Examination of Plant Physiological Monitoring alongside In-Vivo Four-Point-Probe Impedance Spectroscopy of live Tobacco plants

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***Abstract*—Electrical impedance spectroscopy for continuous plant monitoring has been offered as a promising method. This paper attempts to show the feasibility of the application of this method to monitor known physiological parameters. This is achieved by the evaluation of photosynthesis parameters in the plant, and the plant's response of stress values. This feasibility study is a step towards quantifying the impedance spectroscopy data obtained in plant physiology terms - thus moving towards the realization of a new sensing methodology.**

Keywords*—Electrical impedance spectroscopy, plant physiological monitoring, precision agriculture.

I. Introduction

Over the past few years, the need for new sensing technologies in precision agriculture has been well established, based on UN estimations for population growth and environmental changes [1]. This includes a range of advances for crop monitoring and early detection of plant stress in the field.

Impedance spectroscopy, in an in-vivo continuous coupling manner has been suggested as a promising method [2,3]. Results have shown that the method allows to detect changes in plant status. However, in order to further promote this methodology, it needs to be examined in terms used and accepted in the field of plant science. This will allow researchers to study and estimate the plant physiological response that this impedance measurement technology can offer. Here, we present initial tests that permit the quantification of the relationship between the impedance data and known parameters used for plant physiological assessment. This work is carried out using a continuous four-point-probe impedance spectroscopy setup, while simultaneously applying different plant physiology assessment methods.

The paper presents experiments using two different methods in plant studies to assess the possibilities of applying impedance spectroscopy in the field for the evaluation of the plant physiological status.

II. Methodology

A. Plant Physiological Monitoring

Plant monitoring in plant science and biology research is a well-established field. However, only a few non-destructive measurement methods that estimate living whole-plant status are available.

Gravimetry (or gravimetric analysis) [8] is a common method for the estimation of plant health, water usage and response. This measurement is based on continuous weight measurements of the specimen across time. The changes in weight can then be attributed to plant physiological behavior and response. When used for live plants, the measurements include the overall plant weight, taking into account the roots, stem, leaves, as well as the container, the ground and remaining water content. One of the difficulties of this approach, is the independent examination of the plant responses. Different systems have been offered in order to overcome this.

A state-of-the-art whole plant monitoring system was used. It was set up in a greenhouse facility in the Cereal Institute at the Tel-Aviv University. The system chosen is based on the known gravimetric analysis method and incorporates a range of additional sensors in the plant vicinity. This allows the measurement of "whole-plant transpiration" [4,6]. Transpiration defines the amount of water vapor loss from the plant and is usually measured across a single leaf or area. Nowadays using many high precision weight measurements, an estimation of the overall plant water usage can be made to assess plant transpiration. Transpiration is a fundamental and extremely complex process in the plant system [4]. Ongoing gravimetric data collection provides information regarding overall plant transpiration activity, which allows to study water use efficiency, improve the understanding of physiological activity as well as provide an overall view of the plant status. Here, continuous gravimetric measurements are obtained, the plant soil moisture is measured, and the environmental parameters are closely monitored.
The system provides the ability to measure the following parameters:

- Weight changes
- Transpiration rate/ Daily transpiration
- Volumetric water content

These parameters collected from the system allow the evaluation of *theta critical* θ_c, which is defined to be a value where the transpiration limiting factor results from soil moisture. This value provides a threshold that is indicative of plant water stress. This is an accepted parameter and

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explained clearly by Zheng et al. "better knowledge of critical soil moisture thresholds is also important to assess crop yield risks and ecosystem vulnerability from drought exposure" [5].

Figure 1 Tobacco plant monitored by both the impedance system, and the PlantArray gravimetric system set up at a greenhouse in Tel- Aviv.

Therefore, this was chosen as a comparative baseline for inspection of the electrical impedance spectroscopy data acquired. In order to study changes in the impedance values measured, the plants were also monitored using this method simultaneously. In this way, changes could be compared to well-known effects and values and attributed to different physiological phenomena across time.

A. Water Stress/ Drought Conditions

Experiments to study the response of the impedance measurements to water stress situations were carried out. To accomplish this, plants were regularly watered, showing repetitive behavior across the days. This means that the plants were sufficiently watered thus reaching watering saturation. Once this stability was reached, tests to examine of water stress situations could be undertaken. This was done in a monitored environment, where the amount of water given, and timing were planned with precision.

B. Light/ Dark Conditions

Plant growth, development and physiological regulation depend strongly on light [8]. Therefore, exposure to changes in lighting and daylight hour fluctuations are monitored in plant research and taken into account for physiological change studies. Growth chambers are used to control illumination times, as daylight hours fluctuate during the different seasons. Light sources that imitate natural light are widely available.

III. Experimental Setup

Two setups were used in this experimental work. The first was carried out in a growth chamber, where control of lighting conditions and other parameters is possible. Here experiments were run to assess leaf gas exchange parameters in conjunction with the impedance measurements. The second setup was in an outdoor greenhouse, where the plants were exposed to natural light conditions, while being monitored continuously with a gravimetric system.

A. Impedance Spectroscopy Setup

A Hioki IM3705 was used to collect data. The analyzer was coupled to the plant stem using a four-point-probe configuration in an in-vivo manner (this is described in detail in [9]). Measurements were collected at nine-minute intervals at 500mVrms, averaging across four and with a logarithmic frequency sweep between 40 Hz-1MHz.

B. Leaf Measurements

A portable Li-COR 6800 [7] tool for measurement of the leaf was used. It was mounted on to the leaf of a well-watered tobacco plant after calibration as in the user manual. The tool includes a gas exchange chamber, as well as florescence measurements. This allows assessment of different processes in photosynthetic activity detected from the leaf. Here, we inspect the values of stomatal conductance (g_{sw}), photosynthetic carbon assimilation (A) and H_2O release from the leaf (E), which is consistent with leaf transpiration. These parameters are all measured in units of mol m^{-2} s^{-1}. The measurements were collected across approximately 2 hours at nine minute intervals.

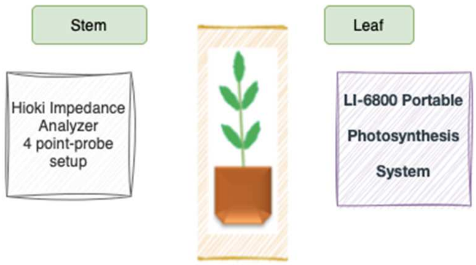

Figure 2 Schematic description of a plant monitored by both the impedance system, and the Licor Leaf monitoring system, set up in an indoor growth chamber.

C. Gravimetric System Description

In this work, the PlantArray ™, which is a whole-plant physiological performance measurement system [10]. The system includes a weights sensory system with accuracy of a few milligrams for gravimetric analysis. In addition, it includes a soil moisture sensor that is placed inside the container as an indicator of changes in the soil water content. Next, the system is connected to a controlled irrigation system where the plant can be hydrated, according to experiment requirements. In addition to the local monitoring of the plant, the greenhouse environment is continuously monitored and maintained. This includes measurements of temperature, light conditions and air humidity. The soil moisture is continuously monitored as well using soil probes combined into the system. The data collected by the system is logged into a soil-plant-atmosphere control (SPAC) software tool [6]. This functional phenotyping system is described in detail in a few publications by Sade et al. [11-12].

D. *Plant Physiological Studies Alongside Impedance Data*

In order to establish whether the impedance data collected might be an indication of the plant status, a range of different conditions were tested experimentally. Simultaneously, data was collected using the presented plant physiology methods, and the responses of the system examined. Here, two known plant physiological changes were introduced: light conditions and hydration status. These are examined in the results below.

IV. Results & Discussion

We start by examining the plant response to change in lighting conditions. Assessment is done with known photosynthetic parameters across time. Here, the measurement method is completed across a leaf of a tobacco plant. Next, we study the critical soil moisture threshold using weight derived plant transpiration values, as well as impedance measurement results.

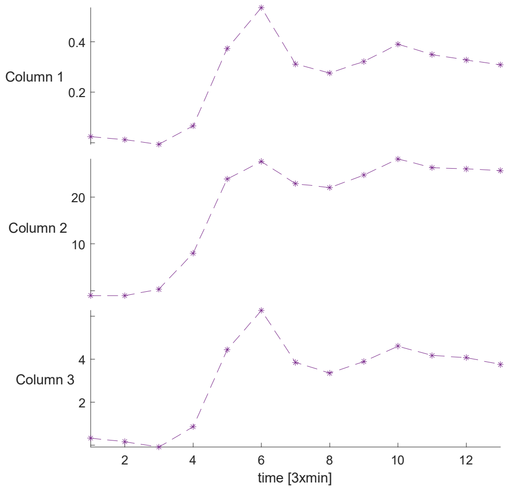

Figure 3 From top: gsw, A, E[mm] (corresponding to Column 1-3) of a tobacco leaf measurements monitored with Li-Cor in the growth chamber , across 2 hours, where initially complete dark conditions were available, and then full illumination was induces. We see a clear change in all gas exchange parameters indication photosynthetic activity.

a. *Leaf measurements*

In the growing chamber, under full watering saturation, illumination was induced from complete dark conditions to full illumination. In this scenario, we expect that the plant monitored will respond with the beginning of a transpiration process. This is measured across the leaf, using LICOR6800, after full calibration.

The measurements begin with dark conditions, and clearly stomatal conductance, photosynthetic carbon assimilation and leaf transpiration values are at low values (near zero). The illumination in the room is introduced after approximately 20 minutes. Results show that all parameters respond to this change. Inspecting both impedance magnitude and phase (shown in figures 4 and 5, across frequencies 100Hz, 10KHz and 100KHz respectively) we see that the impedance shows response to the same change in photosynthesis activity within the plant.

In relation to the time response, both measurements show closely related changes. However, each parameter measured using the Li-COR indicates a different process within the complex photosynthesis response. Therefore, we would suggest further studies using this method to expand the understanding of the electrical response measured.

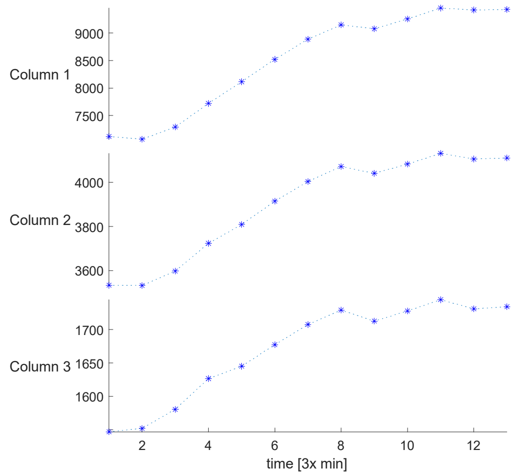

Figure 4 Impedance magnitude measured across time (2 hours), in the growth chamber where initially complete dark conditions were available, and then full illumination was induces. Across frequencies (from top: 10 Hz, 10KHz, 100KHz (corresponding to Column 1-3)) it is visible that the impedance magnitude increases once radiation starts.

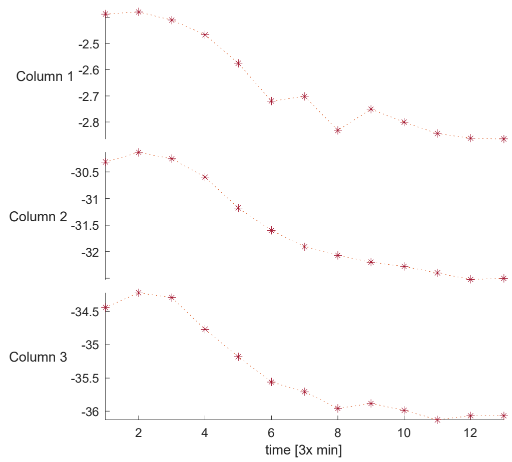

Figure 5 Impedance phase measured across time (2 hours), in the growth chamber where initially complete dark conditions were available, and then full illumination was induces. Across frequencies (10 Hz, 10KHz, 100KHz) (corresponding to Column 1-3) it is visible that the impedance phase decreases once radiation starts.

b. *Theta Critical Evaluation*

Initially, we inspect the known behavior of a tobacco plant monitored using the Plant Array gravimetric system. Examining the θ_c by definition, using a linear extrapolation, we get a value of approximately 0.085. In a similar manner, we examine the impedance spectroscopy data collected for these plants under the same conditions. This can be seen in figure 7. Examining the impedance magnitude values behavior in relation to the water content measured, we can classify three different areas in the plot. They are denoted in figure 7, by A, B and C. We learn from this that the impedance values seemingly show an additional sensitivity to a transition phase, prior to reaching the critical point, where the plant will already be experiencing a large amount of water stress. Once the volumetric water content decreases further, we notice a larger increase in these values. Here, a single frequency has been given as an example, however the

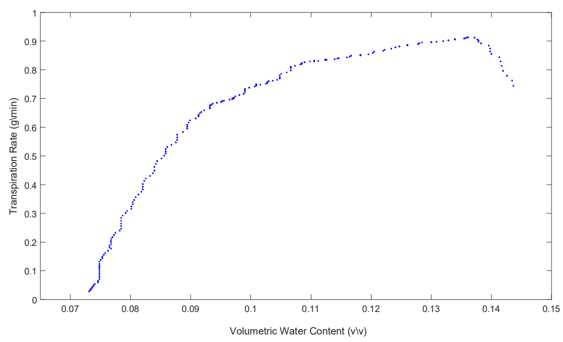

Figure 6 Transpiration rate vs. Volumetric water content measured using the gravimetric Plant Array system across tobbacco plant during dehydration periods. This shows a critical value below VWC of 0.9.

phenomena is expressed across the measured spectrum.

These findings suggest that the impedance measurement could be a convenient detector of early water stress in the plant, indicating changes in its physiological status prior to other available methods.

Overall, we have shown the feasibility of measuring a stress threshold, based on the threshold given by soil moisture limited plant transpiration limiting. The abundance of impedance data, also suggests the possibility of earlier detection, allowing the possibly to improve irrigation in a controlled manner. Further development alongside machine learning methods, such as using neural network approach (shown by Garlando et al. [13]) could promote the implementation of such electrical measurement techniques.

CONCLUSIONS

The electrical impedance spectroscopy method, allowing for continuous live plant monitoring has promising prospects for field implementation and low-cost device manufacturing.

Here, its value has been increased showing that it can offer an indication of critical values for physiological monitoring and can allow earlier sensitivity to stress. This is an initial step towards assessing the suggested impedance spectroscopy method for continuous plant monitoring, moving towards meaningful sensing capabilities. It has shown that the impedance data collected is compatible with other commonly used plant physiological monitoring and could be further studied and developed.

Further research and development are needed for the implementation of such electrical measurement techniques into actual agricultural and plant monitoring tools. This must be done in collaboration with plant scientists and agricultural specialists to tailor the tool and the method to actual needs in the field. Here we offered feasibility of further implementation. This would promote the access and the ease of use for this methodology across a ray of disciplines.

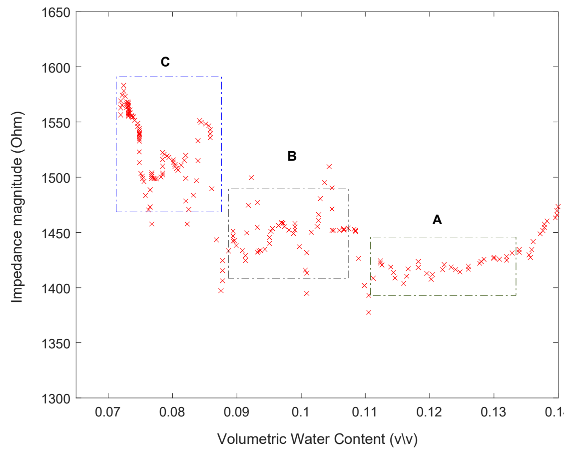

Figure 7 Impedance magnitude (taken at 100kHz as an example) vs. Volumetric water content measured using the gravimetric Plant Array system.

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