impedance values were taken in a dataset based on the given number of samples of each crop (canola and wheat: 48×101 , soybeans and corn: 45×101).

2.2. Relative Water Content (RWC) Measurement

Next, the crop leaves were cut, and the fresh weight of the leaf samples was measured using a weight scale; the leaves were then oven dried at 60 °C for 48 h. Later, the dry weight of the samples was taken on 18–19 March 2019. Using the previous works [5–7], the relative water content (RWC) of the leaf samples for different crops was calculated as follows:

$$RWC = ((\text{Fresh weight} - \text{Dry weight}) / \text{Fresh weight}) \times 100\% \quad (1)$$

The calculated RWCs for the different samples of canola varied from 84.5%–89.6%, for wheat 72.4%–85.6%, for soybean 60.4%–80.6%, and for corn 80.4%–88.7%.

2.3. Statistical Analysis

The correlation coefficient (R) between the leaf impedance (Z) and relative water content (RWC) was determined for single and multiple regressions using the least square method. The results were obtained and validated by analysis of variance (ANOVA) T-test/F-test with the help of PrimaXL Data Analysis ToolPak. The corresponding coefficient of determination (R^2), adjusted R^2 , and root mean square error (rmse) were also determined using the expressions below:

$$R^2 = \frac{\sum_{i=1}^n (\hat{y}_i - \bar{y})^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (2)$$

$$R_{adj}^2 = 1 - \frac{\sum_{i=1}^n (\hat{y}_i - \bar{y})^2 / (n - k - 1)}{\sum_{i=1}^n (y_i - \bar{y})^2 / (n - 1)} \quad (3)$$

$$rmse = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i)^2}{n - k - 1}} \quad (4)$$

where, y is the actual relative water content, $\hat{y}$ is the predicted relative water content, $\bar{y}$ is the mean of the actual relative water contents, n is the number of observations of different crops, and k is the number of features in the given frequency range [27–32].

Initially, the leaf impedance profiles of the crops were observed by varying frequency at different water levels. The RWC was calculated for different applied amount of water to the samples of any individual crop. The correlation between leaf impedance and RWC for different crops was obtained at 101 frequency points using XLMiner Analysis ToolPak. A single regression analysis was made between actual and predicted RWC for the frequency with maximum correlation point. No strong correlation among the leaf impedance and RWC was found at single feature, and therefore, a multiple regression analysis was considered to obtain better correlation results using an increased number of features. From all the features in a dataset, the number of features was selected as $k = n - 2$ considering

the maximum correlation points between RWC and leaf impedance for the given observations (n) of any crop.

Principal component analysis (PCA) is a popular dimensionality reduction (DR) approach of multiple regression, and mostly applicable for feature selection in hyperspectral image analysis, but it works well for the variables that are strongly correlated [33]. Multi-collinearity among multiple features were examined, and most of the highly correlated features with correlation of 95% or above, and the corresponding variance inflation factor (VIF) of 10 or above, were removed. In this work, instead of using PCA, the number of features in a dataset was selected accordingly using the wrapper backward elimination method to perform the multiple regression analysis with the ANOVA tests. From the dataset for each crop, important features were checked sequentially with the threshold probability of rejection of null hypothesis, $p \leq 0.05$ using individual T-test. The probability might be affected with highly correlated features in the model for high values of VIF. The features with the large p -value (i.e., greater than 0.05) were removed from the regression of the dataset, and the features with p -values less than or equal to 0.05 were considered for the prediction. After a few iterations, the training and validations were performed using overall F-test ($p \leq 0.05$), and the optimized multiple regression results were obtained for different crops.

3. Results

3.1. Crop Leaf Impedance Measurement

Three leaves from individual plants of each species were sampled for measuring impedance as shown in Figure 2. Figure 3 shows the average impedance profiles of the leaf samples at varying frequency for the different watering regimes. The leaf impedance profiles were examined at different water status to obtain the correlations with the leaf water contents. It is observed that the leaf impedance decreases with the increase of frequency from 5 to 15 kHz, and with the increase of plant water levels from 50 to 200 mL. The average leaf impedance varies from 6000–10,000 ohm for all the crops in the given frequency range. The impedance profile is high for soybeans and corn compared to canola and wheat in the different watering regimes.

Figure 2. Leaf samples of (a) canola, (b) wheat, (c) soybeans, and (d) corn, at 50 mL water level.

Figure 3. Plots of frequency versus leaf impedance for (a) canola, (b) wheat, (c) soybeans, and (d) corn, at different water levels. Leaf impedance decreased with the increase of frequency and also with the increase of water level in the crops. A good impedance profile was observed for canola compared to the other crops with the equal distribution of water level.

3.2. Single Regression Analysis

At first, with the use of single regression analysis, the correlations between leaf impedance, Z , and RWC for a single feature, were found for canola, wheat, soybeans, and corn as shown in Figure 4. The maximum correlation coefficients (R) of the crops were obtained at 6.3 kHz, 7 kHz, 12.8 kHz, and 5.8 kHz, respectively. The correlation was positive for canola and corn, where the RWC index increased with the increase of leaf impedance. On the other hand, a negative correlation was found for wheat and soybeans, where the RWC index decreased with the increase of leaf impedance. A moderate correlation was obtained for canola and wheat with coefficients of $R = 0.50$ (linear), 0.55 (polynomial) and $R = -0.38$ (linear), -0.47 (polynomial), respectively. Although the correlation was poor for soybeans and corn with coefficients of $R = -0.25$ (linear), -0.26 (polynomial), and $R = 0.20$ (linear), 0.48 (polynomial), respectively.

The predicted RWC index was obtained for the selected features of the crops using single linear regression and the result was compared with the actual RWC index as shown in Figure 5. The coefficient of determination (R^2) for canola was obtained as 0.25 at 6.3 kHz, where $p = 0.0002$ (T-test), and for wheat was 0.15 at 7 kHz, where $p = 0.006$ (T-test). On the other hand, the coefficient of determination for soybeans was obtained as 0.06 at 12.8 kHz, where $p = 0.09$ (T-test) and for corn was 0.04 at 5.8 kHz, where $p = 0.16$ (T-test). The close approximation results were found for soybeans and corn. A good prediction was determined for canola compared to the other crops considering a single feature in the model.

Figure 4. Linear and polynomial correlations for canola with (a) $R = 0.50$ and (b) $R = 0.55$ at 6.3 kHz, for wheat with (c) $R = -0.38$ and (d) $R = -0.47$ at 7 kHz, for soybeans with (e) $R = -0.25$ and (f) $R = -0.26$ at 12.8 kHz, and for corn with (g) $R = 0.20$ and (h) $R = 0.48$ at 5.8 kHz.

Figure 5. Single linear regression for (a) canola with $R^2 = 0.25$ at 6.3 kHz, (b) wheat with $R^2 = 0.15$ at 7 kHz, (c) soybeans with $R^2 = 0.06$ at 12.8 kHz, and (d) corn with $R^2 = 0.04$ at 5.8 kHz.

The obtained correlation and single regression results of all the selected crops are presented in Table 1. The regression models represented the correlation between the leaf relative water contents and measured leaf impedances for a particular feature. The maximum correlation coefficient (R) of 0.50 (linear) and coefficient of determination (R^2) of 0.25 (linear) were obtained for canola at 6.3 kHz compared to the other crops. A high accuracy was found in the model presented for canola with single linear regression, and above 85% RWC was predicted for the measured leaf impedance in the selected feature. On the other hand, less than 85% RWC was predicted for wheat at 7 kHz. Additionally, less than 80% RWC was predicted for soybeans at 12.8 kHz and more than 84% RWC was predicted for corn at 5.8 kHz from the obtained single linear regression models. Overall, the correlations were not strong enough with single regression analysis for the selected crops.

Table 1. Maximum correlations and single regression models for different crops (considering the probability of rejection of null hypothesis, $p \leq 0.05$ with positive ANOVA tests).

Canola	Wheat
Linear: $R = 0.50$ at 6.3 kHz $RWC = 0.0004Z + 84.92$	Linear: $R = -0.38$ at 7 kHz $RWC = -0.0006Z + 85.88$
Polynomial (order 2): $R = 0.55$ at 6.3 kHz $RWC = -4 \times 10^{-8}Z^2 + 0.0011Z + 82.30$	Polynomial (order 2): $R = -0.47$ at 7 kHz $RWC = -1 \times 10^{-7}Z^2 + 0.0012Z + 78.93$
Soybeans	Corn
Linear: $R = -0.25$ at 12.8 kHz $RWC = -0.0016Z + 87.544$	Linear: $R = 0.20$ at 5.8 kHz $RWC = 0.0003Z + 83.146$
Polynomial (order 2): $R = -0.26$ at 12.8 kHz $RWC = 3 \times 10^{-7}Z^2 - 0.0069Z + 108.25$	Polynomial (order 2): $R = 0.48$ at 5.8 kHz $RWC = -2 \times 10^{-7}Z^2 + 0.0043Z + 66.99$

3.3. Multiple Regression Analysis

In multiple regression, all features were taken along with the response variable in the dataset. Correlations were analyzed, not only between dependent and independent variables, but also between all independent variables. The number of features were removed where the independent variables were highly correlated. Multi-collinearity was tested with the calculation of variance inflation factor (VIF), which identified the correlation between independent variables and the strength of that correlation. A high value of VIF (equal or greater than 10) or low value of tolerance ($=1/\text{VIF}$) indicated multi-collinearity among the multiple features, where the correlation between independent variables was 95% or above. The features that were affected with the multi-collinearity problem were removed from the dataset. The final dataset was prepared for multiple regression by the backward elimination of features with individual T-test.

The predicted RWC values were obtained for different observations of each crop using multiple linear regression and the residuals were calculated by comparing with the actual values as shown in Figure 6. Canola was with lower residuals of -0.33 to 0.34 , and those were contributing a better prediction of leaf water contents compared to the other crops. On the other hand, soybeans were with higher residual values of -8.16 to 7.8 , and in turn a moderate prediction was obtained. The residuals for wheat and corn were also obtained from -2.4 to 1.5 and -4.5 to 2.47 , respectively.

Figure 6. Plots of number of observations versus value of residuals for (a) canola, (b) wheat, (c) soybeans, and (d) corn with water contents. The residuals for canola were very low compared to the other crops.

The actual versus predicted RWC for four different crops in multiple regression was obtained as shown in Figure 7. The lower value of residuals of canola and wheat helped in achieving a high value of coefficient of determination (R^2) compared to soybeans and corn. A strong correlation between

RWC and leaf impedance was obtained for all the crops, and the optimized multiple regression results with the overall F-test are summarized in Table 2.

The correlation coefficients (R) of 0.99, 0.96, 0.79, and 0.78 were obtained for canola, wheat, soybeans, and corn with the corresponding rmse values of 0.3%, 1.44%, 3.36%, and 1.8%, respectively. The optimized features were selected as 36 (f_1 to f_{55}) for canola, 31 (f_1 to f_{85}) for wheat, 10 (f_{14} to f_{82}) for soybeans, and 20 (f_3 to f_{101}) for corn. The required number of features was high for canola and wheat to achieve satisfactory regression performance, which contributed in achieving very strong correlation between leaf impedance and relative water contents. A better regression performance was obtained for canola compared to the other crops because of its lower rmse value.

Figure 7. Plots of actual versus predicted relative water content (RWC) using multiple linear regression for (a) canola with $R^2 = 0.98$, (b) wheat with $R^2 = 0.92$, (c) soybeans with $R^2 = 0.63$, and (d) corn with $R^2 = 0.62$. The best predicted results were obtained for canola and wheat by performing the multiple regression.

Table 2. Multiple linear regression analysis for four different crops with water contents (considering the probability of rejection of null hypothesis, $p \leq 0.05$ with positive ANOVA tests).

Canola	Wheat	Soybeans	Corn
$f = 5\text{--}10.4$ kHz ($n = 48$, $k = 36$)	$f = 5\text{--}13.4$ kHz ($n = 48$, $k = 31$)	$f = 6.3\text{--}13.1$ kHz ($n = 45$, $k = 10$)	$f = 5.2\text{--}15$ kHz ($n = 45$, $k = 20$)
$R = 0.99$, $R^2 = 0.98$, adjusted $R^2 = 0.93$, rmse = 0.30%, $p = 4.8 \times 10^{-6}$ (F-test)	$R = 0.96$, $R^2 = 0.92$, adjusted $R^2 = 0.77$, rmse = 1.44%, $p = 0.0001$ (F-test)	$R = 0.79$, $R^2 = 0.63$, adjusted $R^2 = 0.51$, rmse = 3.36%, $p = 5.3 \times 10^{-5}$ (F-test)	$R = 0.78$, $R^2 = 0.62$, adjusted $R^2 = 0.31$, rmse = 1.80%, $p = 0.05$ (F-test)

The proposed coefficients of multiple linear regression models for the prediction of RWC of the four different crops are presented in Table 3. The overfitting was reduced in the model by the backward elimination of features with p -values greater than the threshold. The individual coefficients were multiplied with the measured leaf impedances of selected features and the summation of the results added to the coefficient of intercept to obtain the predicted RWC for the crops.

Table 3. Proposed coefficients of multiple linear regression models for the prediction of RWC of four different crops (considering the probability of rejection of null hypothesis, $p \leq 0.05$ with positive ANOVA tests, and the selected features ranging frequencies from f_1 to f_{101}).

Canola	Wheat
87.08 (intercept), 0.0012 (f_1), 0.0050 (f_2), −0.0074 (f_4), −0.0036 (f_5), 0.0009 (f_7), 0.0137 (f_8), −0.0156 (f_9), 0.0042 (f_{10}), −0.0037 (f_{11}), −0.0024 (f_{12}), 0.0023 (f_{13}), 0.0042 (f_{14}), 0.0042 (f_{15}), 0.0034 (f_{16}), −0.0074 (f_{17}), −0.0058 (f_{18}), 0.0019 (f_{19}), 0.0080 (f_{20}), −0.0095 (f_{22}), 0.0040 (f_{23}), 0.0031 (f_{24}), −0.0023 (f_{25}), 0.0072 (f_{26}), −0.0077 (f_{27}), −0.0093 (f_{28}), 0.0089 (f_{29}), 0.0090 (f_{30}), −0.0097 (f_{32}), −0.0057 (f_{34}), 0.0072 (f_{35}), 0.0074 (f_{36}), −0.0091 (f_{39}), 0.0025 (f_{40}), 0.0030 (f_{42}), −0.0044 (f_{45}), 0.0022 (f_{55})	80.03 (intercept), 0.0068 (f_1), −0.0250 (f_4), 0.0196 (f_5), 0.0253 (f_6), −0.0254 (f_7), −0.0193 (f_9), 0.0309 (f_{10}), −0.0167 (f_{11}), −0.0354 (f_{13}), 0.0523 (f_{14}), −0.0438 (f_{16}), 0.0222 (f_{17}), 0.0332 (f_{18}), 0.0132 (f_{19}), −0.0670 (f_{20}), 0.0216 (f_{21}), 0.0116 (f_{22}), −0.0144 (f_{24}), −0.016 (f_{25}), 0.0700 (f_{26}), −0.0412 (f_{27}), −0.0294 (f_{31}), 0.0341 (f_{32}), −0.0063 (f_{34}), 0.0173 (f_{77}), −0.0197 (f_{78}), 0.0175 (f_{80}), −0.0725 (f_{81}), 0.0914 (f_{82}), −0.0414 (f_{83}), 0.0069 (f_{85})
Soybeans	Corn
70.18 (intercept), −0.0145 (f_{14}), 0.0245 (f_{17}), −0.0133 (f_{21}), 0.0072 (f_{22}), −0.0087 (f_{23}), 0.0053 (f_{27}), −0.0158 (f_{63}), 0.0166 (f_{64}), −0.0093 (f_{79}), 0.0088 (f_{82})	78.63 (intercept), −0.0052 (f_3), 0.02053 (f_5), −0.0182 (f_6), 0.00327 (f_9), −0.003 (f_{72}), 0.00766 (f_{77}), −0.0156 (f_{79}), 0.01044 (f_{80}), −0.0074 (f_{83}), 0.00784 (f_{84}), −0.0123 (f_{87}), 0.02107 (f_{88}), 0.00919 (f_{89}), −0.0282 (f_{90}), 0.02423 (f_{92}), −0.0156 (f_{93}), −0.0104 (f_{94}), 0.01106 (f_{97}), 0.00884 (f_{98}), −0.007 (f_{101})

Next, the multiple regression analysis was performed for a total of 186 combined observations of all the crops with the selection of features in frequency ranges 5–15 kHz. The residuals were obtained from −10.8 to 10.02 for different observations from the difference between actual and predicted RWC as shown in Figure 8. The overall multiple regression results are summarized in Table 4. A maximum correlation coefficient (R) of 0.70 was obtained with rmse of 4.3% for 37 features ranging 5–14.8 kHz. The obtained coefficients were proposed to predict the leaf RWC in combined observations of the crops.

Figure 8. (a) Residuals, and (b) multiple linear regression for canola + wheat + soybeans + corn combined observations. The overall coefficient of determination (R^2) was extracted as 0.50.

Table 4. Multiple regression analysis for the combined observations of the crops with water contents (considering the probability of rejection of null hypothesis, $p \leq 0.05$ with positive ANOVA tests, and the selected features ranging frequencies from f_1 to f_{101}).

Canola + Wheat + Soybeans + Corn	
$f = 5\text{--}14.8 \text{ kHz } (n = 186, k = 37)$	
$R^2 = 0.50, R = 0.70, \text{adjusted } R^2 = 0.37, \text{rmse} = 4.3\%,$ $p = 7.7 \times 10^{-10}$ (F-test)	86.72 (intercept), 0.0029 (f_1), -0.0076 (f_2), 0.0095 (f_3), -0.0064 (f_4), 0.0054 (f_{10}), -0.0067 (f_{11}), 0.0047 (f_{12}), -0.0042 (f_{16}), 0.0043 (f_{19}), -0.0018 (f_{22}), -0.0031 (f_{25}), 0.0042 (f_{28}), -0.0028 (f_{31}), 0.0058 (f_{35}), -0.0053 (f_{36}), 0.0047 (f_{39}), -0.0089 (f_{41}), 0.0095 (f_{44}), -0.0098 (f_{45}), 0.0064 (f_{48}), -0.0080 (f_{52}), 0.0107 (f_{54}), 0.0055 (f_{55}), -0.0193 (f_{57}), 0.0079 (f_{58}), 0.0095 (f_{64}), -0.0216 (f_{65}), 0.0141 (f_{66}), 0.0039 (f_{77}), -0.0103 (f_{80}), 0.0229 (f_{81}), -0.0162 (f_{82}), -0.0108 (f_{86}), 0.0137 (f_{87}), -0.0097 (f_{97}), 0.0168 (f_{98}), -0.0094 (f_{99})

4. Discussion

The regression analysis was performed for the validation of the predicted leaf water contents by comparing the actual water contents. At first, single regression results were found for all the crops and the corresponding regression models were extracted using a single feature. The RWC was related to the measured leaf impedance according to the extracted models. Canola showed a maximum correlation coefficient of 0.50 (linear) between leaf impedances and water contents compared to the other crops, but the overall outcome using single regression was not satisfactory. Important features at frequencies of 6.3 kHz, 7 kHz, 12.8 kHz, and 5.8 kHz were extracted considering single regression to predict the leaf water contents of canola, wheat, soybeans, and corn, respectively. The obtained coefficient of determination (R^2) was too small for all the crops, especially for soybeans and corn, where the results were not satisfactory for a single feature.

Later, multiple regression analysis was employed by increasing the number of features in the given frequency range of 5–15 kHz. The predicted water contents were obtained using multiple regression analysis, and the corresponding residuals were found from the difference between the actual and predicted water contents. Highly predictive results were obtained for the lower residuals considering multiple features. It was found that the correlation coefficient and the coefficient of determination increase with the increase of the number of features for the taken samples of the crops. The improved results were found with the help of positive ANOVA tests. The important multiple features at frequencies of 5–10.4 kHz, 5–13.4 kHz, 6.3–13.1 kHz and 5.2–15 kHz were extracted with individual T-test by employing multiple regression to predict the relative water contents of canola, wheat, soybeans, and corn leaves, respectively. A very high correlation was obtained for canola ($R = 0.99$) and wheat ($R = 0.96$) compared to that of soybeans ($R = 0.79$) and corn ($R = 0.78$), because of lower rmse values. The coefficient of determinations (R^2) of the crops were obtained as 0.98, 0.92, 0.63, and 0.62 with the rmse values of 0.3%, 1.44%, 3.36%, and 1.8%, respectively for $p \leq 0.05$. Lower rmse of canola contributed in achieving better prediction of leaf water contents compared to that of the other crops. The obtained results in this study were satisfactory in comparison with the previous works [5–10]. In those works, leaf water contents of different crops were determined using optical spectroscopy. More than 400 samples were considered for 11 different species and the coefficient of determination of 0.93–0.96 with 7.1% rmse was obtained by Arshad et al. [5]. Different sensitive wavelengths of 11–1041 nm for 624 miscanthus samples were proposed by Jin et al. [6]. The coefficient of determination of 0.92 with 0.73% rmse for 75 samples of epipremnum aureum was obtained by Zhang et al. [7]. The leaf water content in soybeans was also accessed by Kovar et al. [8], and a correlation coefficient of 0.786 with 12.8% rmse was obtained using hyperspectral or infrared spectroscopy. Leaf water contents were also measured using terahertz laser optical spectroscopy by Baldacci et al. [9], and the correlation coefficient of 0.81 with 4.4% rmse for 40 wheat leaf samples was obtained by Li et al. [10]. Soil moisture contents by Umar et al. [18] and carrot moisture contents by Kertész et al. [14]

were also determined by employing EIS. This study was limited to four different crop species with a total of 186 samples in vegetative growth stage only, but very strong correlations were found for the individual crop species, especially for canola (48 samples) and wheat (48 samples) with lower rmse values of 0.3% and 1.44%, respectively for $p \leq 0.05$. The proposed EIS device is cheaper than the optical spectroscopy methods. The overall manufacturing cost including labor and overhead in this study is approximately \$150 USD (EIS board and components: \$100, labor and overhead: \$50), which is much less than optical spectroscopy methods. The leaf water content of corn was determined using a sensor based on the Four-Electrode method by Zheng et al. in 2015, as reported by J6cs6k et al. [11]. A detector was developed to find a correlation between leaf electrical property and relative water content, and the highest negative correlation was obtained in the seedling stage. The moisture content of carrot slices during drying was determined using LCR meter by Kert6sz et al. [14]. A good correlation between carrot impedance and moisture content was obtained for different slices by varying drying time. Although, in this study, a pair of ECG electrodes connected to the AD5933 evaluation board was used to determine the leaf water content of canola, wheat, soybeans, and corn. A strong correlation between leaf impedance and RWC was found considering multiple features under varying water stress in vegetative growth stage.

In order to generalize the EIS model for the prediction of leaf water contents, the combined observations of the crops were considered and employed multiple regression for a dataset of 186×101 impedance values. A strong correlation of $R = 0.7$ and coefficient of determination (R^2) of 0.5 were obtained with 4.3% rmse. The overall correlation was decreased with the increase of rmse in combination with the samples of multiple crops. The physiological properties were different for different crops. It was found in the analysis that the EIS technique is model-dependent, and the optimized models worked better for the individual dataset of any particular crop. The accuracy of the models might be varied with the variation of growth stages of the crops because of different water stress and different physiological properties. For each crop, different features were selected with the model coefficients based on the positive ANOVA tests. In canola and wheat, the additional individual features contributed less in correlation and hence, higher number of features were required compared to soybeans and corn.

Appropriate fitting of the models shows the accuracy of the measurements using the EIS evaluation board. The reduction of overfitting in the model is a challenging issue. To ensure appropriate feature selection, considering p -value as lower than or equal to the threshold is essential. Multi-collinearity among multiple features was considered during the feature selection in a dataset for evaluating appropriate regression models using ANOVA T-tests. Highly correlated features were removed by calculating VIF, and also with the help of the wrapper backward elimination method to obtain the optimized regression model. Therefore, a good correlation was found between leaf impedances and leaf water contents for each crop using multiple features.

5. Conclusions

The proposed study shows that precise measurement of leaf impedance allows the estimation of water content in crops under different water stress conditions. The measurements were carried out in vegetative growth stage. A strong correlation between leaf water contents and leaf impedances was found considering multiple features in the regression model. Multi-collinearity among the features was considered during feature selection using the wrapper backward elimination method. The optimized regression model coefficients were proposed for canola, wheat, soybeans, and corn to determine the leaf water contents rapidly and efficiently using a portable and non-invasive EIS method. A comparative statistical analysis among the four different crops was performed, and the maximum correlation coefficient (R) of 0.99, the coefficient of determination (R^2) of 0.98, and rmse of 0.30% were obtained for canola in the frequency range of 5–10.4 kHz. The proposed model coefficients were sensitive to the leaf water contents of different crops. The study indicated that non-destructive analysis of impedance measurement using the EIS device could be successfully used for the efficient determination of leaf

water contents in multiple crops. The effectiveness of this EIS method can be considered in multiple growth stages of the crops in the future.

Author Contributions: R.B. performed the experiments, analyzed the data, and wrote the draft of the manuscript. K.A.W. and A.D. suggested the experiments and data analysis, helped edit the draft, and provided critical comments to improve the paper. R.S. facilitated the set up of the greenhouse experiment, helped edit the draft, and provided critical comments to improve the paper. R.F. and A.S.M. helped edit the draft and provided critical comments to improve the paper. All authors have read and agreed to the published version of the manuscript.

Funding: This work is supported by the Canada First Research Excellence Fund (CFREF) through the Global Institute for Food Security (GIFS), University of Saskatchewan, Canada. RS received funding support from Agriculture and Agri-Food Canada.

Acknowledgments: The authors would like to extend their appreciation to Shankar Pahari, Agriculture and Agri-Food Canada, Saskatoon, Saskatchewan, Canada, for his assistance during the experimental period.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ihuoma, S.O.; Madramootoom, C.A. Recent advances in crop water stress detection. *Comput. Electron. Agric.* **2017**, *141*, 267–275. [\[CrossRef\]](#)
2. Evans, J.R. Improving photosynthesis. *Plant Physiol.* **2013**, *162*, 1780–1793. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Irmak, S.; Haman, D.Z.; Bastug, R. Determination of crop water stress index for irrigation timing and yield estimation of corn. *Agron. J.* **2000**, *92*, 1221–1227. [\[CrossRef\]](#)
4. Tomkiewicz, D.; Piskier, T. A plant based sensing method for nutrition stress monitoring. *Precis. Agric.* **2012**, *13*, 370–383. [\[CrossRef\]](#)
5. Arshad, M.; Ullah, S.; Khurshid, K.; Ali, A. Estimation of leaf water content from mid- and thermal-infrared spectra by coupling genetic algorithm and partial least squares regression. *J. Appl. Remote Sens.* **2018**, *12*, 22203. [\[CrossRef\]](#)
6. Jin, X.; Shi, C.; Yu, C.Y.; Yamada, T.; Sacks, E.J. Determination of leaf water content by visible and near-infrared spectrometry and multivariate calibration in miscanthus. *Front. Plant Sci.* **2017**, *8*, 1–8. [\[CrossRef\]](#)
7. Zhang, Q.; Li, Q.; Zhang, G. Rapid determination of leaf water content using VIS/NIR spectroscopy analysis with wavelength selection. *Spectrosc. Int. J.* **2012**, *27*, 93–105. [\[CrossRef\]](#)
8. Kovar, M.; Brestic, M.; Sytar, O.; Barek, V.; Hauptvogel, P.; Zivcak, M. Evaluation of hyperspectral reflectance parameters to assess the leaf water content in soybean. *Water* **2019**, *11*, 443. [\[CrossRef\]](#)
9. Baldacci, L.; Pagano, M.; Masini, L.; Toncelli, A.; Carelli, G.; Storchi, P.; Tredicucci, A. Non-invasive absolute measurement of leaf water content using terahertz quantum cascade lasers. *Plant Methods* **2017**, *13*, 1–7. [\[CrossRef\]](#)
10. Li, B.; Long, Y.; Yang, H. Measurements and analysis of water content in winter wheat leaf based on terahertz spectroscopy. *Int. J. Agric. Biol. Eng.* **2018**, *11*, 178–182. [\[CrossRef\]](#)
11. Jócsák, I.; Végvári, G.; Vozáry, E. Electrical impedance measurement on plants: A review with some insights to other fields. *Exp. Plant Physiol.* **2019**, *31*, 359–375.
12. Muñoz-Huerta, R.F.; Ortiz-Melendez, A.J.; Guevara-Gonzalez, R.G.; Torres-Pacheco, I.; Herrera-Ruiz, G.; Contreras-Medina, L.M.; Prado-Olivarez, J.; Ocampo-Velazquez, R.V. An analysis of electrical impedance measurements applied for plant N status estimation in lettuce (*Lactuca sativa*). *Sensors* **2014**, *14*, 11492–11503. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Postic, F.; Doussan, C. Benchmarking electrical methods for rapid estimation of root biomass. *Plant Methods* **2016**, *12*, 1–11.
14. Kertész, A.; Hlaváčová, Z.; Vozáry, E.; Staroňová, L. Relationship between moisture content and electrical impedance of carrot slices during drying. *Int. Agrophys.* **2015**, *29*, 61–66. [\[CrossRef\]](#)
15. Juansah, J.; Budiastra, I.W.; Dahlan, K.; Seminar, K.B. The prospect of electrical impedance spectroscopy as non-destructive evaluation of citrus fruits acidity. *IJETAE* **2012**, *2*, 58–64.
16. Meiqing, L.; Jinyang, L.; Xinhua, W.; Wenjing, Z. Early diagnosis and monitoring of nitrogen nutrition stress in tomato leaves using electrical impedance spectroscopy. *Int. J. Agric. Biol. Eng.* **2017**, *10*, 194–205.
17. Cavalieri, R.; Bertemes-Filho, P. Plant tissue differentiation using electrical impedance spectroscopy with deep neural networks. *Int. J. Biosen. Bioelectron.* **2020**, *6*, 20–23.

18. Umar, L.; Setiadi, R.N. Low cost soil sensor based on impedance spectroscopy for in-situ measurement. *Aip. Conf. Proc.* **2015**, *1656*, 040005.
19. Ozier-Lafontaine, H.; Bajazet, T. Analysis of root growth by impedance spectroscopy (EIS). *Plant Soil* **2005**, *277*, 299–313. [[CrossRef](#)]
20. Greenham, C.G.; Randall, P.J.; Müller, W.J. Studies of phosphorus and potassium deficiencies in *Trifolium subterraneum* based on electrical measurements. *Can. J. Bot.* **1982**, *60*, 634–644. [[CrossRef](#)]
21. Zhao, X.; Zhuang, H.; Yoon, S.C.; Dong, Y.; Wang, W.; Zhao, W. Electrical impedance spectroscopy for quality assessment of meat and fish: A review on basic principles, measurement methods, and recent advances. *J. Food Quality* **2017**, *2017*, 1–16. [[CrossRef](#)]
22. Grossi, M.; Riccò, B. Electrical impedance spectroscopy (EIS) for biological analysis and food characterization: A review. *J. Sens. Sens. Syst.* **2017**, *6*, 303–325.
23. Mizukami, Y.; Yamada, K.; Sawai, Y.; Yamaguchi, Y. Measurement of fresh tea leaf growth using electrical impedance spectroscopy. *Agric. J.* **2007**, *2*, 134–139.
24. Margo, C.; Katrib, J.; Nadi, M.; Rouane, A. A four-electrode low frequency impedance spectroscopy measurement system using the AD5933 measurement chip. *Physiol. Meas.* **2013**, *34*, 391–405. [[PubMed](#)]
25. Hamed, K.B.; Zorrig, W.; Hamzaoui, A.H. Electrical impedance spectroscopy: A tool to investigate the responses of one halophyte to different growth and stress conditions. *Comput. Electron. Agricult.* **2016**, *123*, 376–383. [[CrossRef](#)]
26. Basak, R.; Wahid, K.; Dinh, A. Determination of leaf nitrogen concentrations using electrical impedance spectroscopy in multiple crops. *Remote Sens.* **2020**, *12*, 566. [[CrossRef](#)]
27. Sun, J.; Yang, J.; Shi, S.; Chen, B.; Du, L.; Gong, W.; Song, S. Estimating rice leaf nitrogen concentration: Influence of regression algorithms based on passive and active leaf reflectance. *Remote Sens.* **2017**, *9*, 951. [[CrossRef](#)]
28. El-Mohsen, A.A.A. Correlation and regression analysis in barley. *World Essays J.* **2013**, *1*, 88–100. [[CrossRef](#)]
29. Mokarram, M.; Bijanzadeh, E. Prediction of biological and grain yield of barley using multiple regression and artificial neural network models. *Aust. J. Crop. Sci.* **2016**, *10*, 895–903. [[CrossRef](#)]
30. Liang, L.; Di, L.; Huang, T.; Wang, J.; Lin, L.; Wang, L.; Yang, M. Estimation of leaf nitrogen content in wheat using new hyperspectral indices and a random forest regression algorithm. *Remote Sens.* **2018**, *10*, 1940. [[CrossRef](#)]
31. Riccardi, M.; Mele, G.; Pulvento, C.; Lavini, A.; d’Andria, R.; Jacobsen, S.-E. Non-destructive evaluation of chlorophyll content in quinoa and amaranth leaves by simple and multiple regression analysis of RGB image components. *Photosynth. Res.* **2014**, *120*, 263–272. [[PubMed](#)]
32. Odabas, M.S.; Kayhan, G.; Ergun, E.; Senyer, N. Using artificial neural network and multiple linear regression for predicting the chlorophyll concentration index of Saint John’s wort leaves. *Commun. Soil Sci. Plant. Anal.* **2016**, *47*, 237–245. [[CrossRef](#)]
33. Liu, L.; Song, B.; Zhang, S.; Liu, X. A novel principal component analysis method for the reconstruction of leaf reflectance spectra and retrieval of leaf biochemical contents. *Remote Sens.* **2017**, *9*, 1113. [[CrossRef](#)]