First bioimpedance assessment for detecting effects caused by environmental stimuli on Grapevine (*Vitis vinifera* L.): preliminary results

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First bioimpedance assessment for detecting effects caused by environmental stimuli on Grapevine (*Vitis vinifera* L.): preliminary results

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Abstract. Plants comprise biological tissues where bioimpedance could be measured. Changes in values of these impedance parameters within tissues are the result of changes in the chemical composition of the sap, cellular depolarization, and ion transport in response to external stimuli such as water availability, diseases, and photosynthesis activity, among others. Therefore, the environmental effects on the plant change its morphology and physiology would be related to bioimpedance. In this way, bioimpedance could become a novel and powerful technique used to analyze, in-situ and in real-time, the physiologic activity and status. In this study, we assess the *Evaluation Board AD5933EBZ* as a bioimpedance low-cost measurement device to perform observations of module and phase of the bioimpedance, aimed to correlate them with the effects of environmental stimuli such as irrigation, CO_2 concentration, sun exposure and temperature level in the grapevine.

1. Introduction

Bioimpedance could be used for studying the structures of plant materials. The changes in value of their electrical impedance could be the result of changes in the chemical composition of the sap, cellular depolarization, and ion transport, causing in morphology and physiology in response to external stimuli such as water availability, CO_2 concentration, diseases, and light. For this reason, bioimpedance has started to be used as a technique that allows us to analyze the correlation between environmental stimuli, the electrochemical dynamics of plant activity, and the physiological state in crops [1, 2, 3, 4, 5].

A question arises: could bioimpedance be a reliable *in-situ*, real-time plant state and physiological change indicator due to environmental stimuli ?.

In the grapevine, few studies are related to bioimpedance analysis. It was used in the literature to estimate different resistance diseases by measuring grape berry bioimpedance [2] and the maturity and damages by cell death during grape ripening [6]. However, no study references can be found in relation to trunk or shoot bioimpendance as measurements of the effects of environmental stimuli on this crop. In that sense, an exploratory study is carried out.

The objectives of this study are the following: *i)* to address the study of bioimpedance in a woody crop, taking as a model the grapevine (to the best of our knowledge no previous work in this area reported); *ii)* to correlate qualitatively the bioimpedance Bode graphs to the plant response (physiological variables such as stomatal conductance) related to interaction with the environment (e.g. temperature, solar radiation), under controlled situations (potted plants in a growth chamber) and under outdoor situations (potted plants outdoor) and; *iii)* to make use of the *Evaluation Board AD5933EBZ* [7] which composes of the high precision impedance converter system *AD5933*.

The novel contribution of this work is to assess and confirm, for first time, the possibility of using bioimpedance as a grapevine physiologic biosensor.

2. Materials and experimental setup

2.1. Plant material and physiological measurements

Two-year-old grapevine plants were selected in pots from two different cultivars (one plant each *cv.*) in order to evaluate possible effects of the phenotype on the bioimpedance. The selected cultivars were *cv.* Tannat, the most planted red variety in Uruguay and *cv.* Marselán, a promising red cultivar of interest to the uruguayan vitivinicultural sector. These plotted grapevines were exposed under outdoor situation (potted plants outdoor) and controlled situations (potted plants in a growth chamber).

To measure physiological variables on the grapevines (such as reported in Table 1) under outdoor situation we use an infrared gas analyzer (IRGA, Licor-6400®, LI-COR Biosciences, Inc., Lincoln Nebraska) for CO_2 assimilation measurement, transpiration rate and stomata conductance. The IRGA was placed like a clamp by squeezing the leaf of the grapevine as Figure 1 shows. For light radiation measurements we use a spectroradiometer (USB2000+ spectrometer, Ocean Optics, Duiven, The Netherlands).

A potted plant was left without water irrigation during three weeks to evaluate the effect of water stress on young plants. It was then, that we sacrificed a potted grapevine to the effect of studying the water stress, to observe the variations of the bioimpedance in a plant under stress and irrigation on the one hand, and on the other hand, to evaluate the response speed when changing environmental conditions (in this case through irrigation).

To assess the possible bioimpedance of the two grapevine cultivars, environmental conditions were modified in the potted plants as changes in sun light (shade and 100% exposure) and different CO_2 concentrations (outdoor concentration or reduced). When physiological measures were evaluated, illumination intensity of IRGA was 950.3 $\mu molfotones/m^2s$, environmental relative humidity was 72.0% and air temperature was $25.2°C$.

Moreover, for the controlled conditions, we exposed the grapevines to a growth camera, where it is possible to control the light, temperature and humidity and to measure the bioimpedance curves in a fully controlled experimental setup. We measured the bioimpedance (module and phase) in the petiole of the leaf with the same in-vivo invasive bipolar method and electrodes, alternating situations of total exposure of light and shade and different ambient temperatures (31^{o}C and 25^{o} C). The measured cycle was as follow: A) complete darkness, $T = 31°C$ (measure performed after 10 minutes of light turned off), B) light 100%, $T = 31°C$ (10 minutes after -A- measurement), C) light 100%, $T = 25°C$ (measured after temperature stabilization) and D) complete darkness, $T = 20°C$ (10 minutes after -C- measurement).

2.2. Acquisition system, electrode placement, board calibration and frequency range

The bioimpedance measurements on which this study is based were performed with the *EVAL-AD5933EBZ*. This board was selected because it contains the AD5933 integrated circuit that is used in the literature for a similar application, as in [2]. It is a low cost board, which has

proprietary software that runs under Windows and directly provides the bioimpedance values (module and phase) once it is calibrated.

Figure 1. Potted Grapevine *cv.* Tannat with IRGA at the leaf and electrodes

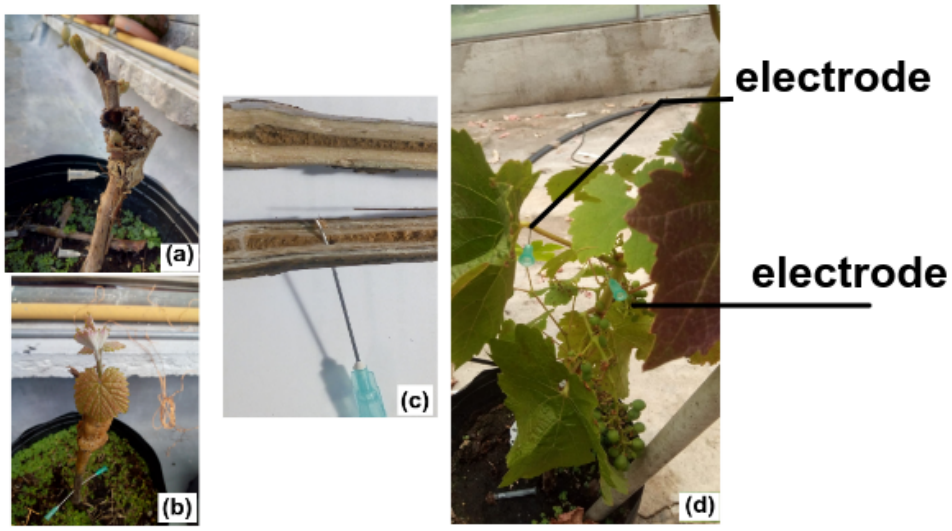

Figure 2. Configuration (a), electrodes in grapevine trunk in transverse configuration; configuration (b) electrodes in grapevine trunk in radial configuration; configuration (c) dry (died or stage of dehydration) grapevine trunk showing that the needles reach the xylem; configuration (d) electrode placed in leaf petiole.

The main challenge was to calibrate the *Evaluation Board AD5933*, a problem that in turn is associated with discovering which plant organ could be punctured with steel needles that function as the electrodes of the bioimpedance system (needle 27 G 1/2, stainless steel of $0.4mm$ diameter). The measurements were performed using the two-electrode method (invasive) and the *Evaluation Board AD5933*.

After several tests, and in a heuristic iterative process, it was determined that the best installation to place needles in the trunk, in a radial configuration or opposite configuration (see in advance (b) in Figure 2). This was because it is intended that the needles maintain an

invariant position, which is not possible on the leaf (the needles move with the leaf and both can fall). Figure 2 shows the different needles (electrodes) placement assessments. The needles in the trunk penetrate and come into contact with the xylem of the plant which is composed of cells and extracellular liquid. Its extracellular environment mostly consists of electrolytes, water and other chemical components. The circular needle electrode had little surface contact with the xylem but it was the only one that allows penetrating the trunk, so we had no alternative but to use and not others.

The resistance of configuration (b) in Figure 2 (radial configuration) was measured by means of a multimeter and it resulted in around 250 $k\Omega$ in average among all potted grapevine. It is important to underline that in this case, the bioimpedance current runs perpendicular to the xylem fibers.

The radial configuration presents less resistance than the configuration (a) of Figure 2, this configuration was chosen it was better to calibrate the *Evaluation Board AD5933* with reduced values, which resulted in a calibration standard resistance of 220 $k\Omega$.

It has been postulated that the impedance of an electrode-electrolyte interface depends on electrode material, electrolyte concentration, temperature, and contact area. Therefore, the tissue contact impedance should be considered but according to [8], the electrode polarization impedance parasitic can be many times larger than the sample impedance at low frequencies (frequencies below $1kHz$), and that is not our. We started the study at frequencies above $5kHz$. On other hand, in all measurements we observed that the asymptotic behavior of the bioimpedance module begins around $30kHz$, so that no relevant effect was reflected beyond this frequency and then the range of frequencies $5kHz - 30kHz$ was considered adequate.

3. Results and discussion of experimental results

3.1. Plotted grapevine response to different water conditions

In Figure 3, the bioimpedance curves (module and phase) are observed when a plant suffers water stress (curves A and B) and how the curves are modified with a high response speed (intervals of 15 minutes) after irrigation (curves C and D respectively).

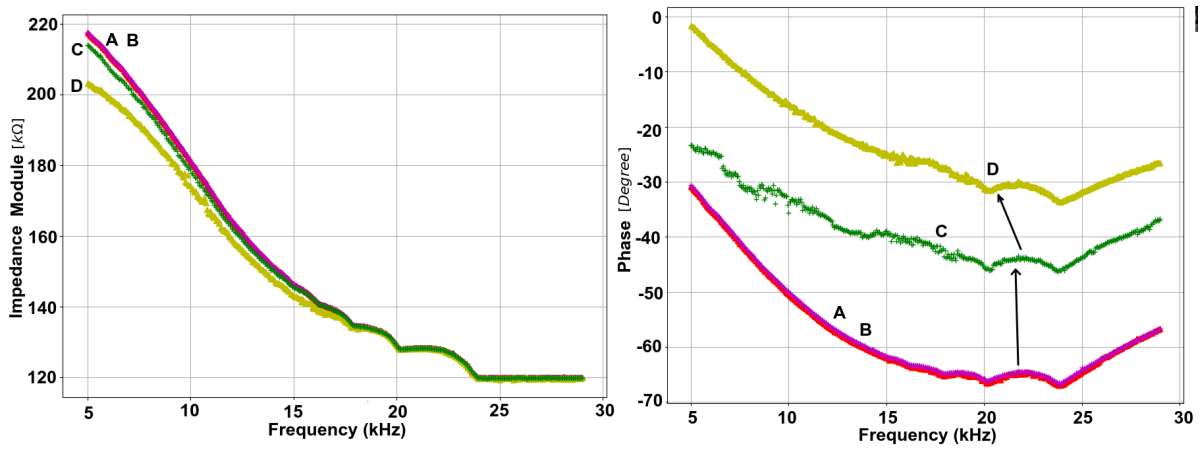

Figure 3. Bioimpedance Bode (module and phase) of dry state and irrigation. A and B correspond to a plant suffering water deficit stress. C and D correspond to a plant irrigated after suffering water deficit stress.

It is also possible to observe the decrease in the phase (curves C and D in Figure 3) in the studied frequency band, which indicates less capacitive effect of the cell membranes. The

decrease in module would indicate more intercellular flow due to sap flow caused by irrigation.

The aforementioned is the first evidence that it was observed as bioimpedance response to the irrigation in grapevines. Semicircular arcs are found in the phase plot. The range of frequencies used for the analysis could be considered adequate when observing the Bode shapes. It could be associated with a Cole model of 1-dispersion where the curve reaches an asymptotic behavior from a certain frequency, in this case around $22kHz$; both curves present a characteristic morphology to impedance measurements on biological systems. This shows that bioimpedance could be used for a smart irrigation system by measuring the biompedance when the automatic irrigation of a plantation begins, and by analyzing it is possible to determine when the plant responded to the irrigation. In this way, it could be possible to create an irrigation system with feedback, without wasting water or irrigating more than necessary. Another relevant preliminary result of the irrigation measurements during budding, is that the information on changes is clearly seen in what are called curves of the Bode diagram (particularly in the phase). At this point it is important to underline that animal cells and plant cells are different in their structure.

The plant cell membrane, named plasmalemma, is a phospholipid bilayer with embedded proteins surrounded by cell wall, which is rigid and thick structure that plays an important role in metabolic processes specific enzymes contained in such cell wall. The cell structure could be simulated by equivalent electrical circuits and the tissue (assumed to be a network or array of cells) as an arrays of mini-circuits. But there is no consensus in literature what electrical circuit based models represent plant tissues blocks: there is the Cole model [9], the Hayden model [10] and the Double-shell Model [11]. None has been tested in-vivo plant trunk and none fits our measurements in the grapevine trunk. This is the first time.

3.2. Plotted grapevines physiological response to different environmental conditions

According to the results shown in Table 1 and Figure 4, it was possible to observe the response of the Bode Diagram of the bioimpedance of the grapevine to different environmental conditions.

Table 1. Physiology variables measured in two grapevines cultivars (M1 and M2 *cv.* Tannat and M3 and M4 *cv.* Marselan) at different CO_2 concentrations and light conditions (shade and sun 100%)

Graph	Cultivar	CO_2 concentration $\mu mol\ CO_2\ /s$	Sun light	Photosynthesis level $\mu mol\ CO_2\ /m^2 s$	Stomata Conductance $milimol\ H_2O\ /m^2 s$	Transpiration Rate $milimol\ H_2O\ /m^2 s$
M_1	Tannat	900	Shade	8.12	0,004	0,04
M_2	Tannat	100	Shade	5.1	0,01	0,1
M_3	Marselan	900	Sun 100%	10.5	0,07	0,4
M_4	Marselan	100	Sun 100%	6.3	0,09	0,7

On the one hand, a response is clearly observed between the sun exposure condition (curves M_3 and M_4 , cultivar Marselan in Figure 4) with respect to the shadow situation (curves M_1 and M_2, cultivar Tannat), which could be due to the more transpiration rate in the former. Within this last condition, a similar bioimpedance behavior was observed when the measured leaf was changed (M_1 vs. M_2). Both leaves presenting a low measured stomatal conductance close to 0, low transpiration rate, and net photosynthesis that changed from one leaf to the other. This is reflected in the phase of the impedance which changes very little, just visibly (M_1 vs. M_2). It is interesting to note that variation is never observed in the bioimpedance module, it was clearly found in the phase. On the other hand, the differences found in the leaves exposed to the sun (M_3 and M_4) varied because they were regulated with different concentrations of external CO_2

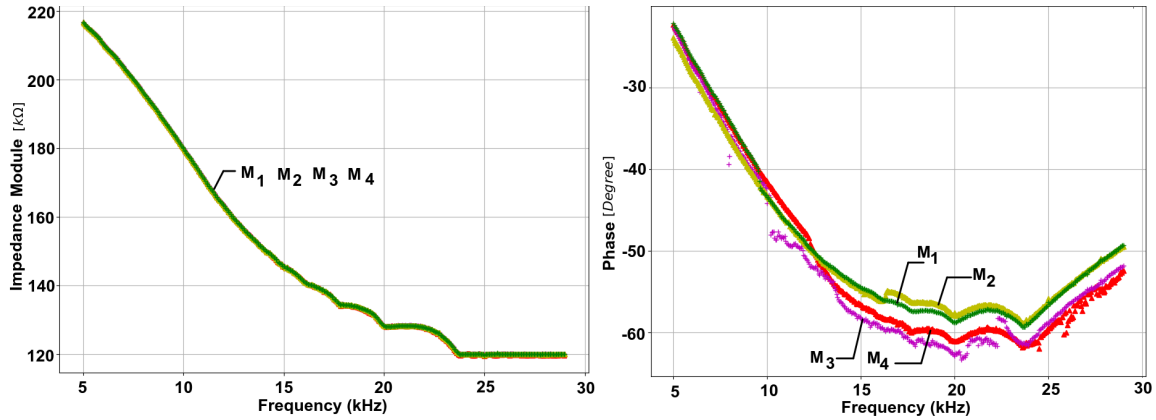

Figure 4. Curves of the module and phase of the bioimpedance of a Tannat grapevine exposed to the shade (green color - M_1 and yellow color - M_2), bioimpedance of a Marselan grapevine exposed to the sun (red color high outdoor CO_2 concentration - M_4 , violet color intermediate / low concentration of external CO_2 - M_3), with simultaneous measurements of physiological activity through the IRGA in the leaf.

through IRGA, with M_3 presenting the highest negative phase values of the bioimpedance Bode curve. This shows that there was an increased phase of the bioimpedance within the frequency range of 12 up to 23 kHz that were possibly a response to light and CO_2 concentrations in the evaluated grapevine leaves. It is interesting to note that effects in the leaf are reflected in the electrodes placed in the trunk.

The bioimpedance dynamics as a function of frequency, coincide with the bibliography that cites that extra-cellular and cellular phenomena govern bioimpedance at low frequencies, while at high frequencies they are governed by tissue structures inside the membranes. During photosynthesis (sun) the extracellular flux increases.

In our results, it was possible to a frequency zone starting at approximately 12 kHz where differences are seen, which translates into differences in the dynamics of extracellular tissues and materials, for example higher concentrations of extracellular ion fluxes. It is in this area, where the curves separate because bioimpendance is sensitive in this range of frequencies to the effect of the environment (light-shadow, different concentrations of CO_2), while for other ranges of frequency the tendency is to join (they remain invariant to the effect of environment stimuli).

3.3. Experimental results under controlled environmental conditions

The results of measuring the bioimpedance (module and phase) in the petiole of the leaf with the same in-vivo invasive bipolar method and electrodes, showed that this plant organ seems to be more sensitive to the different environmental stimuli and, furthermore, it is the phase of the bioimpedance that seems to better reflect these phenomena.

Figure 5 shows the bioimpedance measurements when alternating situations of total light exposure (full light represents 1246 μMoles of Photons per m^2/s of PPFD - (Photosynthetic Photon Flux Density) and power 27019 $\mu W/cm^2$) and shadow, and different ambient temperatures (31ºC and 25º C) and relative humidity 60% a grapevine plant measured through the leaf petiole.

When the curves without light are compared, there is a firm response to temperature change (A vs. D in Figure 5), while when the temperature is constant and high (31ºC in this case), there is a difference in response whether or not there is exposure to light but this is not so marked (A vs. B). After 30ºC, *Vitis vinifera* begins to close its stomata as a protection mechanism against

water loss due to increases in transpiration rates [12].

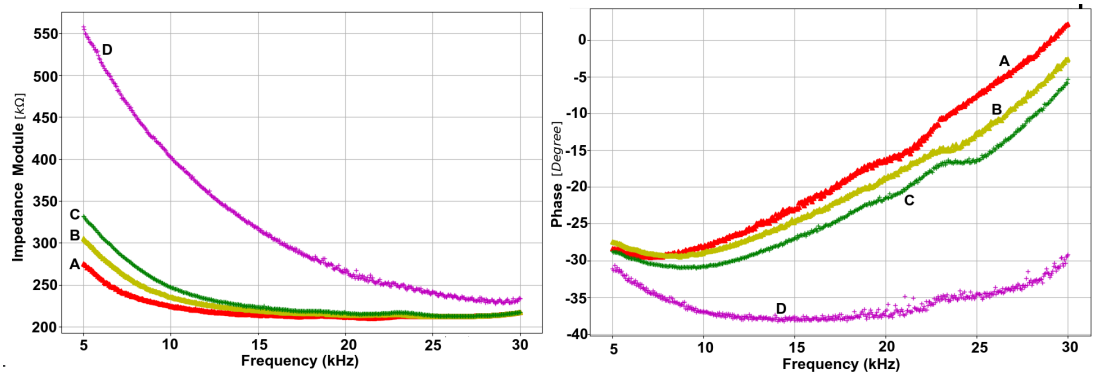

Figure 5. Bioimpedance (Bode) measured in the Grapevine leaf petiole at different temperature and light conditions, where curve A: complete darkness, $T = 31°C$ measure performed after 10 minutes of light turned off, curve B: light 100%, $T = 31°C$, curve C: light 100%, $T = 25°C$ and curve D: complete darkness, $T = 20°C$

However, stomatal closure in grapevines happens in a heterogeneous way [12] so the behavior of curve A -similar to those where light is on- may be due to not waiting the necessary time for all stomata closed, while a transpiration flow still exists causing the decreases in temperature cause decreases in transpiration rates [13] and therefore a lower conductive liquid flow which translates into higher bioimpedance values like observed in curve D. Light causes photosynthetic activity and with it the stomatal opening and ion transport, so due to the ion flux there will be low values of bioimpedance observed in B and C. In addition, higher temperatures increase the transpiration rate [12] contributing to the above mentioned. Despite the fact that temperature in B is above the threshold where grapevine begins to close its stomata, there is the factor of light that could play a predominant role in stomatal regulation and therefore in the transpiration rate and associated bioimpedance values.

Future studies remain to be able to conclude the magnitude of the effect of one variable or another on the changes in the bioimpedance values. The results show that changes in bioimpedance are the consequence of environmental stimuli on the grapevine.

4. Conclusions

This work reports for first time experimental results of bioimpedance variations in grapevine under controlled and uncontrolled environmental stimuli, which demonstrate its capacity and accuracy to provide grapevine physiological state information.

The calibrated *EVAL-AD5933EBZ* works in the grapevine plant, showing bioimpedance changes and good sensitivity when plants are affected by different environmental stimuli. Different cultivars were evaluated and exposed to different environmental situations (humidity, temperature, light and CO_2 concentration) and it can be concluded that bioimpedance variations are a good indicator of the presence of the stimuli. We observe that in general, the bioimpedance phase is more sensitive to change due to the presence of stimuli and an electrical model could be built based on this observation.

This work shows although larger studies are needed to confirm the possibility of using bioimpedance as a grapevine physiologic biosensor. However, it may assist future researches to explore the potential applications in the grapevine cultivar field.

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