Agricultural Water Management 234 (2020) 106109

Contents lists available at ScienceDirect

Agricultural Water Management

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

Stem electrical properties associated with water stress conditions in olive tree

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

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

ARTICLE INFO

Keywords:
Plant electrical signal
Stem resistance
Drought stress
Olea europaea
Signal classification

ABSTRACT

The analysis of the electrophysiological activity of plants permits a real-time information of the plant status (e.g. light availability and water stress). However, even though it is clear that the role of the electrical signals in plant is crucial, especially in processes involving the propagation of rapid signals, a systematic approach for the interpretation of the electrical patterns is still missing. In this work a multi-electrodes approach has been applied to study the electrical signals in olive trees plants subjected to three different level of water stress. In particular, by using specific water irrigation regimes, a control group, a mid/mild stressed group and a high stressed group have been monitored and subsequently subjected to a long period of prolonged drought stress. Physiological parameters and electrical activity have been continuously monitored for the whole experiment to highlight any correlation between the electric signal and the water stress. Our results showed that it has been possible to differentiate the electric signals related to drought conditions of different intensity (i.e. control, mild and high). In particular we have found that the average daily relative electrical resistance change, the opposite of the electrical conductance, is directly related to the drought stress whilst the signal variance increases during the period of main water stress. Additionally, a proposed signal classification system has been successfully able to detect the absence/presence of stress and to effectively recognize daily class samples (93 % control, 76 % mild and 80 % high). The set-up could provide a useful tool for monitoring water conditions in plants and has several potential applications for sensor and automatic system in greenhouse or field able to monitor directly the plant water status.

1. Introduction

1.1. Electrical signaling in plant

The propagation of electrical signals in plant is an important system used to transfer information about the environmental stimuli perceived by the sensory system and spread it along different and remote organs (Fromm and Lautner, 2007). To date, abundant studies have highlighted that the role of the electrical signals in plant is crucial in response to biotic or abiotic stress condition, especially in processes involving the propagation of signals, such as light stimuli (Datta and Palit, 2004; Gurovich and Hermosilla, 2009; Chatterjee et al., 2014), water accessibility (Fromm and Eschrich, 1988; Grams et al., 2007; Oyarce and Gurovich, 2010), osmo-regulation (Schroeder and Hedrich, 1989), temperature (Volkov et al., 2012; Kai et al., 2011), gravity (Masi et al., 2015), mechanical touch/damages and insect attack (Brenner et al., 2006; Volkov et al., 2007).

It has already been proved that different environmental stimuli produce typical responses in living cells generating specific electrical signals (Fromm et al. 2005, Lautner et al. 2005, Fromm and Fei 2009). For that reason, the use of plant as biosensors has been theorized (Volkov and Ranatunga, 2006; Davies, 2004; Gurovich, 2009) and attempts for the classifications of these signals exist (Chatterjee et al., 2015). Moreover, whilst the chemical signalling involving compounds are not appropriated to respond rapidly to environmental stress factors, because are slower and localized (e.g. hormones), electrical signals can be quickly diffused and propagate over long distances throughout different plant organs and their analysis is a promising candidate for the study of early stress responses.

Additional researches, by monitoring the electrophysiological activity of plants, have already performed successful non-destructive methodologies to gather real-time information of the plant status (e.g.

---

* Corresponding author.
E-mail address: dr.comparini@gmail.com (D. Comparini).

https://doi.org/10.1016/j.agwat.2020.106109
Received 10 January 2020; Received in revised form 20 February 2020; Accepted 25 February 2020
Available online 17 March 2020

light availability and water stress) for the construction of automatic irrigation systems (Nadler et al., 2008, Oyarce and Gurovich, 2010; Ríos-Rojas et al., 2014) or have used plant as sensor for particular stimulus (e.g. light, ozone exposure, saline stress) (Chatterjee et al., 2014; Morosi et al., 2015; Dolfi et al., 2015; Wang et al., 2019). However, even though it is clear that the role of the electrical signals in plant is crucial, especially in processes involving the propagation of rapid signals, a systematic approach for the interpretation of the electrical patterns is still missing. The levels of complexity and dynamics of electrical activity, both temporal (when) and spatial (where and how the signal propagates) make data analysis more complicated. For this reason recent researches and findings have been directed to all ionic currents generated at different level of the plant organization that recently have been classified as "plant electrome" (De Loof, 2016 and Souza et al., 2017).

Indeed, several mechanism and responses of different nature (e.g. chemical, hydraulic, electrical, etc.) have been associated with abiotic stress as water stress and it is still very difficult to evaluate the effects derived by different related biochemical and molecular pathways. These modifications involve physiological and/or morphological factors that affect the electrical properties of the plant tissue and have been subjected to numerous investigations. In various cases, the measurement of the electrical resistance of the plant tissues has been used to estimate their physiological features, for example Gora et al. (2017) have reported a correlation between the trunk resistance of tree in tropical and temperate climates and the damages derived from lighting strikes. These studies clearly reported that the resistance values are linked to morphological and physical characteristics of the plants, in particular to the cell dimension and to the presence of water in the tissues (Nadler et al., 2008) and the solutes concentration (Gora et al., 2017). Similar principles have been shown in studies that have tried to detect modifications or damages in cells or plant tissues (Azzarello et al., 2012) or in the whole plant (Ben Hamed et al., 2016) by measuring the impedance.

1.2. Water stress in plant

Water is a fundamental resource for all plants and water shortage is one of the stresses that leads to greater physiological damage and which more compromises the productivity of crops. The main stress factors caused during drought stress influence the internal plant water potential causing the closure of the stomata followed by reduced gas exchanges that implies an altered status of limited photosynthetic activity and metabolism. All these conditions can be translated later in permanent damages if drought last over time or can culminate in the plant death depending on several elements (e.g. plant species, growth stage, duration, etc.) (Jaleel et al., 2008; Tátrai et al., 2016).

The stomatal closure is the simplest defense of a plant to limited water availability as it allows to minimize the loss by transpiration. Plant turgor or leaf water potential have been used in the past as indicators of water status, but it has been demonstrated that some plants can have turgor identical or higher than those of well-watered and still present a reduced growth but have much lower stomatal conductance. For this reason, leaf conductance measurement has been suggested as preferable indicator of plant drought stress (Davies and Zhang, 1991; Thomas et al., 1989).

Several researches have showed a reduced photosynthesis rate in plant subjected to low/medium intensity drought stress that are usually caused by stomatal constrictions and non-stomatal dependent constriction situations of intense drought stress (Wang et al., 2018; Degl'Innocenti et al., 2009; Misson et al., 2010) but relatively few works have investigated the correlation between electrical activity and stomatal conductance at different drought stress levels.

The plant hormone abscisic acid (ABA) is considered to be strongly correlated with the decrease in stomatal conductance in drought stress and it is well known that it plays a crucial role in the regulation of the stomatal behaviour of water-stressed conditions (Wilkinson and Davies, 2002; Jiang and Hartung, 2007; Brunetti et al., 2018). Nevertheless, it should be noticed that both short-term and long-term signaling that occurs during water stress is very complex and ABA is not the only signal related to the process which involves the coordination of numerous elements as plants need to react to various environmental conditions to adjust the stomatal aperture based on the surroundings (Kuromori et al., 2018).

1.3. Objective of the work

The study of electrical signals generated by plants subjected to drought stress is a very innovative topic but still clear methodologies for signal interpretation are missing. The analysis of the electric properties of different zones of the stem/trucks have been used to enhance water utilization and efficiency in open field by their correlation with stomata conductance and plant internal water flux (Gibert et al., 2006; Oyarce and Gurovich, 2010) and could potentially be used for early detection of water stress. This study aims to elucidate long term electrical analysis related to water stress. The analysis of the physiological status of each plant has been used to correlate different drought stress intensities with specific electrical patterns. It is based on a continuous monitoring of several plants *in vivo*, in a non-destructive way, for a long period through a new set-up that consists of a multi-electrodes system able to detect the electrical resistance signal and its variance on several plants (or in different zones of the same plant). Thus, after storing all data for the whole period (days or weeks) a statistical analysis based on a systematic approach of intervals analysis have been used for the interpretation of the stem electrical signal patterns in response to different levels of water stress. Finally the analysis of electric signal resistance and variance in olive trees plants subjected to different level of water stress has been used to simulate an automatic detection of the plant status and evaluate their potential applications in field.

2. Material and methods

2.1. Plant material, growing conditions and experimental plan

Trials have been conducted on rooted plant cuttings of *Olea europaea* L. plants, cv. Picholine, measuring about 80 − 120 cm in height. The plants have been purchased after being grown on small pot (13 × 13 × 18 cm) in a mix of 50 % peat and 50 % pumice added with minced horns and fingernails of animals ("cornunghia") and calcium carbonate (lime) amendments. The plants have been watered and kept a week to adapt to the new growth chamber conditions before starting the experiment. The growing conditions have been maintained at day/night light cycle of 14 h/10 h, PAR of 200 − 250 μmol m^{-2} s^{-1} and temperature of 20.5 ± 2 °C during the day and 18.3 ± 2 °C during the night. The trial has been kept and monitored for about two months. All photosynthesis and conductance measurements have been performed with a portable photosynthesis system for gas exchange LI-COR 6400xt (LI-COR Biosciences, USA) (Fig. 1). These data have been used for the interpretation of the electrical signal by correlating the plant status to specific electrical patterns. During gas exchange measurements, the light level inside the leaf chamber was maintained at PAR of 500 μmol m^{-2} s^{-1} and air flow rate of 400 μmol s^{-1}.

For the water stress treatments, we used a modified methodology used by Sinclair and Brilli (Sinclair and Ludlow, 1986; Brilli et al., 2007). In details, at the start of the experiment, all pots have been saturated with water, kept about 4 h to drain and weighted to determine the initial saturated hydrated weight of the pot (100 % pot water capacity). Subsequently, each pot has been wrapped in a plastic bag that has been bonded around the stem to avoid evaporation from the soil in order to monitor only the amount of water removed by the plant (Fig. 2). Three levels of water stress have been tested by unwrapping the plastic bag and adding water at different quantities and periods

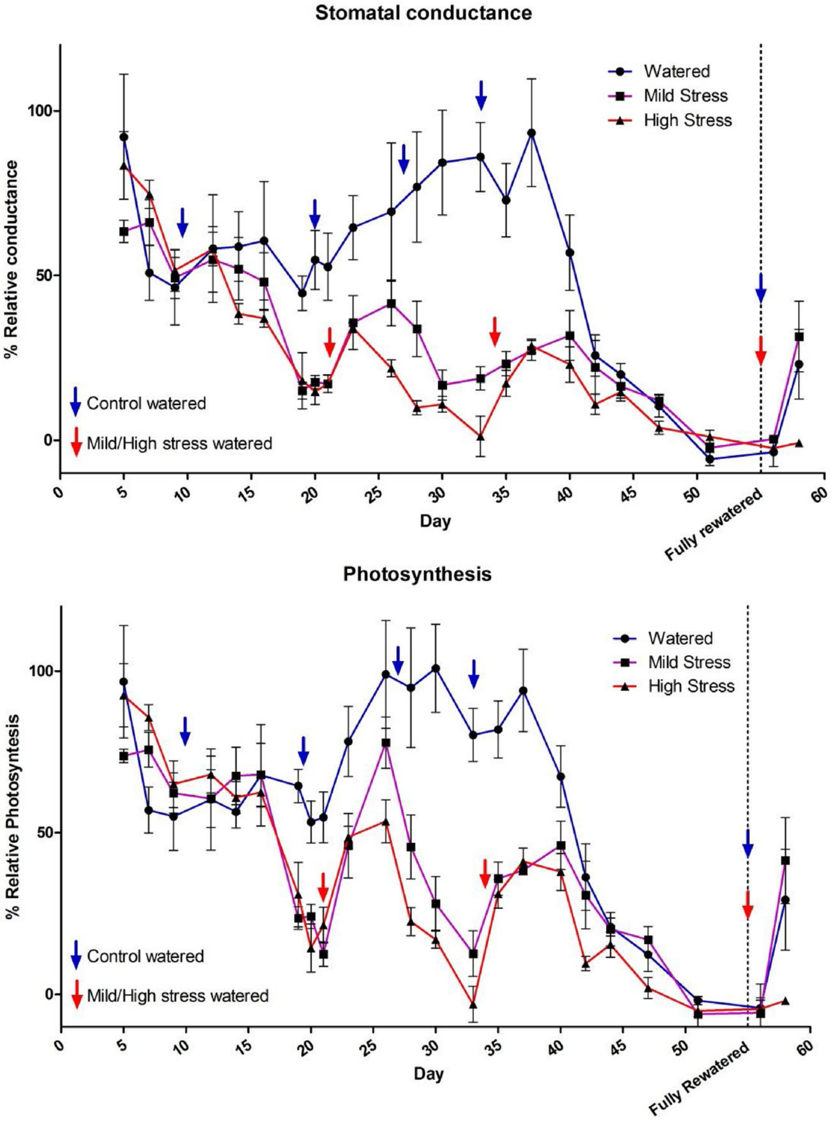

Fig. 1. Average of stomatal conductance (top) and photosyntesis rate (bottom) percent respect the initial measure of the groups of plant subjected at different drought stress level (control, mild stress, high stress) during the whole experiment (n = 5, SEM). The first 33/34 days the plant have been watered with different frequency indicated by the arrows, then a period of 3 weeks of drought stress have been applied to all plants before rewatering.

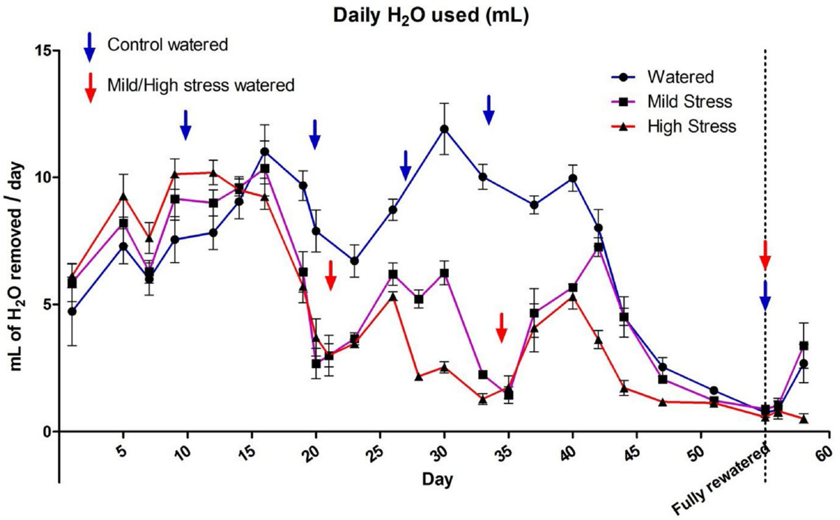

Fig. 2. Average water removed (mL) for day by each plant group (control, mild stress, high stress) subjected to different irrigation regimes (n = 5, SEM).

considering the pot water capacity weight equivalent to 100 % as the water saturated weight (Initial pot weight). To determine the exact amount of water in the soil at which the plant was not anymore able to support transpiration without face water stress we have monitored the conductance to determine the minimum fraction of transpirable soil water (FTSW) defined when value of stomatal conductance comes close to zero (Final pot weight).

Then, the FTSW was calculated for each single pot as: FTSW = (Daily pot weight – Final pot weight) / (Initial pot weight – Final pot weight) (Sinclair and Ludlow, 1986; Brilli et al., 2013).

Following these calculation, fifteen plants have been divided in three groups each of 5 plants: 1) control plants that have been regularly watered up to reach the weight of about 80 % of the FTSW; the other two groups have been watered immediately after FTWS was declining to almost zero (signal of water stress) and restored at different FTSW percent, respectively 2) a group referred as mild stressed plants that have been re-watered at about 40 % of the FTSW and (3) the last group of high stressed plants that have been watered at about 20 % of the FTSW. Control plants have not been subjected to any stress prior the two weeks prolonged drought stress, therefore their stomatal conductance never reached zero. The measure of the whole set of pots was used to estimate the approximate final pot weight of the control group, thus the water content at the stress point.

Finally, after two re-watering regimes described above for the mild and high stress plant, all plants have been subjected to a prolonged period of drought stress at day 33/34 for about 3 weeks to evaluate how the different initial water regimes have influenced the adaptation to a prolonged drought stress. Finally, all plants have been fully re-watered to recover by keeping each pot for one minute immerged in water whilst pipetting 10 mL of water on the soil surface and then wait fifteen minutes to drain (each plant back to about 50–60 % of the FTSW).

2.2. Relative water content

The relative water content measurement has been performed at the end of the experiment, for each plant. In particular, leaves have been collected after 3 days following the recovery phase (plants abundantly irrigated after the final prolonged drought stress). The relative water content (RWC) of the leaves in each plant has been calculated as the ratio of fresh leaves weight (FW) measured immediately after harvesting, the leaves turgid weight (TW) obtained by sinking all leaves in demineralized water for 48 h and dry weight obtained by keeping the leaves at 45 °C for 48 h. The formula used was RWC (%) = 100 [(FW-DW)/ (TW-DW)]. The RWC has been calculated separately for leaves of the upper part and leaves from the lower part of each plant. This approach has been used because it has been observed that the bottom leaves of each plant have been the most affected by the water stress. In particular four leaves for each plant have been collected and used to calculate the RWC (n = 4) from the top part collected between the 2nd and 5th node and another group of leaves (n = 4) from the bottom/mid part collected from the 6th and the 10th node of each plant (Fig. 3). Relative water content has been processed with ANOVA applying a Tukey's post-test ($p < 0.05$) using Prism 8 software (GraphPpad Software, USA).

2.3. Light response curves and chlorophyll fluorescence

The LI-COR 6400xt portable photosynthesis system for gas exchange and chlorophyll fluorescence measurements (LI-COR Biosciences, USA) has been used. The measurements of chlorophyll fluorescence have been performed regularly on all plants to obtain additional information about the physiological status of the plant, namely to highlight any damage to the photosynthetic apparatus and the ability of plant to restore quickly to their normal physiological status from temporary problems that aroused during the water stress (Fig. 6).

All parameters (Fo, Fm, Fs, Fm′, and Fo′), from each plant, for each day of measurements, have been achieved using a leaf chamber fluorometer both in dark (1 h before the start of the day cycle – light off) and in light condition (1 h after the start of the day cycle - light on). The LI-COR by default is able to provide several calculated parameters, among them Fv/Fm, Fv′/Fm′, ΦPSII (PhiPS2), qP, NPQ, and ETR. Basically, the maximum fluorescence in dark (Fm) and the minimum level of fluorescence (Fo) were obtained in dark and variable fluorescence, Fv, was calculated as the difference between Fm and Fo. Then all other parameters have been calculated by the data obtained in light condition (e.g. Fm′, and Fo′) following the formulas that have been already deeply described (see for example Maxwell and Johnson, 2000; Oxborough, 2004) (Fig. 4). Fluorescence analysis has been processed with ANOVA applying a Tukey's post-test ($p < 0.05$) using Prism 8 software (GraphPad Software, USA).

2.4. Electrical activity

The acquisition of the electrical activity has been performed using stainless steel needle (EMG like) non-isolated electrodes (length = 10 mm) which have been connected to a modular data acquisition system (DAQ) consisted of a remote controller chassis with a multiplexer terminal block and a digital multimeter (respectively model PXI-1033; TB-2605; PXI-4065; National Instruments Corporation), which permits AC/DC voltage, and 2wires resistance measurements. In particular, during the data acquisition, each pair of electrodes has been maintained at a fixed distance of 0.9 cm using a plastic support and inserted at the base of the stem of each plant (Fig. 5). This distance is chosen based on the electrical signal measure and is influenced by the stem variability and how the electrodes is implanted, it needs to be previously calibrated to be in the range of the instruments to maintain enough resolution without go out of scale. This set-up has been chosen in order to simplify the complexity that arise from measuring the electrical property of a certain material as it is influenced by its length, structure and content (e.g. electrolytes, solutes, etc…). In this kind of measurements, distance between the two electrodes is a key factor, and it may be subjected to a change due to the plant stem elongation (Wang et al., 2009). In our case, given the plant growth negligible, distance can been assumed as constant.

2.5. Electrical measurements data analysis

Data analysis of the electrical activity has been performed on the whole dataset that included all data collected (one acquisition every 2 min). Each plant initial resistance signal values were different. For this reason, to permit a comparison of all the data, the relative change respect their initial value has been used. In detail, for each plant, a baseline signal has been extrapolated as the average of 3 days resistance signals registered when the plant was in good health; this has been subtracted to the successive data. For example, if a plant when healthy had a 3 days average signal of 2 Ω and it reached 2.5 Ω, a relative change of 0.5 Ω would have been considered. Therefore, the relative changes have been calculated for each plant and the average group signal changes have been reported for each treatment (Fig. 6).

For the daily variance analysis, the data have been grouped for each day, where the initial value was considered the start of the light cycle. In particular, to evaluate the variability of the signal, the daily signal variance has been calculated for every plant and each treatment average has been reported (Fig. 7).

For the cluster analysis (Fig. 8) all average daily values have been represented according to their value of signal resistance and variance. In particular, a threshold limit of 10 Ω for the resistance and 1 for the variance has been set, therefore all points that were over this threshold have been considered exactly 10 Ω for the resistance and 1 for the variance. Each dataset incorporated the average daily resistance and the average daily variance of each plant. The presence or absence of drought stress has been set accordingly to the physiological

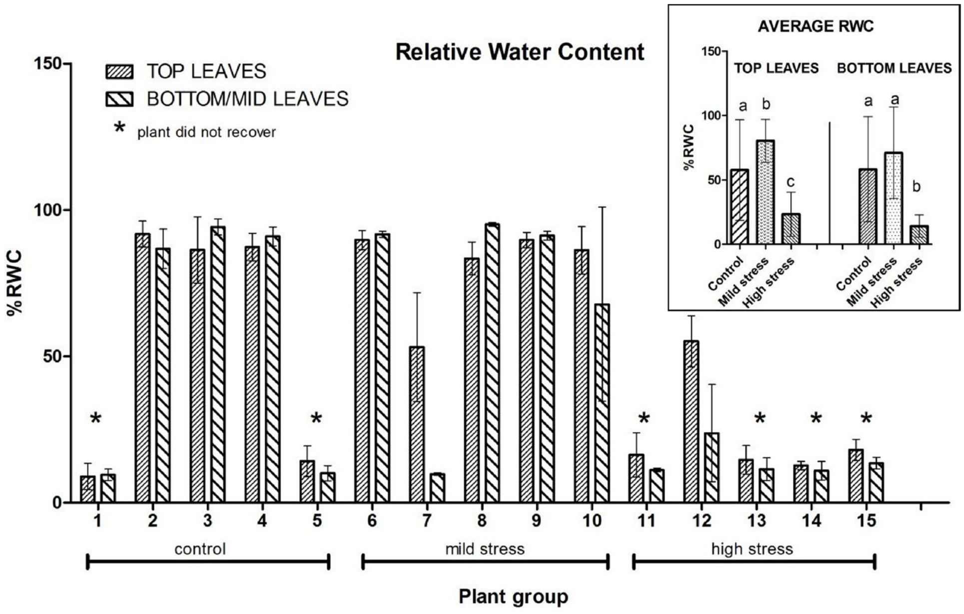

Fig. 3. Relative water content of the leaves from the top part (n = 4, SD) and the bottom part (n = 4, SD) of each single olive tree plant subjected to different irrigation regimes (control, mild stress, high stress); leaves have been collected 3 days after being re-watered subsequently a three weeks prolonged drought stress from internodes 2-5 (TOP) and 6-10 (BOTTOM). Top-right boxed area: RWC values showed as average of each treatment (n = 20, SD). Different letters represent statistical significance ($p < 0.05$).

measurements, in particular a plant was considered stressed when the value of the relative stomatal conductance was lower than 30 % (Fig. 1). A binary classifier has been employed to discriminate and evaluate the days of water stress. The input of the proposed system has been classified into one, and only one, of two non-overlapping classes ("Stress", "No Stress"). To examine the efficiency of the water stress detection classifier, a database of the whole 56 day-long recordings of the three dataset groups was employed and processed using the software MATLAB. Then the correctness of the electrical signals classification has been evaluated by computing the number of correctly recognized class examples (true positives, tp), the number of correctly recognized examples that do not belong to the class (true negatives, tn), and examples that either were incorrectly assigned to the class (false positives, fp) or that were not recognized as class examples (false negatives, fn). According to Sokolova and Lapalme (2009), the following performance measures for classification were reported in Table 1 and considered as: accuracy = (tp + tn) /(tp + fn + fp + tn); precision = tp / (tp + fp); sensitivity = tp / (tp + fn); specificity = tn / (fp + tn).

3. Results

The photosynthesis and stomatal conductance measurements collected regularly during the whole experiment (Fig. 1) have highlighted the three group's plant status during the two months and during the increasing drought stress conditions. All plant parameters (i.e. photosynthesis rate and stomatal conductance) showed a relatively healthy state of plants up to day 16. Then, intense decrease of the photosynthesis activity and stomatal openings has been observed for all plants that have not been watered; in fact, 80 % reduction respect the initial values has been observed in few days. Meanwhile, the control group plant that have been well watered periodically showed good values of photosynthesis and conductance as expected between 50–100 % of the initial value. According to photosynthesis and stomatal conductance data, the mild stress group recovered after few days and showed a peak at day 26, whilst the high stress plants peaked early at day 23 and rapidly weakened. A similar trend has been observed also for the second watering round (at day 34) for the mild and the high stress groups, where recovery peaks have been observed respectively at day 40 and 37, but have highlighted lower restored values of photosynthesis and stomatal conductance peaks likely due to the stress faced till then. Starting from day 33/34 when a prolonged period of about three weeks drought stress has been applied to all groups, a decline of all parameters has been observed in all plants. From day 42 all plants included the control groups faced a reduction over 60 % of the initial value with the high stress group plant being affected the most. At the end of the prolonged drought stress, after all plants had been recovered, the control and mild stress groups showed an increase of the parameters, whilst the high stress struggled to recover.

The water consumption has been also monitored for the whole period and it declined accordingly to the stomatal conductance; in fact, as soon as the latter approaches to lower values, the water consumption was reduced as well (Fig. 2). Interestingly, after being watered subsequently the prolonged drought period, although it was not statistically significant, the mild stress group showed the highest values of photosynthesis, conductance and water consumption. The relative water content (RWC) following the recovery of the high stress group plant has showed the lowest values with an average of 17 % for the upper leaves and 13 % for the lower leaves, with only one plant able to recover and survive the prolonged drought treatment (Fig. 3). Among the other plant, the mild stress plants have been all able to recover and showed the highest level of RWC (80 % top leaves, 71 % bottom leaves) with only one plant with low value in the bottom part. In the control group, two plants have also showed very low values and did not recover from the prolonged drought stress (plant 1 and 5, Fig. 3).

In general, the more intense is the stress condition for a plant, the lower number of reaction centers are available, thus lowering the Fv/Fm and Fv'/Fm' ratio. Our results followed this rule, where the high stress group has been the one with the lowest ratio, in particular for the day prior the irrigation when the water stress was higher; similar results have been shown in other studies (see for example Sofo, 2011). In general, all values declined during the period of intense drought stress and recovered after irrigation. NPQ analysis did not showed any statistical difference among the groups and showed a very high standard deviation and a general declining trend at the end of the prolonged period for all groups. ETR, qP and Phi(PS2) also showed very similar trends respect Fv/Fm and Fv'/Fm' ratio, with the exception that the differences among the group have been greater in the period where the drought stress was more pronounced. Also, at the end of the prolonged drought stress, the mild stress plant adapted showed higher values of ETR and Phi(PS2) that were statistically different from the high stress group plants.

The electrical signal resistance has been reported as average of each plant group and highlighted in three intervals (day 19–29; 30–39; 39–55) (Fig. 6). The average electrical signal of the resistance (as

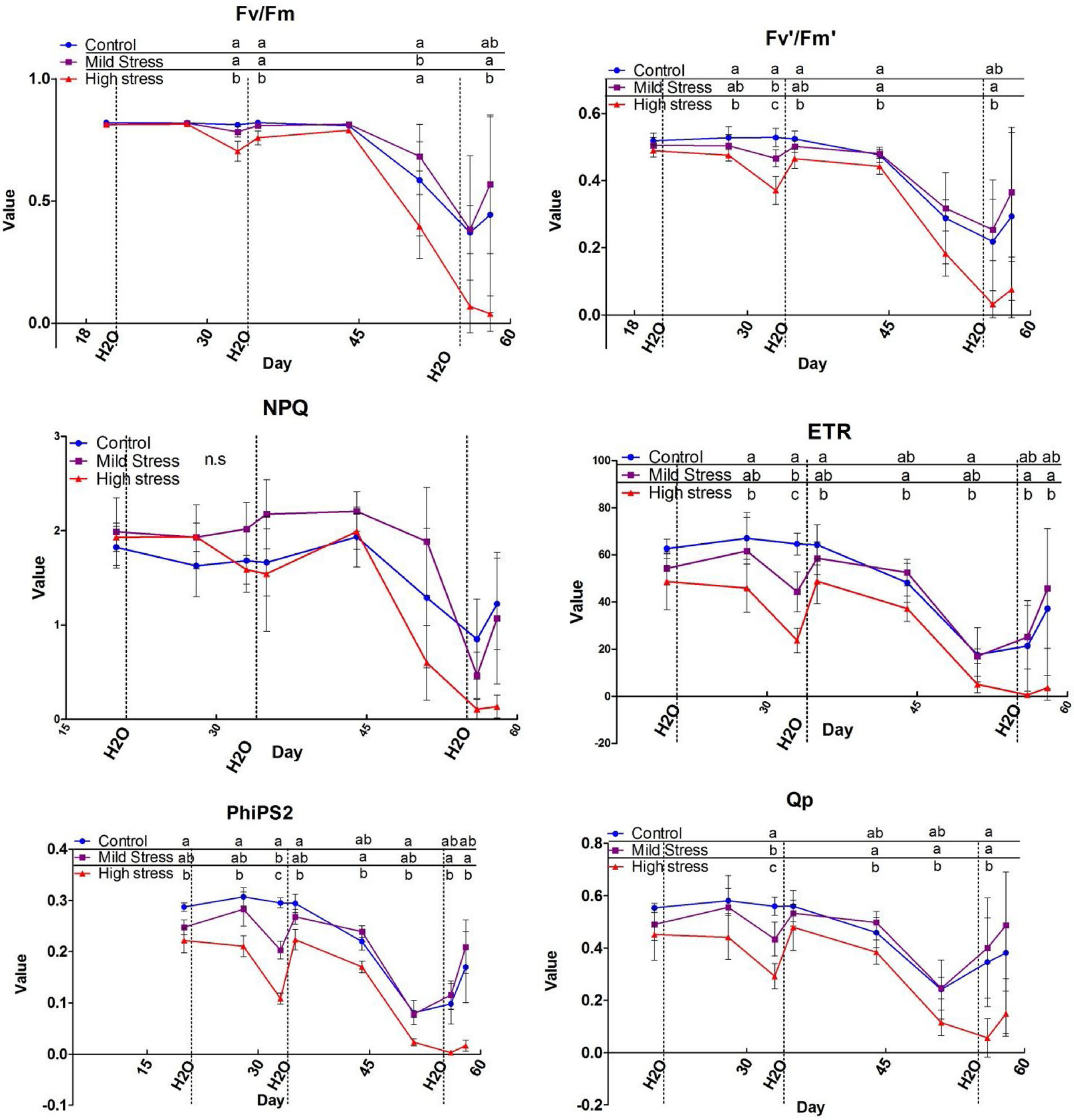

Fig. 4. Quantum yeld and maximum quantum yeld of PSII (Fv/Fm and Fv'/Fm'), non-photochemical quenching (NPQ), electron transport rates (ETR), actual quantum efficiency of photosystem II (Phi(PS2)) and photochemical quenching (qP) for each group of plant subjected to different water regimes (control, mild stress, high stress), (n = 5, SD). Different letters on top of each data mean statistical difference (ANOVA, turkey's test, $p < 0.05$), absence of letters mean no statistical difference.

reciprocal of electrical conductance) of each group highlighted a specific pattern that depends on the day/night cycle and the irrigation regimes. In general the water stress occurred in each plants has been directly correlated with the average value of each plant groups suggesting that the signal was strongly dependent on the water content in the stem. In fact, when the stomatal conductance of the mild and the high stress groups was approaching to zero (see Fig. 1), the relative change in the resistance signal of the two groups increased. On the contrary, no increase in the electrical signal was observed in the control group during the period in which it has been regularly irrigated. These patterns appeared also for the second interval (30–39) for the mild and the high stressed group, where signal has been increasing proportionally to the water stress. Finally, all treatments have intensely increased their resistance during the prolonged water stress period (day 39–55) with a relative signal changes over ten times higher than before.

Furthermore the variance of the resistance of each group has been calculated daily and has highlighted an increment of the electrical signal variability related to the water stress, slightly higher at day 20, medium at day 33 and very high during the prolonged period.

Finally, by plotting together the daily signal of resistance and its variance, it has been possible to observed that the values tend to group in cluster depending on the drought conditions (Fig. 8). In our study an empirical approach was adopted, by considering the leaf stomatal conductance of the control dataset to adjust and validate the threshold values that could indicate a stress based on the electrical resistance and electrical signal variance. A binary classifier has been implemented to distinguish and identify the phase (days) of water stress. The corresponding decision rule has been chosen by setting a threshold value of 3.8 Ω on electrical resistance and 0.01 Ω^2 on signal variance. The detection performance evaluation of the proposed classification system is

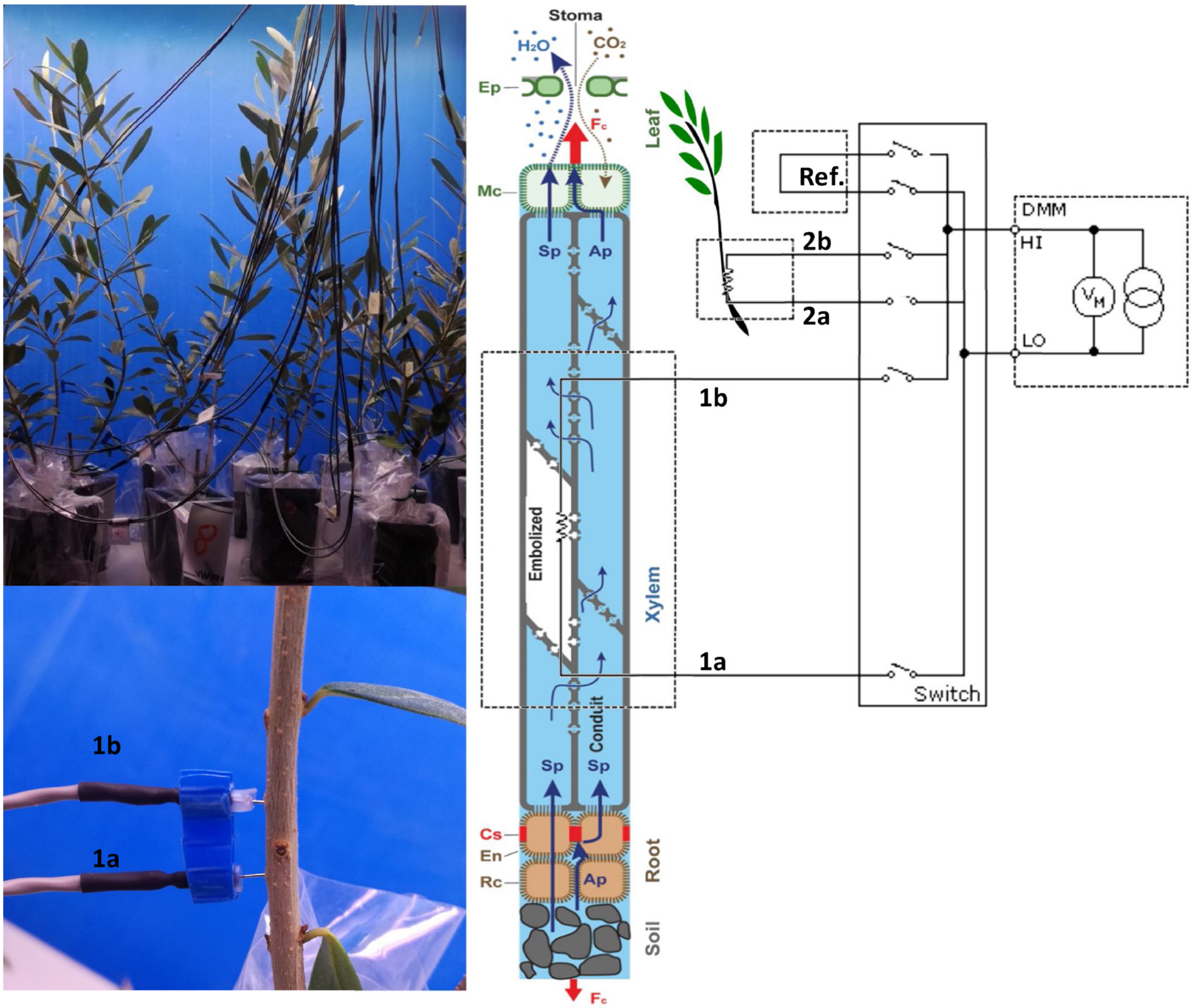

Fig. 5. On the left-top: picture of several olive trees plant with the electrodes; on the left-down corner an enlargement focused on one two electrodes inside a plant. On the right the model where the two wire resistance measurement schematic is shown: electrode 1a and 1b are shown based on the plant stem physiology (picture modified from Venturaes et al. 2017). Additional electrodes can be added controlled by a switch to monitor concurrently several plant as shown (2a and 2b) and a reference electrode is used as an offset adjustment reference (ref.).

Legend: Cell wall capillary forces (Fc arrow), mesophyll (Mc), symplastic and transmembrane pathway (Sp), apoplastic pathway (Ap), root cells (Rc), endodermis (En), casparian strip (Cs), epidermis (Ep), H2O vapor loss (broken blue arrow) and CO2 uptake (broken brown arrow); (Venturas et al., 2017); digital multimeter (DMM), Voltage (VM). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

listed in Table 1 and reported as percent of success. Different classification results have been compared when the signals of resistance (Res) and variance (Var) have been considered alone or associated (Res + Var). The classification system was capable to discriminate the response to drought stress using both signals with an accuracy of 93 % for the control group, 76 % for the mild stressed and 80 % for the high stressed ones. A good performance is achieved also in terms of precision (88 % control, 82 % mild, 83 % high) and specificity (96 %, 78 % mild, 75 % high) and sensitivity (82 % control, 75 % mild, 84 % high). Interestingly the comparison of the performances of the various stress detection methods shows that the combined use of both resistance and variance is not always more efficient than the single signal analysis, but increased notably the performance measures of the sensitivity (the percentage of stressed plants that are correctly identified as unhealthy) and accuracy, especially in monitoring mild and high stressed plants. On the other hand, the performance measures of precision and specificity (the percentage of healthy plants that are correctly identified as not having the condition) have shown higher performances on single signal classifiers. In general, when both variance and resistance values increase, it means that plants are entering in a phase of stress and the use of both signals helped to limit false negative detection.

4. Discussion

The experiment showed the stomatal conductance and photosynthesis activity of three different groups of plant elucidating the effect of repeated mild and high water stress condition on olive tree plants. As expected, the high stress group has suffered the most and was not able to recover a subsequent long period of water privation. Instead, the mild stress plant group, even presenting symptoms of stress condition, has been able to face the prolonged period and recover. In fact, both considering the relative water content and the photosynthetic apparatus status, a clear trend suggested that the mild stress group plant has better tolerated and recovered from the prolonged drought treatment. This suggests that plants that have been previously subjected to a mild stress are able to adapt and react promptly to the prolonged stress period and recover faster, attributable to an acclimation effect. This hypothesis is particularly enforced by the RWC values measurements showed in Fig. 3. It is likely that the drought stress duration had irreversibly damaged the plants of the high stress group by affecting the

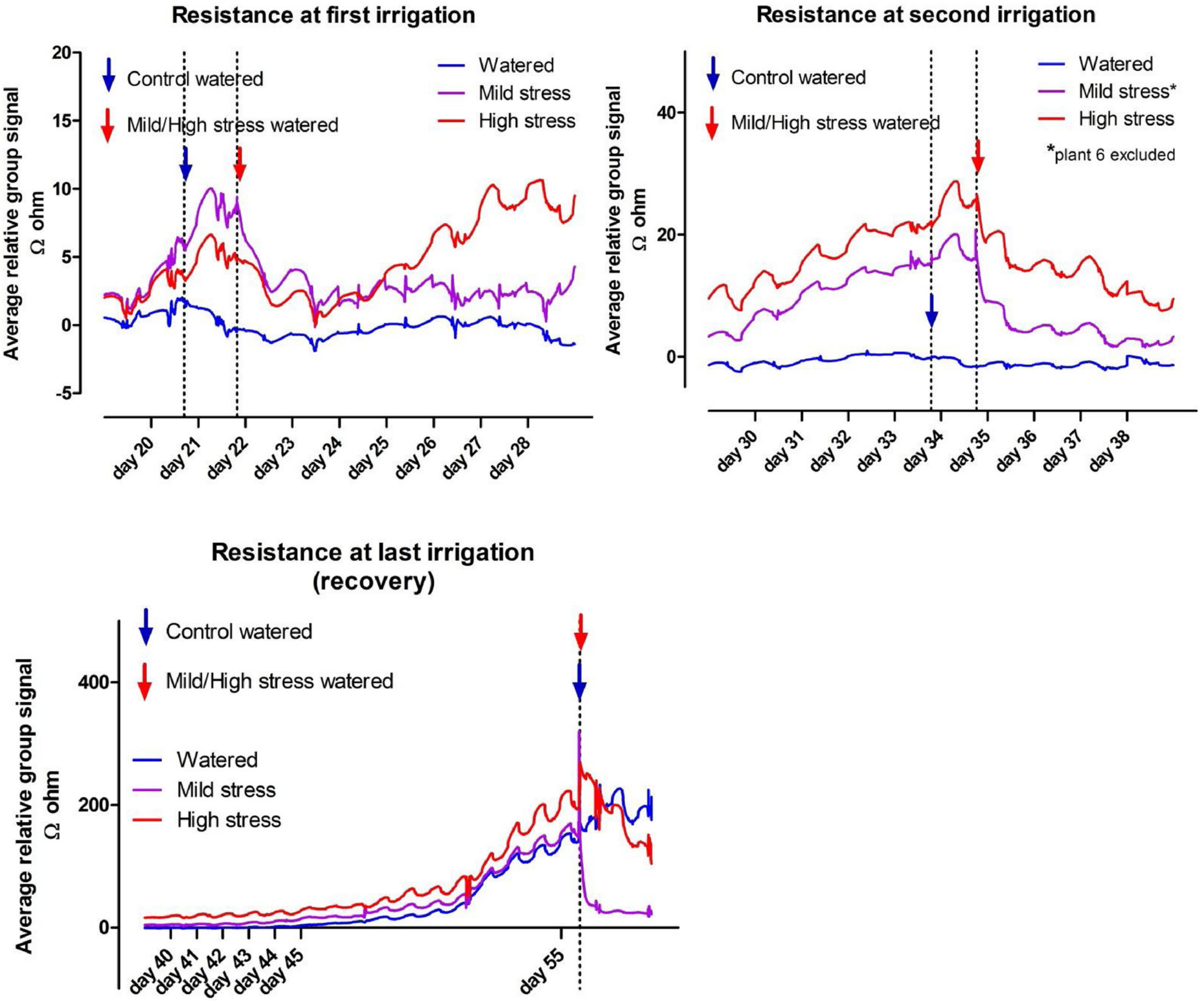

Fig. 6. The average signal changes of each group (control, mild stress, high stress) is showed for the intervals prior irrigation. Each signal have been adjusted for each plant based on the average of 3 days registered signal at the begin of the experiment when the plants were in good health (n = 5).

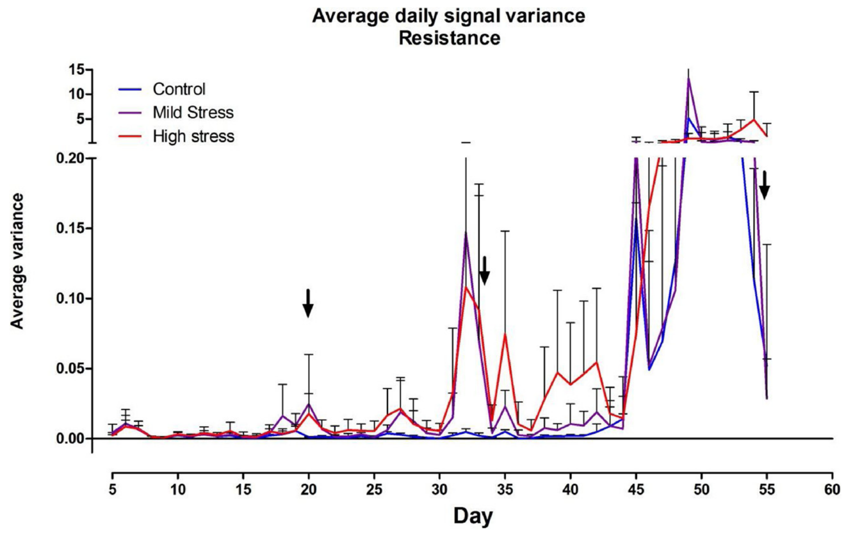

Fig. 7. The picture shows the variance of the electric signal (resistance) calcolated on a daily interval (n = 5, SEM) for all the groups of plant subjected at different drought stress level (control, mild stress, high stress) during the whole experiment. Black arrows represent the day of re-watering.

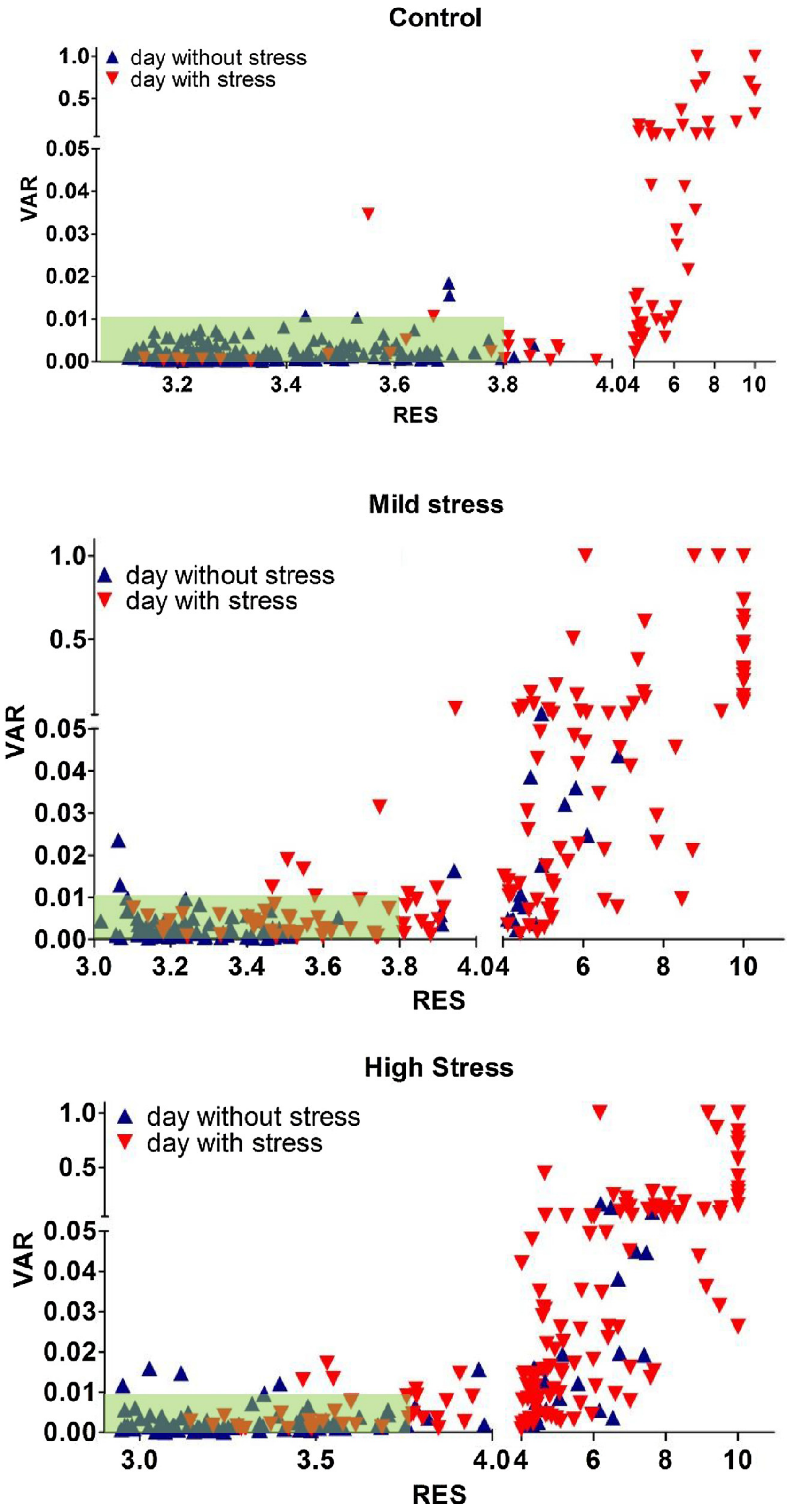

Fig. 8. In the graph above each single plant average daily value of the electrical signal variance (VAR) and resistance (RES) have been plotted for each treatments (control, mild stress, high stress). According to the value of the stomatal conductance monitored during the whole experiment data have been divided in day with or without stress (stress has been considered when the conductance was less than 30 % respect the value monitored at good health).The green rectangles represent the classification used to determine the presence/absence of stress. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

biochemical activities. In fact it has been already observed that severe water stress causes permanent injury to tissue cells, in particular the accumulation of reactive oxygen species (ROS) that cause permanent peroxidation damage (Xu et al., 2010). Additionally the root apparatus ability to recover weakened with increasing stress (Stasovski and Peterson, 1991). Furthermore the fluorescence analysis showed how the different plants have managed the surplus of energy trying to limit damage and how the photosynthesis rate has been due to stomatal limitation or how temporary or permanent damage affected the photosystem. These results showed that also the photoprotection is dependent on the prolonged drought stress condition and plays an important role during the recovery phase for the photosynthetic productivity.

The electrical properties measurement (i.e. resistance) has been

Table 1
Proposed computing system detection results (accuracy, precision, sensitivity, specificity) reported as percent of success of distinguish and identify the phase (days) of water stress for each drought stress treatment.

Treatment	Accuracy			Precision			Sensitivity			Specificity		
	Res + Var	Res	Var	Res + Var	Res	Var	Res + Var	Res	Var	Res + Var	Res	Var
Control	0.93	0.94	0.86	0.88	0.97	0.87	0.82	0.79	0.53	0.96	0.99	0.97
Mild stressed	0.76	0.75	0.72	0.82	0.84	0.88	0.75	0.71	0.60	0.78	0.81	0.89
High stressed	0.80	0.81	0.74	0.83	0.85	0.87	0.84	0.82	0.65	0.75	0.79	0.86

utilized to monitor the water status of each plant and to evaluate the possibility to establish when the plant was facing the stress, the timing of the recovery and eventually to differentiate the intensity of the stress (control, mild and high). The comparison between the physiological parameters of the plants and the electrical resistance measurements has highlighted the possibility to use such electrical signal to detect the plant water status. In fact, by using a multi-electrodes system and analyzing the signal of several plants subjected to the same treatment, it emerged that electrical resistance signal was strongly related to the water plant status; accordingly, the stress level was detected as well as it was possible to differentiate between mild and high stress, in particular this was particularly clear when considering the average signals of a group of plant subjected to the same conditions. Indeed the combined use of a multi-electrodes approach and of the relative changes of signal respect to the initial value has turned out to be promising. This is in accordance with other studies where the electrical resistance has been related to the stem water content; at the same time it suggests that stem variability could affect direct comparison of different plant and recommends the analysis of the relative signal changes (Nadler et al., 2008). Several studies focus on the analysis of the electrical potentials and all agree that the stress condition increase the complexity of electrical oscillations, making harder to find a proper way to interpret and analyze for specific signals (De Toledo et al., 2019; Choi et al., 2017). In our case it has been possible to obtain information also by observing the daily resistance signal variability and this has been possible by recording the data at high frequency (every 2 min). An increment in the signal variance has been usually associated with a stress condition as already cited by other authors (e.g. Nadler et al., 2008). Furthermore, there are hypothesis that suggest that the signal propagation is a combination of hydraulic and chemical signals oscillations (Vodeneev et al., 2015) and thus it is possible to hypothesize that water stress has affected part of the signaling mechanism. The physiological mechanism implied in this phenomenon is still unclear and the increase in the resistance is probably related to the amount of water present in the tissues or due to embolized tissues (Fig. 5), but the meaning of a signal variance deserves a better understanding. The electrodes (Fig. 5) are inserted in the stem and are in contact with several different cells and cells' compartments that could increase the interactions and complexities of observed phenomena. The electric resistance signal variability could be easily influenced by the interruption of the water flow in the xylem caused be phenomena of cavitation or embolism. In fact the xylem in olive tree is mainly made of fibers for structure support and vessels for water transport (Tyree and Zimmerman, 2002; Carlquist, 2012). As most angiosperm, olive tree is very vulnerable to embolism because vessels easily cavitate, nevertheless it has a superior ability to react and repair tissue affected by embolism. In particular, olive tree responds to water stress by increasing xylem vessel frequency or reducing their diameters (Bacelar et al., 2007; D'Odorico et al., 2006). It can be supposed that these physiological modifications contribute to the variability of the electrical resistance, as highlighted in our results: in fact, although smaller or less xylem vessels can reduce embolism, also the hydraulic conductivity decreases (D'Odorico et al., 2006). Furthermore, given that the conducting sieve tubes can be a possible connection between root-shoot electronic-coupling and could be a site for the sorting and modulation of the electricals signals (De Toledo et al., 2019), then their modification may influence also the tissue resistance signal.

Drought-induced accumulation of ABA has been reported to cause the decrease of the membrane turgor pressure and induce stomatal closure (Fromm and Fei, 1998; Du et al., 2018). Increase of ABA in response to stress-specific signal has been also linked to ROS waves associated with the generation and propagation of electric signals (Zimmermann et al., 2009, Oyarce and Gurovich, 2011, Suzuki et al., 2013). This observation confirms that several responses involved in water stress are linked together and we can speculate that several overlapping signaling in response to a stress cause an increment of the resistance signal variability.

In particular, in the clusters analysis, the values of stomatal conductance have been used to confirm whenever each plant was suffering for the water stress; this has allowed to observe the area of the graph where the plant were not subjected to stress (green rectangles, Fig. 8). The proposed stress detection system takes advantage of both daily electrical resistance and signal statistical dispersion (variability) related to water stress in plants. The daily variance can be used as additional confirmation about the plant water stress in addition of the resistance increase. The implementation of a binary classifier to distinguish and identify the different period of stress has demonstrated that according to each specific case it is possible to refine the algorithm to improve the performances in terms of sensitivity and limit the false negative detection results. Due to environmental or physiological fluctuations, it is often possible that the electrical signal may be affected in terms of measured resistance, without an effective water stress affecting the plant. In this case the associated analysis of the signal variance can be crucial and exploited to support or disconfirm the hypothesis derived from the analysis of the signal resistance alone. These features have been highlighted by the clusters distribution of the daily resistance/variance of each plant during the whole experiment (Fig. 8) and can be used as a base to develop more complex detection algorithms and to improve the interpretation of the stem electrical properties related to drought stress.

4.1. Conclusions

Concluding, the results obtained in this study are promising and the methodology presented has a good potential for empirical applications. Electrical resistance measurements can be used at early stage to select the most drought tolerant cultivars for increasing productivity in semiarid conditions. Additionally, the multi-electrodes approach can be easily applied for monitoring a few plants as "biosensor" to check the status of a more numerous group of plants subjected to the same irrigation regime. Future experiments based on this methodology on outdoor mature vegetable or trees are required, in particular accompanied with other measurements, such as stem water potential, and to evaluate their implementation. The use of several plants as well as the analysis of both the signal resistance and its variance can be used to avoid false negative or false positive for develop faultless automatic algorithm to establish when the plants are effectively in a stress condition. This approach, if refined, has the advantage of obtaining data directly from the plant instead of an indirect measurement of the soil or the environment and could be possibly applied for the interpretation of the signals of other stress as well. The measurement of the change in the signal resistance is much cheaper and easy to perform compared to the impedance methodology and the signal itself has a good reliability. The whole system, probes and the cable are flexible and relatively economics. Furthermore the data storage, even if collected at high frequency can be pruned by saving only the average at specific interval of signal and variance (e.g. hourly or daily) and accomplish for long period thus permitting automatic analysis of the signals with numerous electrodes for a scalable and personalized set-up that controls irrigation depending on the real plant needs.

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.

Acknowledgments

This work has received funding from the European Union's Horizon 2020 research and innovation programme (Marie Sklodowska-Curie grant agreement 750807) and the support from Fondazione CR Firenze, project "Identificazione tramite Analisi Numerica dello Stress Idrico nelle Piante - IANSIP" ("Ricerca e Innovazione" programme 2018).

References

Azzarello, E., Masi, E., Mancuso, S., 2012. Electrochemical impedance spectroscopy. Plant Electrophysiology. Springer, Berlin, Heidelberg, pp. 205–223.

Bacelar, E.A., Moutinho-Pereira, J.M., Gonçalves, B.C., Ferreira, H.F., Correia, C.M., 2007. Changes in growth, gas exchange, xylem hydraulic properties and water use efficiency of three olive cultivars under contrasting water availability regimes. Environ. Exp. Bot. 60 (2), 183–192.

Brenner, E.D., Stahlberg, R., Mancuso, S., Vivanco, J., Baluška, F., Van Volkenburgh, E., 2006. Plant neurobiology: an integrated view of plant signaling. Trends Plant Sci. 11 (8), 413–419.

Brilli, F., Barta, C., Fortunati, A., Lerdau, M., Loreto, F., Centritto, M., 2007. Response of isoprene emission and carbon metabolism to drought in white poplar (Populus alba) saplings. New Phytol. 175 (2), 244–254.

Brilli, F., Tsonev, T., Mahmood, T., Velikova, V., Loreto, F., Centritto, M., 2013. Ultradian variation of isoprene emission, photosynthesis, mesophyll conductance, and optimum temperature sensitivity for isoprene emission in water-stressed Eucalyptus citriodora saplings. J. Exp. Bot. 64 (2), 519–528.

Brunetti, C., et al., 2018. Metabolic plasticity in the hygrophyte Moringa oleifera exposed to water stress. Tree Physiol. 38 (11), 1640–1654.

Carlquist, S., 2012. How wood evolves: a new synthesis. Botany 90, 901–940. https://doi.org/10.1139/b2012-048.

Chatterjee, Shre Kumar, et al., 2014. Forward and inverse modelling approaches for prediction of light stimulus from electrophysiological response in plants. Measurement 53, 101–116.

Chatterjee, S.K., Das, S., Maharatna, K., Masi, E., Santopolo, L., Mancuso, S., Vitaletti, A., 2015. Exploring strategies for classification of external stimuli using statistical features of the plant electrical response. J. R. Soc. Interface 12 (104), 20141225.

Choi, W.G., Miller, G., Wallace, I., Harper, J., Mittler, R., Gilroy, S., 2017. Orchestrating rapid long-distance signaling in plants with Ca2+, ROS and electrical signals. Plant J. 90 (4), 698–707.

D'Odorico, P., Porporato, A., Runyan, C.W. (Eds.), 2006. Dryland Ecohydrology Vol. 9 Springer, Dordrecht, The Netherlands.

Datta, P., Palit, P., 2004. Relationship between environmental factors and diurnal variation of bioelectric potentials of an intact jute plant. Curr. Sci. 680–683.

Davies, E., 2004. New functions for electrical signals in plants. New Phytol. 161 (3), 607–610.

Davies, W.J., Zhang, J., 1991. Root signals and the regulation of growth and development of plants in drying soil. Annu. Rev. Plant Biol. 42 (1), 55–76.

De Loof, A., 2016. The cell's self-generated "electrome": the biophysical essence of the immaterial dimension of Life? Commun. Integr. Biol. 9 (5), e1197446.

De Toledo, G.R., Parise, A.G., Simmi, F.Z., Costa, A.V., Senko, L.G., Debono, M.W., Souza, G.M., 2019. Plant electrome: the electrical dimension of plant life. Theor. Exp. Plant Physiol. 31 (1), 21–46.

Degl'Innocenti, E., Hafsi, C., Guidi, L., Navari-Izzo, F., 2009. The effect of salinity on photosynthetic activity in potassium-deficient barley species. J. Plant Physiol. 166, 1968–1981.

Dolfi, M., Colzi, I., Morosi, S., Masi, E., Mancuso, S., Del Re, E., et al., 2015. Plant electrical activity analysis for ozone pollution critical level detection. In: In 2015 23rd European Signal Processing Conference (EUSIPCO). IEEE. pp. 2431–2435.

Du, H., Huang, F., Wu, N., Li, X., Hu, H., Xiong, L., 2018. Integrative Regulation of Drought Escape through ABA Dependent and Independent Pathways in Rice Molecular Plant. https://doi.org/10.1016/j.molp.2018.01.004.

Fromm, J., Eschrich, W., 1988. Transport processes in stimulated and non-stimulated leaves of Mimosa pudica. I. The movement of 14C-labelled photoassimilates. Trees 2, 7–17.

Fromm, J., Fei, H., 1998. Electrical signaling and gas exchange in maize plant of drying soil. Plant Sci. 132, 203–213.

Fromm, J., Lautner, S., 2007. Electrical signals and their physiological significance in plants. Plant Cell Environ. 30 (3), 249–257.

Gibert, D., Le Mouël, J.L., Lambs, L., Nicollin, F., Perrier, F., 2006. Sap flow and daily electric potential variations in a tree trunk. Plant Sci. 171 (5), 572–584.

Gora, E.M., Bitzer, P.M., Burchfield, J.C., Schnitzer, S.A., Yanoviak, S.P., 2017. Effects of lightning on trees: a predictive model based on in situ electrical resistivity. Ecol. Evol. 7 (20), 8523–8534.

Grams, Thorsten E.E., et al., 2007. Distinct roles of electric and hydraulic signals on the reaction of leaf gas exchange upon re-irrigation in Zea mays L. Plant Cell Environ. 30 (1), 79–84.

Gurovich, L., 2009. Real-time plant Water potential assessment based on electrical signalling in Higher plants. ASABE Joint Annual Meeting, 7th World Congress on Computers in Agriculture. Conference Proceedings. https://doi.org/10.13031/2013.29106.

Gurovich, L.A., Hermosilla, P., 2009. Electric signalling in fruit trees in response to water applications and light–darkness conditions. J. Plant Physiol. 166 (3), 290–300.

Hamed, K.B., Zorrig, W., Hamzaoui, A.H., 2016. Electrical impedance spectroscopy: a tool to investigate the responses of one halophyte to different growth and stress conditions. Comput. Electron. Agric. 123, 376–383.

Jaleel, C.Abdul, et al., 2008. Alterations in morphological parameters and photosynthetic pigment responses of Catharanthus roseus under soil water deficits. Colloids Surf. B Biointerfaces 61 (2), 298–303.

Jiang, F., Hartung, W., 2007. Long-distance signalling of abscisic acid (ABA): the factors regulating the intensity of the ABA signal. J. Exp. Bot. 59 (1), 37–43.

Kai, L., Gang, X., Lin-lin, F., Xiao-li, M., Xiang, G., Qing, L., 2011. The changes of electrical signals in corn at different temperatures. Procedia Environ. Sci. 10, 39–44.

Kuromori, T., Seo, M., Shinozaki, K., 2018. ABA transport and plant water stress responses. Trends Plant Sci. 23 (6), 513–522.

Masi, E., Ciszak, M., Comparini, D., Monetti, E., Pandolfi, C., Azzarello, E., et al., 2015. The electrical network of maize root apex is gravity dependent. Sci. Rep. 5, 7730.

Maxwell, K., Johnson, G.N., 2000. Chlorophyll fluorescence—a practical guide. J. Exp. Bot. 51 (345), 659–668.

Misson, L., Limousin, J.M., Rodriguez, R., Letts, M.G., 2010. Leaf physiological responses to extreme droughts in Mediterranean Quercus ilex forest. Plant Cell Environ. 33, 1898–1910.

Morosi, S., Dolfi, M., Del Re, E., Masi, E., Colzi, I., Mancuso, S., et al., 2015. A WSN for ground-level ozone monitoring based on plant electrical activity analysis. In: 2015 International Wireless Communications and Mobile Computing Conference (IWCMC). IEEE. pp. 715–720.

Nadler, A., et al., 2008. Detecting water stress in trees using stem electrical conductivity measurements. Soil Sci. Soc. Am. J. 72 (4), 1014–1024.

Oxborough, K., 2004. Imaging of chlorophyll *a* fluorescence: theoretical and practical aspects of an emerging technique for the monitoring of photosynthetic performance. J. Exp. Bot. 55 (400), 1195–1205.

Oyarce, P., Gurovich, L., 2010. Electrical signals in avocado trees: responses to light and water availability conditions. Plant Signal. Behav. 5 (1), 34–41.

Ríos-Rojas, L., Tapia, F., Gurovich, L.A., 2014. Electrophysiological assessment of water stress in fruit-bearing woody plants. J. Plant Physiol. 171 (10), 799–806.

Schroeder, J.I., Hedrich, R., 1989. Involvement of ion channels and active transport in osmoregulation and signaling of higher plant cells. Trends Biochem. Sci. 14 (5), 187–192.

Sinclair, T.R., Ludlow, M.M., 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Funct. Plant Biol. 13 (3), 329–341.

Sofo, A., 2011. Drought stress tolerance and photoprotection in two varieties of olive tree. Acta Agric. Scand. 61 (8), 711–720.

Sokolova, M., Lapalme, G., 2009. A systematic analysis of performance measures for classification tasks. Inf. Process. Manag. 45 (4), 427–437.

Souza, G.M., Ferreira, A.S., Saraiva, G.F., Toledo, G.R., 2017. Plant "electrome" can be pushed toward a self-organized critical state by external cues: evidences from a study with soybean seedlings subject to different environmental conditions. Plant Signal. Behav. 12 (3), e1290040.

Stasovski, E., Peterson, C.A., 1991. The effects of drought and subsequent rehydration on the structure and vitality of Zea mays seedling roots. Can. J. Bot. 69 (6), 1170–1178.

Suzuki, N., Miller, G., Salazar, C., Mondal, H.A., Shulaev, E., Cortes, D.F., et al., 2013. Temporal-spatial interaction between reactive oxygen species and abscisic acid regulates rapid systemic acclimation in plants. Plant Cell 25 (9), 3553–3569.

Tátrai, Z.A., et al., 2016. Morphological and physiological plant responses to drought stress in Thymus citriodorus. Int. J. Agron 2016.

Thomas, A., et al., 1989. Cell expansion rate, temperature and turgor pressure in growing Lolium temulentum leaves. New Phytol. 112 (1), 1–5.

Tyree, M.Y., Zimmerman, M.H., 2002. Xylem Structure and the Ascent of Sap. Springer, Berlin.

Venturas, M.D., Sperry, J.S., Hacke, U.G., 2017. Plant xylem hydraulics: what we understand, current research, and future challenges. J. Integr. Plant Biol. 59 (6), 356–389.

Vodeneev, V., Akinchits, E., Sukhov, V., 2015. Variation potential in higher plants: mechanisms of generation and propagation. Plant Signal. Behav. 10 (9), e1057365.

Volkov, A.G., Ranatunga, D.R.A., 2006. Plants as environmental biosensors. Plant Signal. Behav. 1 (3), 105–115.

Volkov, A.G., Adesina, T., Jovanov, E., 2007. Closing of Venus flytrap by electrical stimulation of motor cells. Plant Signal. Behav. 2 (3), 139–145.

Volkov, A., et al., 2012. Circadian rhythms in biologically closed electrical circuits of plants. Plant Signal. Behav. 7 (2), 282–284 doi:10.4161/psb.18798.

Wang, Z.Y., Leng, Q., Huang, L., Zhao, L.L., Xu, Z.L., Hou, R.F., Wang, C., 2009. Monitoring system for electrical signals in plants in the greenhouse and its applications. Biosyst. Eng. 103 (1), 1–11.

Wang, Z., et al., 2018. Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. Biol. Open 7 (11). https://doi.org/10.1242/bio.035279. bio035279.

Wang, Z.Y., Qin, X.H., Li, J.H., Fan, L.F., Zhou, Q., Wang, Y.Q., et al., 2019. Highly reproducible periodic electrical potential changes associated with salt tolerance in wheat plants. Environ. Exp. Bot. 160, 120–130.

Wilkinson, S., Davies, W.J., 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. Plant Cell Environ. 25 (2), 195–210.

Xu, Z., Zhou, G., Shimizu, H., 2010. Plant responses to drought and rewatering. Plant Signal. Behav. 5 (6), 649–654.

Zimmermann, M.R., Maischak, H., Mithöfer, A., Boland, W., Felle, H.H., 2009. System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. Plant Physiol. 149 (3), 1593–1600.