Sensing and Bio-Sensing Research 32 (2021) 100420

Contents lists available at ScienceDirect

Sensing and Bio-Sensing Research

journal homepage: www.elsevier.com/locate/sbsr

Electrical signaling related to water stress acclimation

Marco Dolfi [a,*], Caterina Dini [a], Simone Morosi [a], Diego Comparini [b], Elisa Masi [b], Camilla Pandolfi [b], Stefano Mancuso [b]

[a] Department of Information Engineering (DINFO), University of Florence, Italy
[b] Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Italy

ARTICLE INFO

Keywords:
Plant electrical signal
Stem resistance
Water stress acclimation
Brassicaceae
Statistical analysis

ABSTRACT

Nowadays, the debate involving climate change concerns safeguarding the water resources of the planet, an extremely important topic also in agriculture. In particular, the problem of scarce water availability becomes more and more pressing every year and at the agronomic level it is necessary to make intelligent use of water without affecting the quality and quantity of production. The aim of this work is to understand when the plant is under water stress conditions at the electrical signal level, in order to achieve the information of true needs of water in plants. The proposed methodology considered the plant black kale cultivated in hydroponic condition: the plants were based in an electrically isolated environment, with electrodes inserted in their stems to acquire and study the electrical activity associated with determined irrigation cycles. By using different stop/flow water irrigation regimes, an acclimated and a not-acclimated group of plants have been selected to experience different periods of prolonged water flow stress. Results indicated that the evaluation of a measure of statistical dispersion, the daily variance of the electrical resistance, can be closely related to the induced water stop/flow stress. Moreover, experiencing an acclimation stage has shown to allow a better recovery response for the plant.

1. Introduction

1.1. Electrical excitability and signaling in plants

A fundamental property of all living organisms is the ability to carry out a continuous collection of environmental information as well as the possibility of physiological responses to new or unusual environmental conditions. The generation and conduction of electrochemical impulses through tissues and organs, resulting from abiotic (lifeless ecosystem components) and biotic (life-threatening components) in environmental conditions, have made it interesting to study the transmission of signals over intra/inter cellular, long/short distances in plants and animals [1]. The conduct of electrical signals has been studied in order to provide a translation of certain parameters and stimuli from the external environment or perceived through sensory systems. In fact, because of their immobility, plants are forced to live with the various stresses that nature presents to them; frequently they alter their growth and development to cushion any of the changes perceived as unfavourable in the environment around them. For a long time, plants were thought to be living organisms whose limited ability to move and respond were linked to relatively limited detection capabilities [2]; on the contrary, today we know that plants monitor continuously the surroundings and react to it, and process this information to improve growth. For this reason, electrical signals play a key role in internal signaling induced by environmental stimuli, and in plant physiological responses [3]. In fact, over the years, it has been discovered that plant cells become excited in bioelectrophysical terms when they undergo environmental changes and the reason why plants have developed pathways for electrical signal transmission is likely related to their need to respond quickly to environmental stressors [4]. Since electrical signals have been referred to as a widespread phenomenon in the plant kingdom [5], it is certainly important to study physiological processes as a whole as they could affect the electrical phenomena in certain plant tissues. A recent study described Mimosa's responses to the light and dark reactions of photosynthesis, highlighting the importance that electrical signals have in triggering a photosynthetic response over long distances within the plant and thus providing evidence of a link between electrical signal and photosynthetic response [6]. Moreover, other studies have exploited the monitoring of the electrical activity in plants to use their sensing capabilities for specific stimuli (e.g. light, ground-level ozone pollution, salinity stress [7–10]). Although electrical signals are known to play a crucial role in regulating the system response in plants, there has been

* Corresponding author.
E-mail address: marco.dolfi@unifi.it (M. Dolfi).

https://doi.org/10.1016/j.sbsr.2021.100420
Received 28 December 2020; Received in revised form 30 March 2021; Accepted 12 April 2021
Available online 16 April 2021

no attempt to associate the interpretation of electrical patterns to external stimuli in a systematic way.

1.2. Water stress in plants

Since plants are sessile organisms (i.e. they live anchored to the substrate and are not able to move), they developed multiple strategies to cope with the constraints that nature presents to them. They are therefore exposed to various environmental conditions, which affect photosynthetic performance, plant growth and development and can consequently damage crop productivity. One of the most common and harmful environmental stress is that of soil drought: soil characteristics in such conditions can range from scarcity and water depletion to prolonged periods of water deprivation. There is also the inadequate availability of water in drought-prone environments, which negatively affects crop growth and productivity by lowering the water level of tissues and cellular turgor (i.e. the pressure that a restless fluid exerts on the surface unit with which it is in contact). Plants act to remove water from the soil and absorb it: when soil moisture is low, plants need more energy to absorb water, and when the soil is dry, the plant can no longer absorb water and begins to show symptoms of stress. Plants, however, have a variety of physiological and biochemical responses at the cellular and whole organism level, which make the phenomenon of drought, in particular, even more complex. The stimuli generated by an electrical signal, as a response to changes in light and water availability conditions, result in a rapid response in the opening and closing of the stomata: in drought conditions the rate of photosynthesis is reduced due to stomatic closure. This closure operation undertaken by the plant itself represents a simple defense that is put in place to minimize the transpiration of water. In fact, the opposite situation, or open stomata, means that the plant can achieve optimal gaseous exchanges of carbon dioxide and oxygen for the photosynthesis but it also results in higher transpiration. For this reason, the action of closing the stomata is considered a defense against the drought of the soil. Although drought tolerance is a complex phenomenon, plants show a range of mechanisms to resist drought, as well as stomatic closure, such as reducing water loss through increased resistance or extracting more water from the soil. In addition, plants also undergo some morphological and physiological changes in order to minimize stress-induced losses; however, all these defense mechanisms vary depending on the different plant organism. Thus, summing up, plant resistance to drought depends on adaptive strategies based in turn on certain mechanisms, including: increased water absorption and translocation; increase or decrease the elasticity of the walls; reducing water loss during periods of water scarcity; mechanical reinforcement of tissues to prevent withering which can in turn lead to irreversible damage [11]. It is easy to understand that simply measuring the amount of water available in the soil cannot be a universal indicator to establish that this quantity will be sufficient, or else excessive, for the correct vegetative development of the plant. Monitoring plant conditions through a network of sensors allows to have detailed information about critical situations and apply timely irrigation where required or simply to properly manage the irrigation program. This means encouraging water savings and deciding intervention policies and thus promoting a green economy.

1.3. Electrical activity measurements

In plants the electrical properties and their changes caused by environmental stimuli can be studied from multiple points of view: in this work the electrical signal that has been acquired and examined is electrical resistance [3]. The electrical resistance of the tissues of the plant undergoing the passage of a continuous current consists mainly of two components: the resistance of the apoplast and the resistance of the symplast. With the term apoplast we refer to the continuous system of cell walls and inter cellular air cells of the plant's tissues, for example the vascular system. In the apoplast the water can move through the cell walls and any extracellular space stuffed with water (without crossing the membrane). The symplast instead indicates the set of cells that are in continuity with each other, where the water moves travelling from cell to cell via the membrane. The symplast is the main component of the resistance of the tissue, in fact about 75–93% of the current that is generated flows through the symplast itself [12]. When a noticeable variation of electrical resistance values in plant tissues is recorded, we can affirm that physiological changes have occurred to the tissues themselves, and it is possible to identify also the main causes like wounds or stresses related to environmental conditions [13]. The study of the acquired electrical resistance signal therefore can also be associated with the water condition of the plant, by carrying out a monitoring operation that does not adversely affect the analysed tissue, and especially does not affect the normal physiological functions of the plant itself. Regarding the proposed approach, the statistical analysis of the electrical resistance data has shown to represent a very promising tool to study and assess the water status of the plant during its development. By observing the trend and the statistical dispersion of the acquired data, we may be able to establish that some event has occurred, since the electrical resistance, which is the reciprocal of the electrical conductance, is affected by the suffered acclimation to water deficits. There is a close relationship between the moisture content and the electrical resistance itself: in fact, a reduction in the moisture content can determine an increase in electrical resistance [14]. The association between electrical signal variance and water stress condition has already been observed in literature [15,16]. In addition, some state-of-the-art hypotheses indicate that signal propagation is due to the combined variation of both hydraulic and chemical signals [17], so it can be assumed that water stress represents a fundamental aspect of the signaling process. The underlying physiological cause of this mechanism is not yet completely clear: as well as the conductance, the trend of the reciprocal electrical resistance may be related to the water amount in plant tissue. However, in order to have a complete explanation of the factors that induce the variation of the electrical signal, more in-depth analysis is necessary. Furthermore, in our experiment the electrodes inserted in the plant (Fig. 3) remain in contact with different cell compartments, so that the variations in electrical resistance can also be associated with more complex interactions, such as cavitation or embolism phenomena, which can reduce the hydraulic capacity of the plants [18]. In general, it can be assumed that the trend of the electrical resistance signal in plants is associated with some specific physiological mechanisms. For example, the presence of smaller xylem vessels can lead to a lower hydraulic conductivity [19]. In addition, the sieve tubes, the conducting elements of the phloem, can also represent a potential pathway for transmission of electrical signals [20]. To be physiologically important, water stress usually causes a significant change in turgor pressure within a cell: the turgor will change only when a significant inflow (or outflow) of water occurs. The use of real-time information on the electrochemical behaviour of plants can provide a strategy to quantitatively correlate the physiological reactions of plants to environmental changes, in order to also improve the self-programmed operation of irrigation systems, aimed at preventing water stress conditions. State-of-the-art techniques to measure electrical potentials in plant tissues include a non-invasive surface (extracellular) approach and a more invasive one, through the insertion of electrodes (intracellular) [4]. Measurements of extracellular potential on the surface of plants have been widely performed in the past, and they offer the advantage of being able to detect differences in electrical potential over long periods of time (several days). They are based on measurements of the total sum of bioelectric activity in large groups of cells: as examples of this procedure we can cite electrocardiograms (ECGs) and electroencephalograms (EEGs), widely used in medical practice. In addition, surface measurements often appear more suitable as they are non-invasive and physically stable [4], but less accurate. On the other hand, intracellular measurements with penetrating microelectrodes, placed in different positions along the plant, are effective for short periods of time as 1–2 h, because some of the electrolyte inside the electrode usually spreads into the cell and changes its original bioelectric condition. However, this methodology causes wound reactions when inserting the electrode and therefore the electrodes must consist of thin metal wires. Electrodes can be connected via shielded cables to a high-impedance electrometer/amplifier with many input channels. Moreover, an electrode can be placed in the soil where the plant is located, in order to be used as a reference electrode.

1.4. Objective of the work

All the experiments carried out in this work have shown the presence of a consistent and relatively rapid mechanism of generating and transmitting electrical signals in plants, directly proportional to the intensity and duration of the induced stimuli, such as the intensity of light and the availability of water. While many studies in literature have associated the effect of water stress, deficient irrigation, light cycles and mechanical lesions with electrical signals in different fruit tree species (e.g. avocado, lemon [1–3]), there are few experiments in confined environments, where it is possible to isolate abiotic factors and more accurately control the physiological responses of plants. Therefore, this work considered the simultaneous monitoring of small plants in a controlled environment. The correlation between different stop/flow irrigation cycles and specific electrical patterns has been evaluated by observing the physiological condition of each group of plants. In order to obtain the simultaneous monitoring of multiple plants for long periods of time, a new multi-electrodes set-up has been implemented. The implemented system is able to simultaneously acquire the electrical signal of multiple plants and store the data for the actual duration of the proposed experiment (days/weeks). The statistical analysis of the acquired data has been performed in a systematic way, by evaluating specific time intervals related to determined irrigation regimes and consequent water stress acclimation.

2. Materials and methods

2.1. Data acquisition system and plant material

The starting point of the experimental study is the data acquisition system, which allows the electrical activity of the plant to be recorded in a controlled environment. The implementation of the simulated acclimation to water stress has been determined by building a specific electrically isolated set-up, to prevent external electromagnetic signals from interfering with tension and internal implantation measurements. The humidity of the air and the temperature were kept stable, and through artificial lights a predefined day and night cycle was set. The plants were then subjected to different water regimes. They were classified into two distinct groups, and physiologically analysed by measuring leaf gas exchanges (stomatic conductance and photosynthetic activity), with an infrared gas analyser LICOR Li-6400XT. The type of plant under consideration is the *Brassica oleracea* var. *acephala*, commonly known as black kale. This species is considered as a perennial and vigorous plant. The characteristic that distinguishes it from other species of kale is the inability to produce the head (typical of black broccoli and cabbage) and inflorescence (such as cauliflower); the black kale, on the other hand, produces many leaves that persist for a long time on the plant, while the stem is simple in young plants, with some lateral ramifications in the most adult. The diagram shown in Fig. 1 is a general representation of the data acquisition system.

The acquisition system consists of an integrated remote controller PXI-1031, a TB-2605 multiplex terminal, a PXI-4065 digital multimeter and the LabVIEW software (all National Instrument devices, Fig. 2). The signal acquisition card was connected to 15 pairs of electrodes (30 electrodes in total). Monopolar needle electrodes (10 mm long) in stainless steel were used for the experiments to measure AC/DC voltage (measured in Volt) and two-wire resistance (measured in Ohm). There are also 4-wire resistance measurement systems, but in this study it was chosen to use the 2-wire one as electrode pairs were used, one for measurement and one as a reference: in practice the instrument generates a small test current that serves to measure the voltage drop that occurs inside the plant, in order to determine the unknown resistance value.

As you can see from Fig. 3, the electrode pairs thanks to a support were maintained at a fixed distance of 0.9 mm and inserted into the

Fig. 2. National Instruments data acquisition card connected to the electrodes for measuring plant electrical activity.

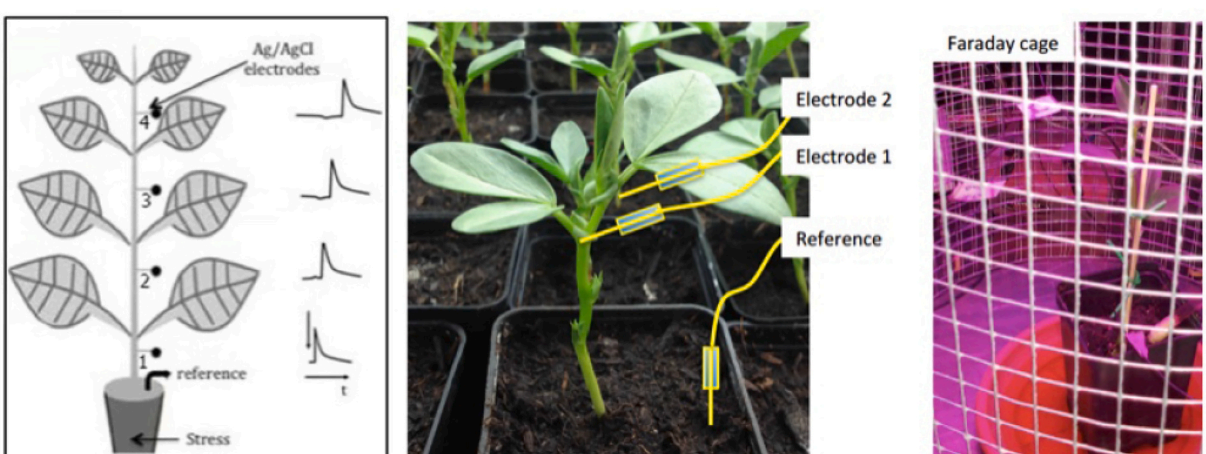

Fig. 1. Data acquisition system example.

Fig. 3. Detail view of the inserted electrodes into the plant's stem.

plant's stem. Then the electrodes were first connected by insulation cables to high-impedance amplifiers, and finally connected to a data logger. Through the use of this data acquisition system, multiple phenomena can be evaluated and studied for long periods, such as the day/night light cycle, the succession of drought cycles during the stop/flow irrigation cycles and the effects of mechanical wounds of the plant's organs (removal of the trunk, pruning, removal of leaves). The use of ultra-thin needles limits tissue damage within 24–48 h after the insertion. The main goal of this work is to detect electrical activity in plants that are exposed to different cases of water availability and periods of darkness.

The plants under study were grown in peat discs, then transferred and acclimated in hydroponics for a week by thoroughly washing the roots in sterile water and transferring them in drilled containers filled with expanded clay. Hydroponics is a method of above-ground cultivation: the land is replaced by an inert substrate (generally expanded clay) and the plant is irrigated with a nutrient solution composed of water and some nutrient compounds necessary to make all the essential elements for a good mineral nutrition. Hydroponic cultivation allows controlled production from a qualitative and hygienic point of view throughout the entire year. The plants were divided into two groups, each placed in a 8 l tank equipped with an aerator, and fertilized with a commercial NPK mix (2.6–1.9-3.6). After a week, a pair of electrodes was inserted into the plants' stem and electrical signals from the two groups were constantly monitored (one acquisition every 1 min). The water level in each tank was automatically adjusted through a water stop/flow system, consisting of two holes with different flow rate (major or less than the pump flow) to allow the tank to be emptied when the pump was off (discharge flow) or to maintain a constant level using an overfilled system (too full runoff) when the pump was on. The electrical activity of the two groups of plants was then analysed in relation to a prolonged range of stop/flow irrigation cycles, observing the differences between the first group of plants previously acclimated with a shorter cycle of stop/flow irrigation scheme and the second group of plants which was instead regularly watered.

2.2. Experimental plan

Within the isolated hydroponic system, 12 plants (one plant per pot) have been installed each with a pair of electrodes. This provision was chosen to avoid interference between electrodes as well as the possible mutual influence between plants. The plants under consideration have experienced three time periods with different water conditions: *I. Preparation regime; II. Mild Stress regime; III. High Stress regime.* In particular, the preparation regime involves normal and continuous water supply and nutrition of the plant, in order to adapt the plant to the environment in which it was located. Subsequently only a subset of 6 plants were applied a period of mild water stress: every 4 h the water supply was interrupted, thus alternating 4 h of continuous irrigation and 4 h of water deprivation. This first group will be referred to as acclimated plants. Subsequently in a similar way the last period of prolonged water stop/flow to induce repeated high water stress was represented by the alternation of 12 h of continuous irrigation and 12 h of water absence. The second group of plants that suffered only this last type of stress will be referred to as not-acclimated plants.

The choice to carry out a period of mild water stress for some plants is related to the objective of stimulating acclimation mechanisms to the hydric conditions. Acclimated plants are expected to be better prepared and therefore able to better cope with the regime of high water stress, reaching the final stage of the experiment in better conditions than the other group of not-acclimated plants. The experimental study was performed on 12 plants divided equally into two groups of 6 plants for a total duration of 22 days of data acquisition. The irrigation and light regimes applied are specified below:

- Light cycle: light ON from 6 a.m. to 10 p.m. every day (14/10 day/night).
- Preparation regime: continuous watering (days 1–3).
- Mild water stress regime (4 h): water ON from 2 p.m. to 6 p.m., from 10 p.m. to 2.00 a.m. and finally from 6 a.m. to 10 a.m. (days 4–9).
- High water stress regime (12h): water ON from 3.30 a.m. to 3.30 p.m. (days 10–22).

Fig. 4 shows the experiment set-up of black kale plants, highlighting the wired connection of the electrodes.

2.3. Data analysis

Statistical analysis has been performed on the whole data set, composed by the electrical signal measurements acquired on each plant. In order to evaluate all the procedures carried out on our experiment, we have exploited the interpretation of descriptive statistics, evaluating the distribution of data in a concise way. In particular, so called variability or dispersion indices were considered, including average, median, variance, standard deviation, range and interquartile range. The mean is a single numeric value that succinctly describes a set of data. The variance of a statistical or random variable is a function that provides a measure of the variability of the values assumed by the variable itself; specifically, the measure of how much they deviate squarely from the

Fig. 4. The black kale plants under study equipped with the wired connection of the electrodes.

arithmetic mean or expected value. In addition to these parameters, a method for graphically depicting groups of numerical data through their quartiles, the so-called box plot, was also used. A box plot is a standardized way of displaying the dataset based on a five-number summary: the minimum, the maximum, the sample median, and the first and third quartiles. Given a distribution, the median (or median value) is defined as the value assumed by the statistical units that are in the middle of the distribution, while the quartile are those values that divide the data set into four parts of equal size. The distance between the upper and lower quartiles is defined interquartile range, which is a measure of variability, based on dividing a data set into quartiles. The box dimensions tend to increase as the dispersion (variance) of the data increases [21]. Moreover, Principal Component Analysis (PCA) was used to reduce the dimensionality of the statistical feature space and better evidence the relationship between the considered descriptive statistics and the distribution of the plants under study in an ordination plot. The PCA of statistical parameters was done using normalized (-score) data in MATLAB software.

3. Results

In order to achieve the objective of the study aimed at assessing the hydric status of the plant through the statistical evaluation of the electrical resistance signal, different water flow regimes have been applied, monitored and compared. The initial signal values of each plant dataset were slightly different. In order to compare multiple groups of data, an estimated baseline has been subtracted from each dataset. In particular, the estimated baseline corresponds to the mean of the signal calculated in the first 3 days of acquisition, when each plant was in good health. The relative trend of the electrical resistance signal has been acquired for each plant and the average relative group signal has been calculated and depicted in Fig. 5, indicating the different irrigation regimes. The average electrical resistance signal shows a specific trend, strongly correlated to the stop/flow water cycles. Note that the first mild stop/flow water stress in the acclimated group is directly associated with the average signal value, while the second hard stop/flow water stress, shared by both plant groups, showed a reduced impact in terms of average signal value and stability on the acclimated group compared to the not-acclimated group, suggesting that the plant group reaction to the stop/flow water stress was dependent on the conditioning of the acclimation stage.

These patterns appear more evident considering the statistical box plot analysis. The average daily statistics of each group of plants (acclimated and not-acclimated) have been represented in Figs. 6 and 7. The average daily statistical distribution of data shows that not only the median value of the signal, but rather the variance and the interquartile range of the electrical resistance can clearly highlight the stop/flow water stress reactions of each plant group: in particular, it can be noted the remarkable response of both groups on the day after the first 12 h stop/flow water cycle. On the other hand, the acclimated group results in a less intense reaction to the long-term 12 h stop/flow water cycle in terms of average daily signal variance compared to the not-acclimated group. Moreover, it should be noted that observations beyond the whisker length in the box plot are marked as outliers: a mild outlier is a value that is more than 1.5 times the interquartile range away from the bottom or top of the box (Tukey's inner fence), while an extreme outlier is a data point that is more than 3.0 times the interquartile range distant (Tukey's outer fence) [22]. In our case, outliers have been investigated carefully to exclude measurement or recording errors. On one hand, the detected outliers in Figs. 6 and 7 mainly represent mild outliers related to physiological baseline variations of the electrical signals observed within each group of plants. On the other hand, in both groups the most extreme observations are strongly correlated with the beginning of the water stress regimes or with a prolonged period of high water stress. Indeed, there is some evidence to suggest that such data points may contain valuable information about the process under investigation and

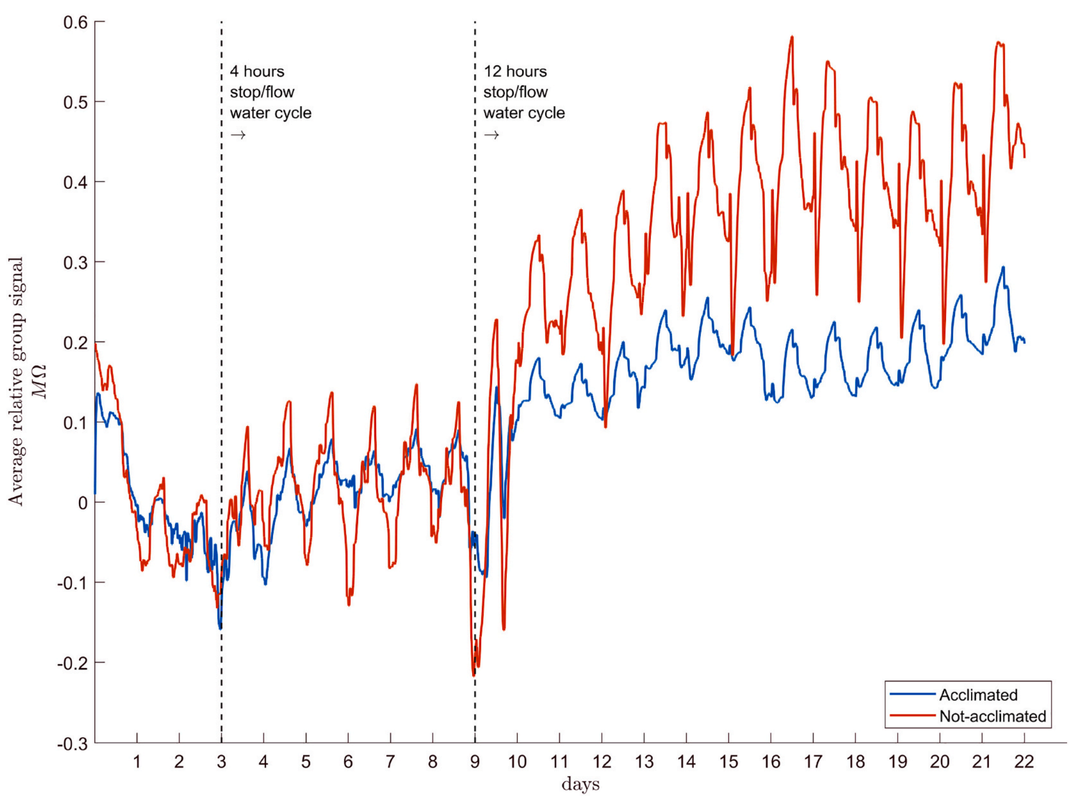

Fig. 5. The average signal trend of the two plant groups (acclimated and not-acclimated) is shown with the time indication of the different stop/flow irrigation schemes applied.

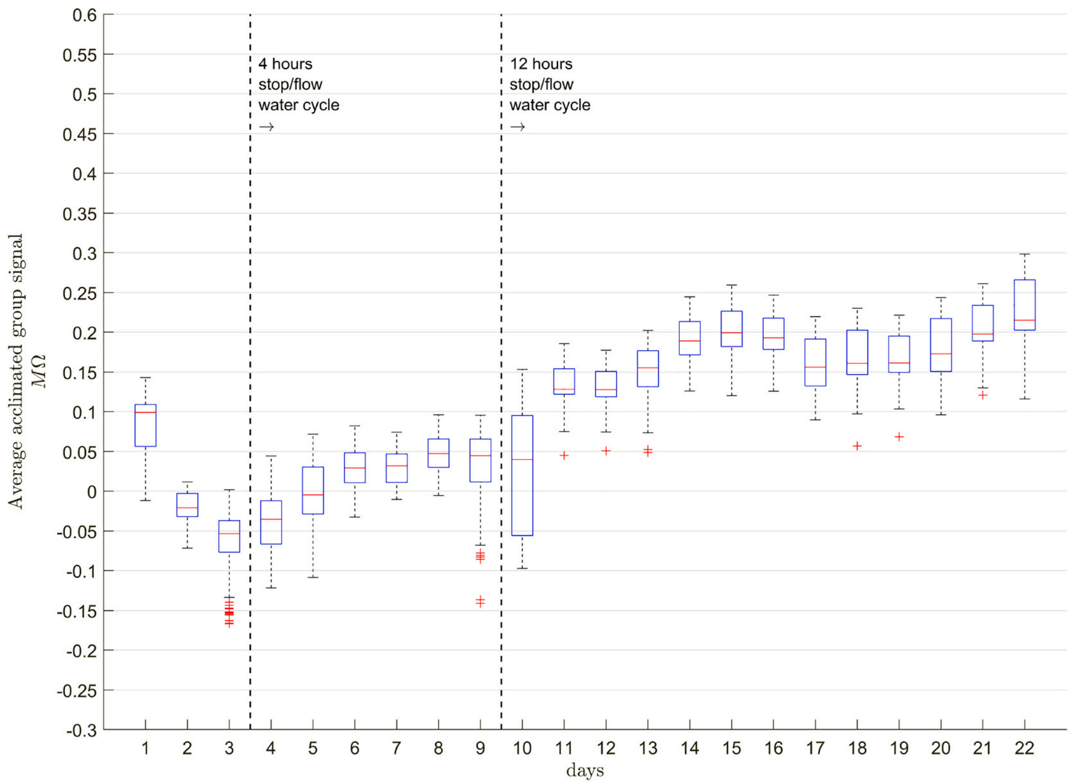

Fig. 6. The box plot of the electrical resistance data, evaluated on a daily interval, of the acclimated group of plants is shown with the time indication of the different stop/flow irrigation schemes applied.

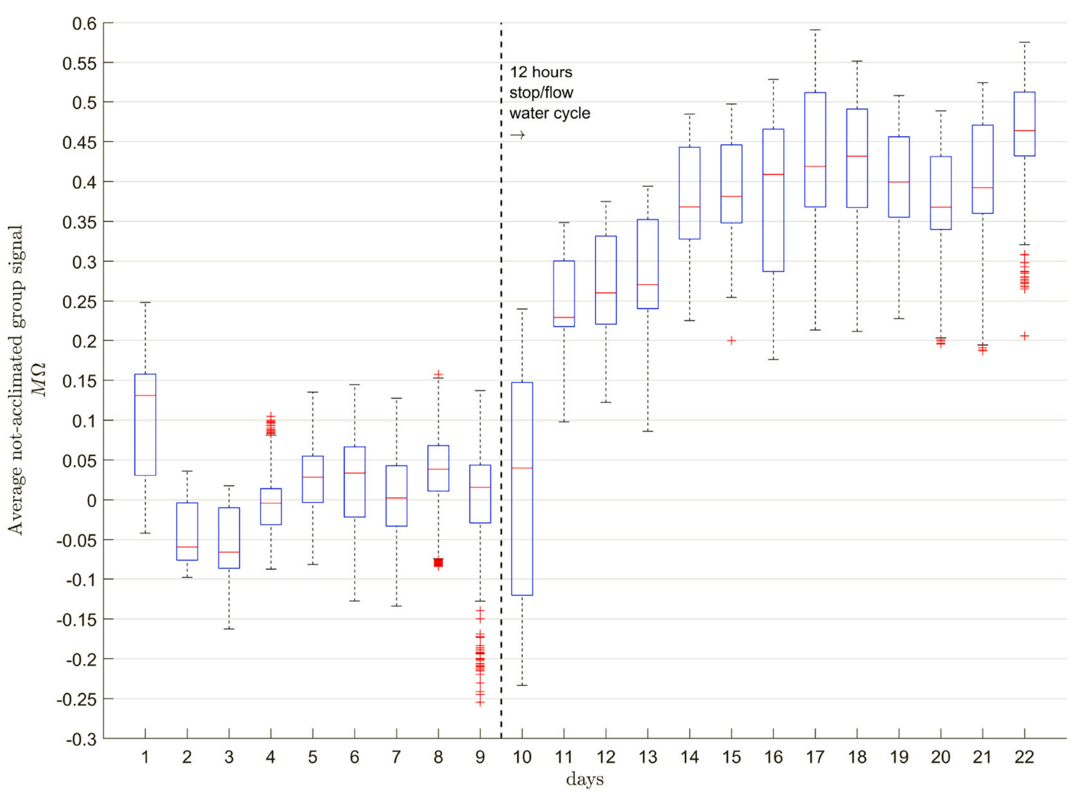

Fig. 7. The box plot of the electrical resistance data, evaluated on a daily interval, of the not-acclimated group of plants is shown with the time indication of the different stop/flow irrigation schemes applied.

therefore the observations have been included in the statistical analysis. To evaluate in detail the statistical dispersion of the electrical signal, the variance of the acquired data has been calculated on a daily basis, where the beginning of the light cycle was considered as the starting time of the day. In particular, the daily data variance of the two groups is shown in Fig. 8, with the time indication of the different stop/flow irrigation schemes applied. As stated before, a low baseline variation of the electrical signals can be observed, but it reflected a normal physiological oscillation in the groups under study, with relatively low dynamic range values. For example, that occurred for the acclimated group of plants on days 3–5 and for the not-acclimated one on days 6–8. Moreover, the graphs show an evident increase in the variability of the electrical signal, directly associated with the stop/flow water cycle and particularly noticeable during the prolonged 12 h regime. Once again, the acclimated group experienced a better ability to promptly react to the most stressful long-term regime, proven by the much more stable trend of its electrical activity. By analysing all the measured statistical variables in combination, the resultant ordination plot shows distinct partitioning of the plants under study across acclimated and not-acclimated groups (Fig. 9). The ordination plot could explain 98.7% of total variation in statistical data. The first principal component, on the horizontal axis, has positive coefficients for all six statistical variables. The largest coefficients are represented by the measures of statistical dispersion, corresponding to the variables interquartile range, range, standard deviation and variance. These parameters were most crucial in the partitioning of plant groups along the PC1. The second principal component, on the vertical axis, has positive coefficients for the central tendency variables (mean and median) and negative coefficients for the statistical dispersion variables. This indicates that the second component could distinguish among plants that have high values for the first set of variables and low values for the second one, or that express the opposite. Overall, the PCA biplot shows that the variation of the measured statistical parameters between the two groups of acclimated and not-acclimated plants is clearly related to the mechanisms involved in the acclimation process.

4. Discussion

The proposed experiment evaluated the acquisition of the electrical resistance signal of two groups of kale plants, subject to determined irrigation regimes to better interpret how plant physiology affect its electrical properties, and also to simulate the acclimation process to water stress conditions. By observing the trend of the acquired electrical signal, as expected the not-acclimated group suffered the most from the prolonged stop/flow irrigation phase, resulting in greater difficulty in recovering during longer periods of water deprivation. On the other hand, the group of acclimated plants showed similar difficulty in reacting to the immediate aftermath of prolonged stress (on the next day), but was also able to first recover more stability in the acquired electrical activity signal. Several studies have already highlighted the plants' ability to face repetitive drought stress at physiological and molecular level by improving their tolerance/resistance. The mechanisms involved in the acclimation process are several and observable from the early priming process, including physiological, biochemical, and molecular responses. These studies have revealed the flexibility of plant metabolism and have shown that its capacity of acclimation is essential in response to the pressure of environmental stress. Specifically, early phenotypic changes like reducing stomatal conductance, improving antioxidant defense and increasing the range of gene expression patterns, have evolved to cope with drought stress [23,24]. Therefore, the electrical signal trend largely confirms the physiological process by which the plants that were already affected by previous mild stress can better adapt to following prolonged periods of increased water stress, recovering their regular electrical activity due to the simulated acclimation process [15,16]. The electrical resistance data analysis then could be also exploited to evaluate the plant's water status, in order to assess when the plant is in a state of water stress, as well as to establish the recovery time and even to recognize its belonging group (acclimated

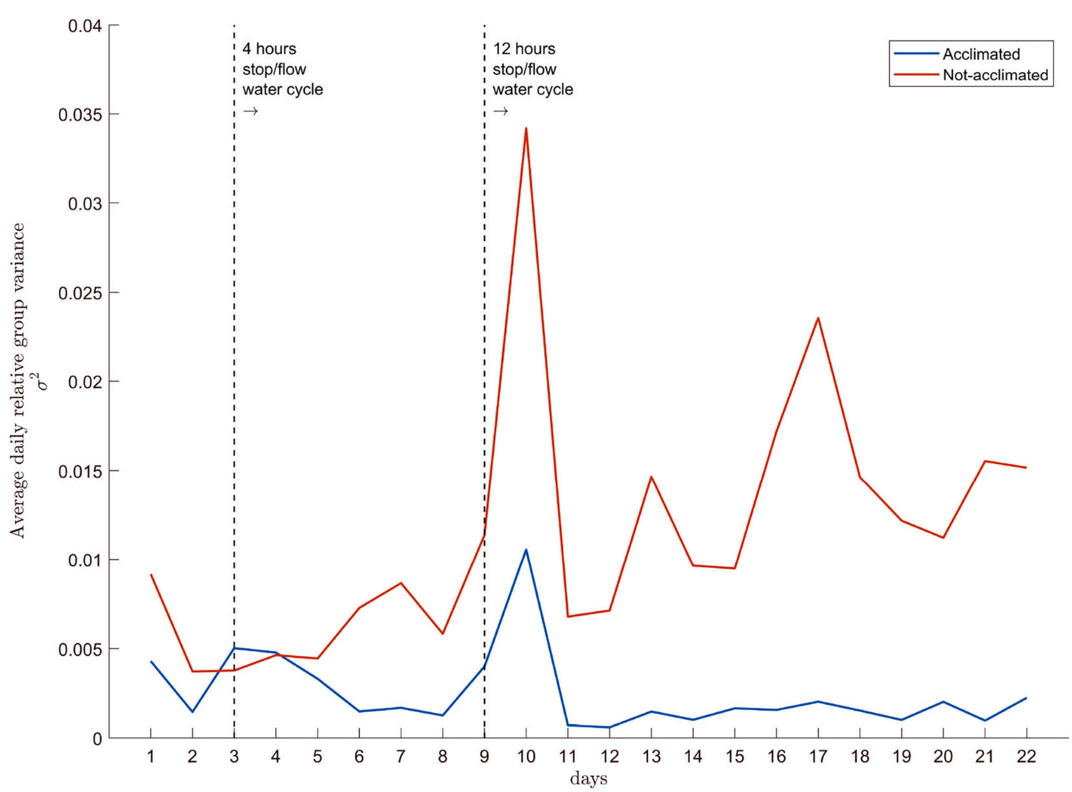

Fig. 8. The variance of the electrical resistance data, evaluated on a daily interval, of the two groups of plants (acclimated and not-acclimated) is shown with the time indication of the different stop/flow irrigation schemes applied.

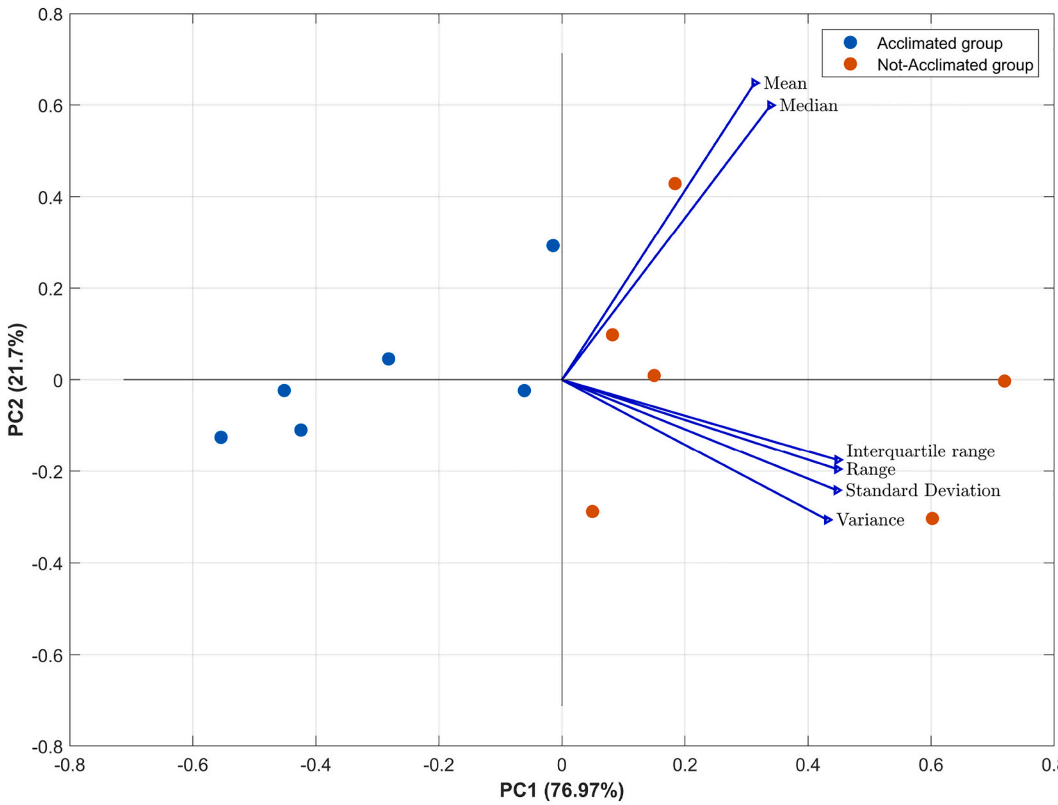

Fig. 9. Biplot of the Principal Component Analysis (PCA) for statistical parameters in the observed plants. The two principal components (PC1 and PC2) explained 98.7% of total variation in statistical data, and show clear partitioning of the Acclimated group of plants from the Not-Acclimated one along the PC1. Blue arrows represent statistical parameters, and circles in color represent the plants under study. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

or not-acclimated). The correlation between the time intervals of the different irrigation cycles and the consequent trend of the electrical signal, as well as the distribution of the observed plants in the PCA ordination plot, clearly show the potential for using the proposed methodology as a consistent tool for the early water stress detection in plants. Accordingly, the different acclimation process applied could be identified as well by evaluating the statistical characteristics of the electrical signal of the two groups of plants, in particular by considering the measures of dispersion of the acquired data, such as the average daily variance. The results of the proposed methodology, composed of multi-electrodes acquiring system and statistical signal analysis, are in accordance with the findings of other studies, where the water stress has been associated with stem electrical conductivity measurements [15]. Several studies in literature have shown the strong perturbation of electrical signaling induced by different stress conditions in plant, causing more difficulty in the interpretation and analysis of specific signals [20,25]. This is confirmed by the assumption that water stress involves multiple signaling responses related to the physiological activity of the plant, which can lead to a significant increase in the variability of the electrical resistance values [3,26,27]. Our results show that the observation on a daily basis of the statistical dispersion of the acquired data can be crucial to obtain information on the hydric condition of the plant under study; moreover, this analysis was achieved by adopting a relatively high sampling frequency (1 sample/min) for the data acquisition system. The different acclimation response of the two groups of plants, highlighted by the statistical evaluation of the electrical signal, could be further exploited by implementing a water stress detection system. Such a tool, based on the proposed electrical signal analysis, could take advantage of both trend and statistical dispersion (variability) evaluation of the electrical resistance, directly associated with the water status in plant, to detect the electrical patterns associated with early water stress. In particular, the measures of statistical dispersion, such as the daily variance and interquartile range, could be calculated to evaluate and classify the plant water stress condition in case of a remarkable electrical resistance variation, compared to baseline average conditions. In fact, environmental or physiological fluctuations may affect the acquired signal in terms of electrical resistance, even if the plant is not affected by water stress. In such cases, the proposed evaluation of the dispersion of the electrical signal data can help establish the actual water status of the plant with more accuracy than by observing the signal trend only. Moreover, the proposed approach can be exploited for the implementation of automatic detection systems that may be improved to effectively acknowledge the early water stress condition in different plant species.

5. Conclusions

In conclusion, the methodology and results presented in this study look promising, in order to develop empirical applications. The plant electrical activity study related to water stress acclimation has the potential to increase productivity control in semiarid conditions. Further experiments based on the presented methodology are required to confirm the early results through the evaluation of other parameters, such as stem water potential and stomatal conductance. In order to develop automatic algorithms for the early detection of water stress in plants, the proposed approach of combining the evaluation of both the electrical signal trend and the measures of statistical dispersion has proven to be effective. Moreover, unlike indirect soil suction or environmental measurements, the proposed methodology manages to extract information directly from the electrical activity of the plant itself. The same approach of interpreting the electrical signal statistics can be applied for the assessment of other stress conditions in plants. In fact, the acquisition and statistical evaluation of the electrical resistance signal represents a simpler and less expensive approach than the impedance methodology, and the presented results have shown a stable trend in terms of signal reliability. Furthermore, the proposed approach can accomplish for prolonged time acquisitions of the multi-electrode data, thus laying the foundations for the implementation of automatic detection and classification methods.

Funding

This research was funded by the European Union's Horizon 2020 research and innovation programme, Marie Sklodowska-Curie grant number 750807, and supported by Cassa di Risparmio di Firenze foundation, project"Identificazione tramite Analisi Numerica dello Stress Idrico nelle Piante – IANSIP", 2018 research and innovation programme.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] L. Gurovich, P. Hermosilla, Electric signalling in fruit trees in response to water applications and light-darkness conditions, J. Plant Physiol. 166 (2009) 290–300, https://doi.org/10.1016/j.jplph.2008.06.004.
[2] L. Gurovich, Electrophysiology of Woody Plants, 2012, https://doi.org/10.5772/28600.
[3] P. Oyarce, L. Gurovich, Electrical signals in avocado trees, Plant Signal. Behav. 5 (2010) 34–41, https://doi.org/10.4161/psb.5.1.10157.
[4] J. Fromm, S. Lautner, Electrical signals and their physiological significance in plants, Plant Cell Environ. 30 (2007) 249–257, https://doi.org/10.1111/j.1365-3040.2006.01614.x.
[5] B. Pickard, Action potentials in higher plants, Bot. Rev. 39 (1973) 172–201, https://doi.org/10.1007/BF02859299.
[6] C. Koziolek, T. Grams, U. Schreiber, R. Matyssek, J. Fromm, Transient knockout of photosynthesis mediated by electrical signals, New Phytol. 161 (2004) 715–722, https://doi.org/10.1111/j.1469-8137.2004.00985.x.
[7] S. Chatterjee, S. Das, K. Maharatna, E. Masi, L. Santopolo, S. Mancuso, A. Vitaletti, Exploring strategies for classification of external stimuli using statistical features of the plant electrical response, J. Royal Soc. Interface 12 (03 2015), https://doi.org/10.1098/rsif.2014.1225.
[8] S. Morosi, M. Dolfi, E. Del Re, E. Masi, I. Colzi, S. Mancuso, F. Francini, R. Magliacani, A. Valgimigli, L. Masini, A wsn for Ground-Level Ozone Monitoring based on Plant Electrical Activity Analysis, 2015, pp. 715–720, https://doi.org/10.1109/IWCMC.2015.7289171.
[9] M. Dolfi, I. Colzi, S. Morosi, E. Masi, S. Mancuso, E. Del Re, F. Francini, R. Magliacani, Plant Electrical Activity Analysis for Ozone Pollution Critical Level Detection, 2015, pp. 2431–2435, https://doi.org/10.1109/EUSIPCO.2015.7362821.
[10] Z. Wang, X.-H. Qin, J. Li, L. Fan, Q. Zhou, X. Zhao, C.-J. Xie, Z.-y. Wang, L. Huang, Highly reproducible periodic electrical potential changes associated with salt tolerance in wheat plants, Environ. Exp. Bot. (01 2019), https://doi.org/10.1016/j.envexpbot.2019.01.014.
[11] M. Farooq, A. Wahid, N. Kobayashi, D. Fujita, S. Basra, Plant drought stress: effects, mechanisms and management, Agr. Sustain. Dev. 29 (03 2009), https://doi.org/10.1051/agro:2008021.
[12] R. Spanswick, Electrical coupling between cells of higher plants: a direct demonstration of intercellular communication, Planta 102 (1972) 215–227, https://doi.org/10.1007/BF00386892.
[13] B. Luyet, Variation of the electric resistance of plant tissues for alternationg currents of different frequencies during death, J. General Physiol. 15 (1932) 283–287.
[14] W. Barkas, R. Hearmon, G. Pratt, Electrical resistance of wood, Nature 151 (1943) 83, https://doi.org/10.1038/151083a0.
[15] A. Nadler, E. Raveh, U. Yermiyahu, M. Lado, A. Nasser, M. Barak, S. Green, Detecting water stress in trees using stem electrical conductivity measurements, Soil Science Society of America Journal - SSSAJ 72 (07 2008), https://doi.org/10.2136/sssaj2007.0308.
[16] D. Comparini, E. Masi, C. Pandolfi, L. Sabbatini, M. Dolfi, S. Morosi, S. Mancuso, Stem electrical properties associated with water stress conditions in olive tree, Agric. Water Manag. 234 (2020) 106109, https://doi.org/10.1016/j.agwat.2020.106109.
[17] V. Vodeneev, E. Akinchits, V. Sukhov, Variation potential in higher plants: mechanisms of generation and propagation, Plant Signal. Behav. 10 (08 2015), https://doi.org/10.1080/15592324.2015.1057365.
[18] M. Tyree, M. Zimmermann, Xylem Structure and The Ascent of Sap, 2002, https://doi.org/10.1007/978-3-662-04931-0.
[19] P. D'Odorico, A. Porporato, C. Runyan, Dryland Ecohydrology, 2019, https://doi.org/10.1007/978-3-030-23269-6.
[20] G. Toledo, A.G. Parise, F. Simmi, A. Costa, L.G. Schultz Senko, M.-W. Debono, G. Souza, Plant electrome: the electrical dimension of plant life, Theor. Exp. Plant Physiol. 31 (02 2019), https://doi.org/10.1007/s40626-019-00145-x.
[21] R. MacGill, J. Tuckey, W. Larsen, Variation of box plots, Am. Stat. 32 (1978) 12–16.
[22] J.W. Tukey, et al., Exploratory Data Analysis vol. 2, Mass, Reading, 1977.
[23] A. Harb, A. Krishnan, M. Ambavaram, A. Pereira, Molecular and physiological analysis of drought stress in arabidopsis reveals early responses leading to acclimation in plant growth, Plant Physiol. 154 (2010) 1254–1271, https://doi.org/10.1104/pp.110.161752.
[24] I. Yordanov, V. Velikova, T. Tsonev, Plant responses to drought, acclimation, and stress tolerance, Photosynthetica 38 (2012) 171–186, https://doi.org/10.1023/A:1007201411474.
[25] W.-G. Choi, G. Miller, I. Wallace, J. Harper, R. Mittler, S. Gilroy, Orchestrating rapid long-distance signaling in plants with ca 2+, ros, and electrical signals, Plant J. 90 (01 2017), https://doi.org/10.1111/tpj.13492.
[26] M. Zimmermann, H. Maischak, A. Mithöfer, W. Boland, H. Felle, System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding, Plant Physiol. 149 (2009) 1593–1600, https://doi.org/10.1104/pp.108.133884.
[27] N. Suzuki, G. Miller, C. Salazar, H. Mondal, E. Shulaev, D. Cortes, J. Shuman, X. Luo, J. Shah, K. Schlauch, V. Shulaev, R. Mittler, Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants, Plant Cell 25 (09 2013), https://doi.org/10.1105/tpc.113.114595.