Scientia Horticulturae 173 (2014) 71–78

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Quantifying effects of irrigation and soil water content on electrical potentials in grapevines (*Vitis vinifera*) using multivariate statistical methods

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ARTICLE INFO

Article history:
Received 27 September 2013
Received in revised form 12 March 2014
Accepted 12 April 2014
Available online 20 May 2014

Keywords:
Plant electrophysiology
Variation potentials
Water stress
Multivariate analysis

ABSTRACT

Several studies have shown that physiological responses in plants, including fruit crops, are associated with changes in electrical potentials (EP), but it is often difficult to statistically quantify these responses. This study tested the effects of irrigation on EP in grapevines (*Vitis vinifera*), taking into account vapor pressure deficit (VPD) and position of electrodes along the stem by using multivariate analytical methods and a suite of statistical pretreatments. In two separate experiments, plants were exposed to one of two irrigation treatments in a greenhouse: (T1) irrigation once per day (Experiment 1), or no irrigation (Experiment 2); or (T2) irrigation three times or twice per day (Experiments 1 and 2, respectively). In each experiment, EP at three positions along the stem, soil (potting medium) water content, and VPD were continuously measured. In Experiment 2, stomatal conductance (gs) and stem water potential (SWP) were also measured for plants in each irrigation treatment as indicators of plant water status. Data were analyzed by Principal Component Analysis (PCA) to determine the effects of irrigation treatment on EP and difference in EP between pairs of electrodes (ΔEP) at various locations along the stem. Data were also analyzed by partial least squares (PLS) analysis to determine if EP or ΔEP could be used as predictors of changes in soil water content due to different irrigation treatments. Significant differences in soil water content due to irrigation treatments could be readily detected by difference in EP or ΔEP using PCA with Orthogonal Signal Correction pre-processing. Also, PLS showed that differences in soil moisture can be predicted by EP and/or ΔEP measurements at specific locations along the stem. Thus, the use of multivariate statistical methods was effective for relating EP and ΔEP measurements in grapevines to soil moisture due to differences in irrigation.

1. Introduction

Plants respond to external stimuli by generating electrical signals, often originating at the roots and travelling through the vascular system to the leaves, or vice versa (Fromm and Eschrich, 1993; Volkov, 2000; Mishra et al., 2001; Volkov et al., 2004). The electrical signaling mechanism in plants has been extensively described by several researchers (Trebacz et al., 2006; Stahlberg et al., 2006; Davies and Stankovic, 2006; Fromm and Lautner, 2006; Stahlberg, 2006; Fromm, 2006; Davies, 2006; Fromm and Lautner, 2007) who summarized the bases and recent advances in the field of electrical signaling related to the generation and propagation of signals, signal transmission pathways and physiological responses in different plant tissues. Two types of electrical signals have been reported in plants as transient propagating depolarizations using vascular bundles to cover long within-plant distances: (1) action potentials (AP) which are rapid propagating electrical pulses

Abbreviations: EP, electrical potential; AP, action potential; VP, variation potential; PCA, principal component analysis; PLS, partial least squares; OSC, orthogonal signal correction.

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http://dx.doi.org/10.1016/j.scienta.2014.04.014
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traveling through phloem cell membranes at a constant velocity and maintaining a constant amplitude (Davies, 2004; Fromm, 2006), and (2) variation potentials (VP) which are long-range pulses (Davies, 2004; Stahlberg et al., 2006) that vary with the intensity of the stimulus, with amplitude and speed decreasing as distance from the generation site increases (Davies, 2004, 2006; Stahlberg et al., 2006).

It has been postulated that AP and VP each serve as communication pathways between roots and leaves in response to certain abiotic stresses such as water deficits, light intensity, osmotic pressure, temperature, mechanical stimulation and salinity (Fromm and Fei, 1998; Fromm and Lautner, 2007). In response to changes in these environmental variables, electrical signals are often generated at the site of stimulation and can travel rather quickly to adjacent cells (Volkov, 2000; Volkov et al., 2004). These electrical potential differences are often followed by changes in stomatal behavior, photosynthesis and/or respiration (Fromm and Eschrich, 1993; Mishra et al., 2001). For example, osmotic stress suddenly applied to *Helianthus annuus* roots generated electrical potential differences between roots and leaves, which were accompanied by decreases in stomatal conductance (Zawadzki et al., 1991). AP may serve as general stress signals, but provide little information about the type of stress that caused them (Zimmermann et al., 2009). On the other hand, VP are likely generated as the result of xylem pressure changes due to different external stimuli such as changes in plant water uptake (Stankovic et al., 1998) and transmit information about local stimuli to distant cells, promoting physiological responses (Brenner et al., 2006). For example, VP changes in avocado (*Persea americana*) trees were associated with plant water deficits and subsequent stomatal closure due to withholding irrigation water from the potting medium (Gil, 2008; Gil et al., 2008a,b). Thus, in perennial woody species such as grapevine, the potential exists to use real-time tree electrochemical responses as early indicators of plant stress due to insufficient irrigation (Gurovich, 2012).

While numerous studies of AP and VP have been conducted with herbaceous plants, including *Vicia faba* (Roblin and Bonnemain, 1985), *Zea mays* (Fromm and Bauer, 1994), *Solanum lycopersicum* (Roblin, 1985), *Cucumis sativa* (Stahlberg and Cosgrove, 1994), *Pisum sativum* (Stahlberg and Cosgrove, 1994) and *Helianthus annuus* (Dziubinska et al., 2001), there have been relatively few studies of electrical signaling in woody perennial trees or vines. In addition to the above-mentioned VP changes in avocado evoked by water stress, it was also demonstrated in avocado that VP changes occur when trees were exposed to osmotic shock (Gil, 2008; Gil et al., 2008a,b). In olive (*Olea europea*), blueberry (*Vaccinum* sp.), lemon (*Citrus limon*), and avocado, root to shoot electrical signals (AP and VP) were generated in response to changes in light intensity and vapor pressure deficit (Gurovich and Hermosilla, 2009; Oyarce and Gurovich, 2010).

While several studies have shown that physiological responses are associated with changes in EP (AP or VP), it is often difficult to statistically quantify these responses. There have been few attempts to statistically quantify the relationship between EP and external (environmental or edaphic) factors. In *Cucumis sativa*, univariate statistical methods such as coefficients of variability (CVs), and Pearson's coefficient of correlation and intra-class correlation (ICC) procedures helped to quantify the relationship between changes in EP and environmental factors (i.e., light, temperature, relative humidity) (Wang et al., 2009). However, quantifying the effects of specific environmental variables on plant electrical potentials is complex because of the simultaneous and interacting effects of external factors (i.e., light, temperature, vapor pressure deficit) on plant electro-chemical responses.

The use of real-time EP monitoring can be developed as a physiological indicator of plant stress such as plant water deficit due to insufficient irrigation. However, for this to be a viable indicator of plant stress, it is essential to be able to clearly isolate specific abiotic factors from each other and changes in EP. Also, the location of electrodes within the plant used to measure EP can cause variations of the recorded data, considering that the EP response is not equal along the relatively large and complex stem of woody plants. Therefore electrode position must also be taken into account when quantifying the effects of external factors on EP.

Multivariate statistical analytical methods such as principal component analysis (PCA) and Partial Least Squares Analysis (PLS) allow simultaneous comparison of multiple factors and therefore are more powerful than univariate statistical methods for analyzing the relationship between EP and multiple external factors. These multivariate approaches are commonly used to describe the variance within a dataset by weighting each variable according to its absolute variance (in the case of PCA) or the variance that is correlated with other variables (in the case of PLS) (Cozzolino et al., 2006).

The purpose of this study was to determine if multivariate statistical tests such as PCA, PLS and a suite of statistical pretreatments can be used to quantify irrigation and soil water content effects on EP in *Vitis vinifera* (grapevines) taking into account vapor pressure deficit and position of electrodes along the stem. A second objective was to determine if EP or ΔEP could be used as predictors of changes in soil water content due to different irrigation treatments.

2. Materials and methods

Two experiments were conducted in an air-conditioned, polycarbonate greenhouse with 50% shade cloth covering the roof to avoid excessive solar radiation. The greenhouse was located at the experimental center of the Plant Sciences School of the Universidad Viña del Mar, Viña del Mar, Chile (33°04′15.9″S 71°33′03.8″W). The experiments were conducted from December 2011 through April 2012.

2.1. Plant material

One-year-old, non-grafted *V. vinifera* (grapevine) cv. Sauvignon Blanc plants obtained from a commercial nursery were used in this study. Plants were grown in a medium of 100% compost in 7 L plastic containers.

2.2. Experimental set-up

Experiment 1: Plants were exposed to one of two irrigation treatments over a 3-day period: irrigation once per day in morning (T1), or irrigation three times per day at midnight, in morning and afternoon (T2). The same quantity (4 L) of water was applied during each irrigation event. There were 3 plants (replications) per treatment.

Experiment 2: Based on the results of Experiment 1, a second experiment was conducted where irrigation treatments were applied for a longer duration and with different regimes. In Experiment 2, irrigation treatments were applied over a 10-day period and irrigation treatments were: no irrigation (T1), or irrigation 2 times per day in early morning and early evening (T2). The same quantity (4 L) of water was applied during each irrigation event. There were 6 plants (replications) per treatment.

2.3. Measurements

2.3.1. Electrical potentials (EP)

In each experiment, electrical potentials were measured at 1 min intervals in plants placed in a grounded metal “Faraday”

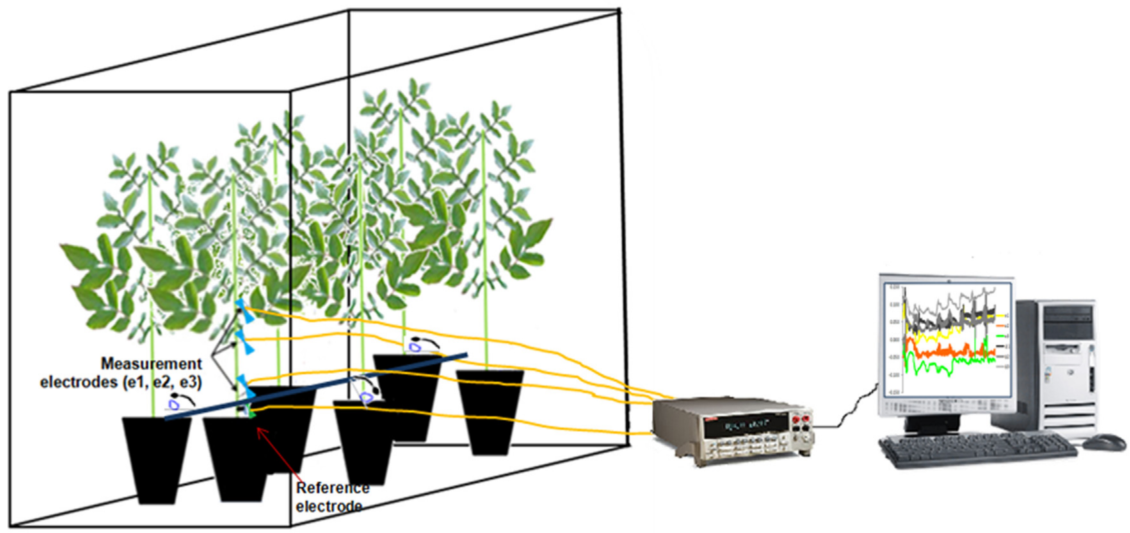

Fig. 1. Schematic diagram of the electrical potential measurement and data acquisition system.

cage to prevent external electromagnetic signals in the greenhouse from interfering with electrical measurements. The cage was constructed of iron mesh grounded to the soil with copper wires and a copper grounding rod.

Electrodes were constructed using a similar technique to that described by Gil et al. (2009), Gurovich and Hermosilla (2009) and Oyarce and Gurovich (2010) based on the original technique reported by Sawyer et al. (1995). Briefly, microelectrodes were made from scalpvein stainless steel syringe needles (0.6 mm of diameter and 1.8 cm length) containing Ag/AgCl electrodes (0.1 mm in diameter, 99.9% Ag) immersed in 1 M KCl. The needle was sealed with heat-fused polyethylene coated insulation at the upper end and with metal–epoxy glue at the tip end of the needle to prevent the conduction solution (KCl) from leaking into the plant or out of the system. The electrodes were inserted into the stem at three sites for each plant: at 8.5 cm, 32.5 cm and 37.5 cm above the surface of the potting medium. Thus, EP was measured at 3 different heights along the stem: at the base of the stem, near the center of the trunk below the canopy, and in a trunk section with active leaves (within the canopy). The insertion depth ranged from 1.0 to 1.3 mm in order to reach the xylem. The depth required to reach the xylem was determined from a previous histological study. In each plant, a reference electrode was inserted into the potting medium at the base of the trunk. The reference electrode was also constructed with scalpvein stainless steel syringe needles (0.8 mm diameter and 1.8 cm length) using the same technique described for the measurement electrodes. Thus, EP was recorded at three points within each plant. Electrical potential differences (ΔEP) between each set of electrodes were determined from EP measurements.

Electrodes were connected to one (Experiment 1) or two (Experiment 2) Keithley 20-channel differential multiplexer model 7700 (Keithley Instruments, Inc., Cleveland, Ohio, USA). The multiplexers were operated using a Keithley Multimeter/Data Acquisition System model 2701/E (Keithley Instruments, Inc., Ethernet Multimeter/Data Acquisition System, Cleveland, Ohio, USA) with high input resistance ($>10^9\ \Omega$), a DC-60 Hz bandwidth, voltage recording from 100 nV to 1000 V and AC/DC converter. The signal was analyzed with ExceLINX-1A software, an add-on utility provided by Microsoft© Excel. A schematic diagram of the electrical potential measurement and data acquisition system is shown in Fig. 1.

2.3.2. Soil water content

In each experiment, soil (potting medium) water content was continuously monitored with frequency domain reflectometry (FDR) probes (Decagon Devices, Inc., Pullman, Washington, USA) and an EM-50 Datalogger (Decagon Devices, Inc.) using Data Trac 3 software (Decagon Devices, Inc.). The FDR probes were inserted to 15 cm below the surface of the potting medium in each container. Water content ($m^3\ m^{-3}$) of the potting medium was recorded at 1-min intervals.

2.3.3. Plant physiological variables

In Experiment 2, stem water potential (SWP) and stomatal conductance (gs) were measured in plants in each treatment to provide physiological indicators of plant water status and plant stress. Stomatal conductance (gs) was measured with a leaf porometer (SC-1, Decagon Devices Inc., Pullman, Washington, USA) during midday (1100–1500 HR) at 2-day intervals. Measurements were made on 3 mature, sun-exposed leaves per plant. SWP was determined periodically at the same intervals as gs measurements. For SWP determinations, 3 sun-exposed leaves per plant were covered with plastic and aluminum foil and then excised 30 min after covering them (Meyer and Reickosky, 1985). SWP of the excised leaves was measured with a pressure chamber as described by Scholander et al. (1965). Leaves were excised and SWP was determined during midday (1100–1500 HR).

2.3.4. Environmental condition in the greenhouse

Vapor pressure deficit (VPD) in the greenhouse fluctuated naturally throughout the day. Air temperature and relative humidity were continuously monitored at 1 min intervals throughout each experiment with a Hobo datalogger (Onset Computer Corporation, Pocasset, Massachusetts, USA) and VPD was calculated from those variables. Air temperature during the measurement period ranged between 13.0 and 39.6 °C.

2.4. Experimental design and data analyses.

In each experiment, EP and soil water content were monitored for 3 plants (replications) per treatment (Experiment 1) or 6 plants (replications) per treatment (Experiment 2) inside the Faraday cage. In Experiment 2, to prevent physiological measurements (i.e., removing leaves for SWP measurements) from

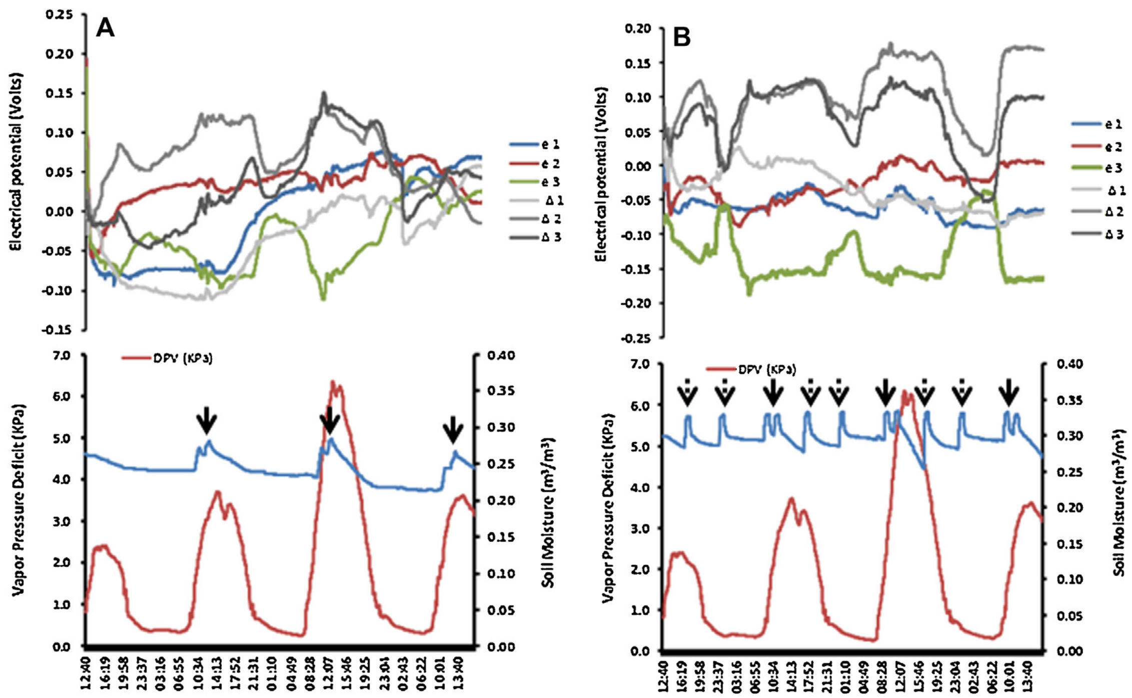

Fig. 2. *Electrical potential for Vitis vinifera plants, soil (potting medium) water content and vapor pressure deficit of the ambient air for (A) plants irrigated once per day or (B) plants irrigated twice per day.* Electrical potentials (EP) and soil moisture are from one representative plant (replication) in each treatment. In the graphs above, lines e_1, e_2 and e_3 (blue, red and green lines respectively) represent EP from electrodes located at base of the trunk (e_1), ~ center of the trunk (e_2) and within the canopy (e_3); the electrical potential difference (ΔEP) was calculated as the EP difference between each pair of electrodes [base of trunk and ~ center of the trunk (Δ_1), ~ center of the trunk and canopy (Δ_2) and base of trunk and canopy (Δ_3)]. ΔEP are shown in gray. Solid arrows indicate an irrigation event for the both T1 and T2 treatments and dotted lines indicate irrigation only for the T1 treatment.

interfering with EP signals, SWP and gs were measured in 6 companion plants (replications) per treatment located outside the Faraday cage. Thus, in Experiment 2, there were 12 plants per treatment.

Data were organized into datasets considering the variables: time (min); electrical potential (EP, volts) at the base of the trunk (e_1), ~ center of the trunk (e_2) and within the canopy (e_3); the electrical potential difference (ΔEP, volts) between each pair of electrodes [i.e., base of the trunk and center of the trunk (Δ_1), ~ center of the trunk and within the canopy (Δ_2) and base of the trunk and within the canopy (Δ_3)]; VPD in the greenhouse (kPa), and soil water content ($m^3\ m^{-3}$).

Statistical pre-processing of data was done by Multiplicative Scatter Correction (MSC), Extended Multiplicative Scatter Correction (EMSC), Savitzky–Golay derivatives, standard normal variate (SNV) (Varmuza and Filzmoser, 2009) and orthogonal signal correction (OSC) (Wold et al., 1998; Trygg and Wold, 2002) to remove random error in the data set caused by external interference with EP signals and not by physiological variables. Data were then analyzed by Principal Component Analysis (PCA) with and without pre-processing treatments to determine the relationship among soil moisture content, VPD and electrode placement on EP and ΔEP. Data were also analyzed by partial least squares regression (PLS) analysis to determine if EP or ΔEP could be used as predictors of changes in soil water content due to irrigation treatment, taking into account VPD. Statistical computations were performed using Unscrambler 10.2 software (CAMO AS, Oslo, Norway). All analyses were validated by full cross-validation routines, minimizing the prediction residual sum of squares function (PRESS) to avoid over fitting the models, according to the methodology described by Yañez et al. (2012). In Experiment 2, differences between irrigation treatments for SWP and gs were determined by Tukey's studentized range test using SAS Statistical Software (SAS Institute, Cary, North Carolina, USA).

3. Results

Experiment 1: Electrical potentials in *V. vinifera* fluctuated during the experiment. Plants irrigated once daily (T1) or 3 times per day (T2) exhibited clear changes in EP that were related to fluctuations in VPD, with higher EP values at the highest VPD (between 12:00 and 19:00 h). However, there was more signal fluctuation for plants irrigated once per day (T1) compared with plants irrigated 3 times per day (T2). The EP values differed among electrode locations, where EP values at e_3 were different from those at the e_2 and e_1 electrodes located lower on the stem. The pattern of signal fluctuation was similar at each electrode location for plants in T2, whereas in T1 the magnitude and direction of the signals differed among electrodes (Fig. 2).

Principal component analysis for EP and ΔEP without pre-processing, and after MSC, EMSC, Savitzky–Golay derivatives, or SNV pre-processing did not clearly separate data according to the irrigation treatments (data not shown). However, after OSC pre-processing, PCA separated EP and ΔEP into two distinct clusters according to the irrigation treatment (Fig. 3).

Principal component analysis with OSC pre-processing showed that the total variance in *V. vinifera* data was explained by two principal factors accounting for 72% of the variance. The main factors explaining the variance of data were soil (potting medium) water content (explaining 42% of the variance) and electrode position on the stem (explaining 30% of the variance) (data not shown).

Partial Least Squares with OSC pre-processing indicated that EP and ΔEP are good predictors of soil moisture content ($R^2 = 0.95$) and that EP measured near the center of the trunk below the canopy (e_2), ΔEP measured between ~ center of the trunk and within the canopy (Δ_2), EP measured with electrodes located at base of the trunk (e_1), and ΔEP measured between the base of trunk and the canopy (Δ_3) were the best predictors of soil moisture resulting from the two different irrigation treatments (Fig. 4).

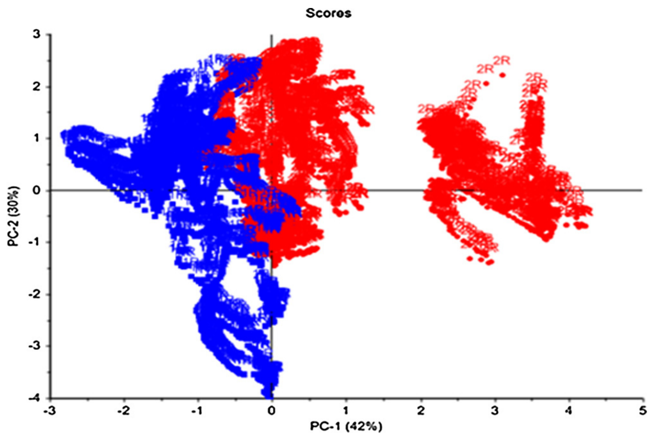

Fig. 3. *Principal Component Analysis score plot of the entire data set for Vitis vinifera after OSC preprocessing in Experiment 1.* Points represent every EP and ΔEP measurement for plants irrigated three times per day (in red) and plants irrigated once per day (in blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Experiment 2: EP fluctuated during the experiment. Plants that were not irrigated (T1) or irrigated 2 times per day (T2) showed clear changes in EP that were related to VPD fluctuations, where the highest EP values corresponded to the maximum VPD (between 1100 and 1800 HR). There were no clear differences in the pattern of EP between irrigated plants and non-irrigated plants. For trees in the irrigated treatment (T2) the pattern of EP fluctuation was similar for electrodes at each location, whereas in the non-irrigated treatment (T1) there was a difference in the magnitude and direction of the EP signal between electrodes (Fig. 5).

After OSC pre-processing, PCA separated EP and ΔEP into two distinct clusters according to irrigation treatment. The separation was not as clear as in Experiment 1. However the total variance for EP and ΔEP was explained by two principal factors accounting for 70% of the total variance; soil moisture and electrical potential difference between the ~ base and the center of the trunk (Δ_1) (Fig. 6).

Partial Least Square with OSC pre-processing indicated that EP and ΔEP are good predictors of soil moisture content ($R^2 = 0.99$) and that EP measured near the center of the trunk below the canopy (e_2), ΔEP measured between ~ center of the trunk and within the canopy (Δ_2), and VPD were the best predictors of soil moisture resulting from the two different irrigation treatments (Fig. 7).

Table 1
Soil water potential (SWP) and stomatal conductance (*gs*) in *Vitis vinifera* plants that were not irrigated (T1) or irrigated 2 times per day (T2). Data are means. An asterisks indicates a significant difference between treatment means and ns indicates no significant difference between treatment means (Tukey's studentized range test, $P < 0.05$).

	Treatment	Day 1	Day 3	Day 5	Day 9
SWP (MPa)	T1	−0.66	−0.87	−0.98	−1.20
	T2	−0.72	−0.71	−0.78	−0.65
	Significance	ns	ns	**	**
gs (μmol m^{-2} s^{-1})	T1	199.5	413.4	133.0	68.3
	T2	176.1	602.5	730.9	323.2
	Significance	ns	ns	**	**

SWP and gs were significantly lower for plants in the non-irrigated (T1) treatment than plants in the irrigated (T2) treatment ($P < 0.05$) beginning five days after treatments were initiated (Table 1).

4. Discussion

Variation potentials (VP) are transmitted in the xylem, probably as a result of changes in pressure or tension and ion transport (Davies et al., 1991; Malone et al., 1994; Mancuso, 1999). Thus in the present study, electrical potentials (EP) measured in *V. vinifera* were determined to be VP and not action potentials (AP) based on the placement of the electrodes within the vascular system and descriptions of the differences between the two types of electrical potentials (Malone et al., 1994; Stankovic et al., 1998; Mancuso, 1999; Davies, 2006).

In both experiments, regardless of irrigation regime, changes in EP were related to daily fluctuations in VPD. However, there were significant differences in EP and ΔEP between well-irrigated plants grown in potting medium with high water content and those receiving reduced or no irrigation. In both experiments, there was a different pattern of variation in EP signals in plants in the non-irrigated or less frequently irrigated plants compared to plants that were well irrigated; EP in non-irrigated or less frequently irrigated

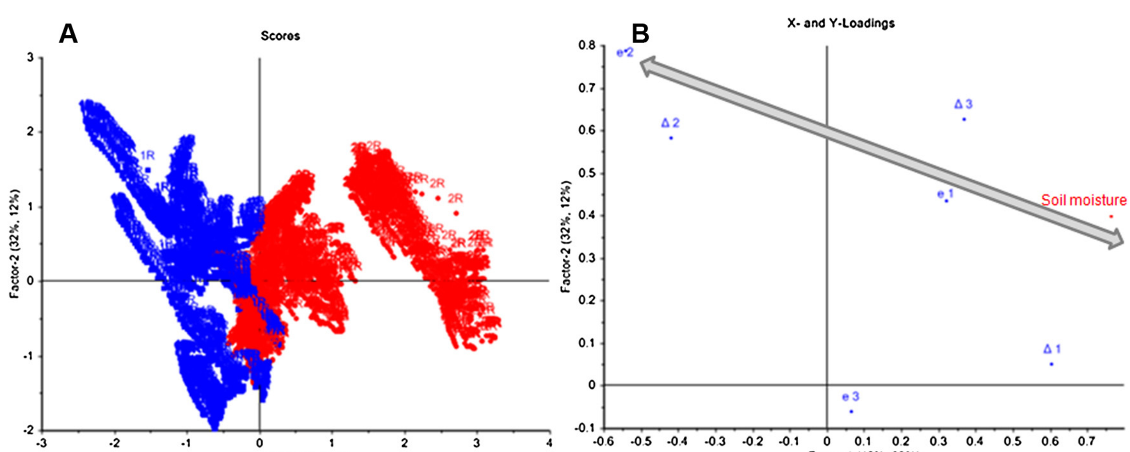

Fig. 4. *Partial Least Squares Analysis after OSC preprocessing for Vitis vinifera in Experiment 1.* In the score plot (A), points represent every EP and ΔEP measurement for plants in the irrigated (in red) and non-irrigated (in blue) treatments. The loading plot (B) shows the relative contribution of each independent variable [EP from electrodes located at base of the trunk (e_1), ~ center of the trunk (e_2), and within the canopy (e_3); and ΔEP between the base of the trunk and canopy (Δ_1), ~ center of the trunk and canopy (Δ_2), and base of trunk and canopy (Δ_3)] to the response variable (soil moisture). Independent variables e_1 and Δ_3, were closest (most closely related) to the response variable (soil moisture) and had the highest positive loading values and therefore were good predictors of soil moisture. Independent variables e_2 and Δ_2 had high negative loading values indicating a strong inverse relationship to the response variable and therefore were also good predictors of soil moisture in the PLS model. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

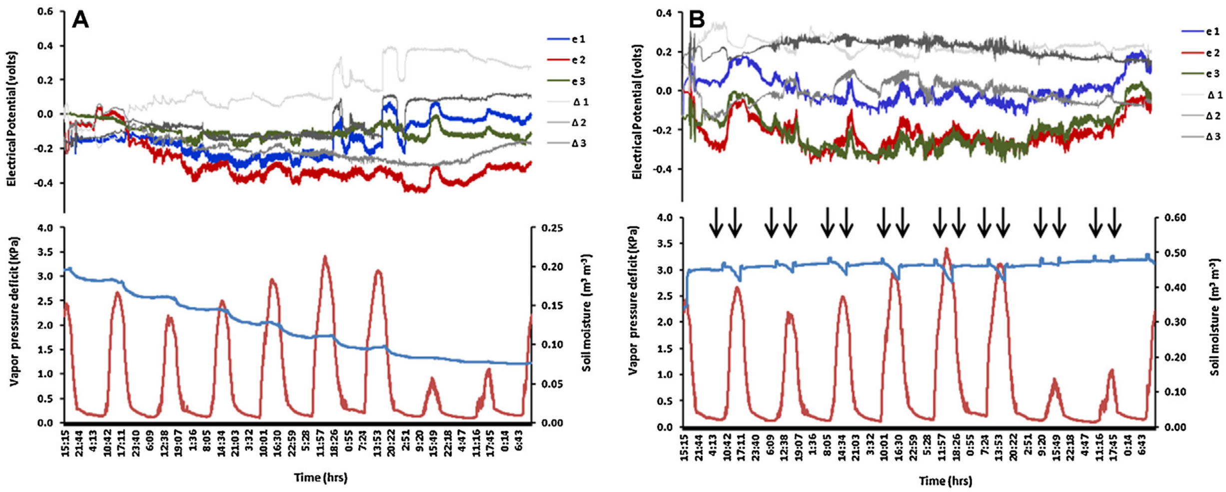

Fig. 5. *Electrical potential for Vitis vinifera plants, soil (potting medium) water content and vapor pressure deficit of the ambient air for (A) plants that were not irrigated or (B) plants that were irrigated twice per day in Experiment 2.* Electrical potentials (EP) and soil moisture are from one representative plant (replication) in each treatment. In the graphs above, lines e_1, e_2 and e_3 (blue, red and green lines respectively) represent EP from electrodes located at base of the trunk (e_1), ~ center of the trunk (e_2) and within the canopy (e_3); the electrical potential difference (ΔEP) was calculated as the EP difference between each pair of electrodes [base of trunk and ~ center of the trunk (Δ_1), ~ center of the trunk and canopy (Δ_2) and base of trunk and canopy (Δ_3)]. ΔEP are shown in gray. Solid arrows indicate an irrigation event for the T1 treatment; the T2 treatment was not irrigated.

plants tended to change amplitude and slope during the experimental period, which was concomitant with physiological changes (reductions in gs and SWP) in Experiment 2. This could possibly be explained by reduced ion transport in the non-irrigated or poorly irrigated plants as a result of less soil water available for plant uptake. Gurovich (2012) suggested that changes in hydraulic pressure and resultant changes in xylem tension provide an extended pathway for long-distance transmission of electrical signals in xylem vessels via changes in ion transport. Thus, in the present study, reduced ion transport as a result of insufficient irrigation presumably resulted in the VP differences observed between plants in the well-irrigated treatment and those in the treatment receiving no or reduced irrigation.

Volkov et al. (2013) recently described different signals causing leaf movements in *Mimosa pudica*. Movement of *M. pudica* leaves is generated in response to external stimuli such as thermal stress, touch, and variations in light intensity. The pathway between the external stimulus and the leaf movement is likely due first to electrical signals and then to hydrodynamic and chemical signal transduction. Electrical, hydrodynamic and chemical signals appear to also be a good explanation for interpreting VP differences measured in *V. vinifera* in response to water content of the potting medium. In the present study, water content of the potting medium was reflected in the EP measurements. Since xylem water flow is largely dependent on water potential gradients, the greater water availability in the potting medium presumably resulted in increased plant ion uptake. According to Volkov et al. (2013), the movement of an electrolyte solution along a capillary generates a streaming potential and a streaming electrical current between the upstream and downstream ends of a liquid flow, which is called an "electrokinetic" force. The electrokinetic movement of the liquid would be impossible without the presence of charges, which are affected by an electrical field, because water movement depends not only on cohesion but also adsorption properties that

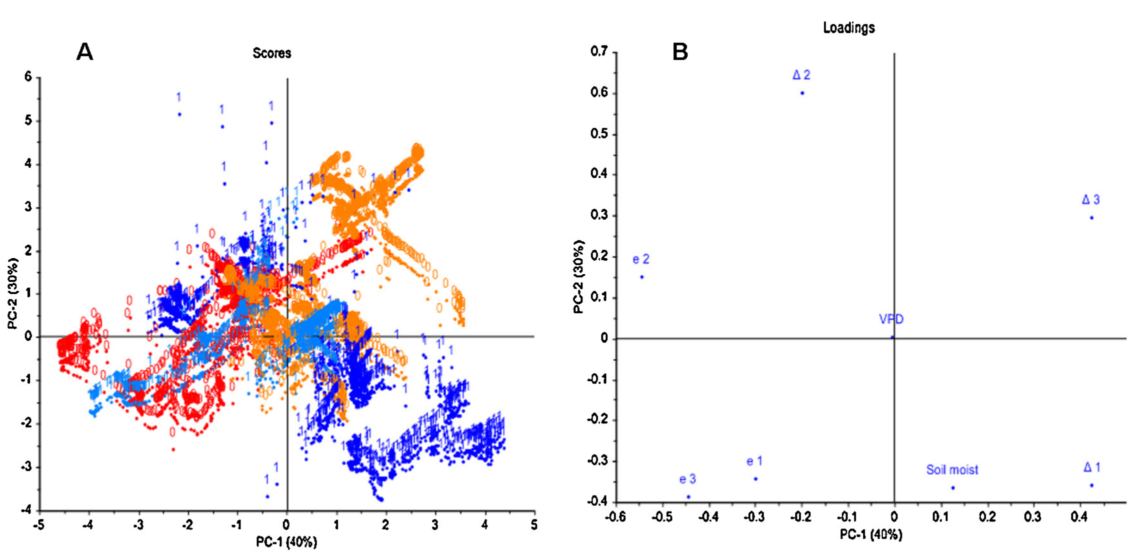

Fig. 6. *Principal component analysis (PCA) score plot after OSC preprocessing in Vitis vinifera plants in Experiment 2.* Points represent electrical potential (EP) and electrical potential difference (ΔEP) measurements every 5 observations for plants in the non-irrigated (red and orange dots) and irrigated (blue and light blue dots) treatments. Red vs. orange dots, and blue vs. light blue dots represent data from multiplexers one and two respectively.

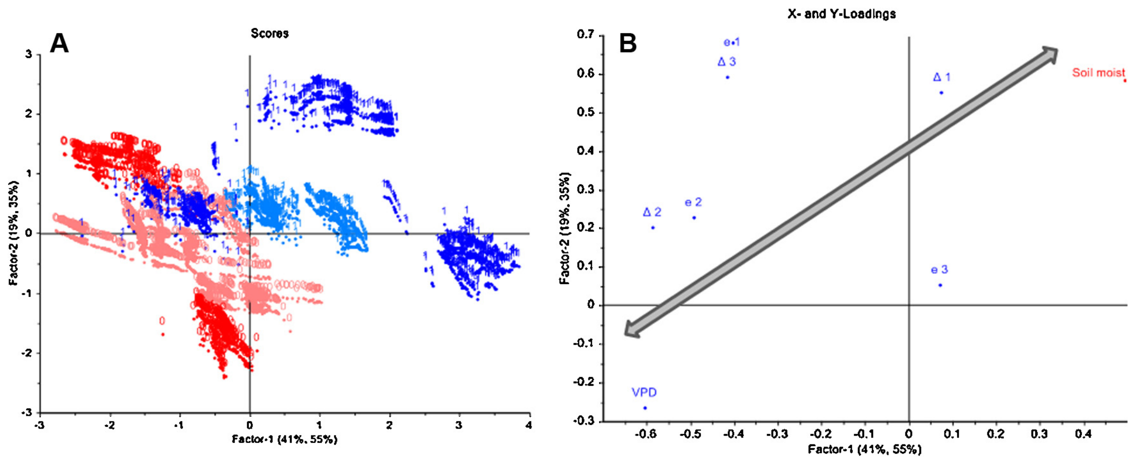

Fig. 7. *Partial Least Squares analysis after OSC preprocessing for Vitis vinifera plants in Experiment 2.* In the score plot (A), points represent EP and ΔEP measurement every fifth observations for plants in the non-irrigated (red and pink dots) and irrigated (blue and light blue dots) treatments. The loading plot (B) shows the relative contribution of each independent variable [EP from electrodes located at base of the trunk (e_1), ~ center of the trunk (e_2), and within the canopy (e_3); and ΔEP between the base of the trunk and canopy (Δ_1), ~ center of the trunk and canopy (Δ_2), and base of trunk and canopy (Δ_3); vapor pressure deficit (VPD)] to the response variable (soil moisture). Independent variables e_2, Δ_2 and VPD, were closest (most closely related) to the response variable (soil moisture) and had the highest positive loading values and therefore were good predictors of soil moisture. Independent variables e_2, Δ_2 and VPD had high negative loading values indicating a strong inverse relationship to the response variable and therefore were also good predictors of soil moisture in the PLS model. Red vs. pink dots, and blue vs. light blue dots represent data from multiplexers one and two respectively.

are dependent on electrical charges of the sap and the capillary (xylem vessels) components. Changing the water content in the potting medium of *V. vinifera* presumably resulted in changes in electrokinetic forces that can be measured as EP changes.

In both experiments, the position of the electrodes along the stem strongly affected EP and ΔEP. For electrodes at each location, EP fluctuations were related to changes in VPD. However, differences in the slope and direction (increase or decrease in amplitude), and daily magnitude of EP were observed among electrodes. The magnitude of EP changes due to VPD variation was generally greater at e_3 or e_2 than at e_1. Since VP are most likely the result of changes in pressure or tension and ion transport in the xylem, differences in EP at each electrode location would be expected because xylem pressure varies along the length of the vascular tissue (Davies, 2006). As transpiration increases in the morning, plant water absorption from the soil does not begin to increase until the decreasing water potential produces enough tension in the xylem (Pallardy, 2008). Therefore, the location of the electrode inserted in the xylem may exert a significant effect on EP as a result of a differential sap flow rate of water in different portions of the plant.

For *V. vinifera*, PCA was an effective analytical statistical method for quantifying the effects of soil moisture and electrode position on EP and ΔEP. However, OSC pre-processing of the data was required for PCA to be effective. OSC is a preprocessing method for removing unwanted variation in spectral data. PCA analysis with OSC pre-processing separated EP and ΔEP into two distinct and clearly defined groups based on differential irrigation treatments. Also, PLS after OSC pre-processing indicated that soil moisture can be strongly predicted by and EP and/or ΔEP data. Thus, the result of the present study indicate that PCA and PLS with OSC preprocessing is an effective statistical method for relating EP or ΔEP to irrigation regime and soil water content.

Previous studies (Gil et al., 2008a,b; Gurovich and Hermosilla, 2009) indicate that measuring EP in fruit trees may have potential for real-time phytomonitoring of soil water content of potted fruit trees in nurseries or in mature, bearing plants in the field. However, for this to be realized, there first must be an effective method of quantifying soil moisture based on EP determinations. The present study indicates that multivariate statistical procedures such as PCA and PLS with Orthogonal Signal Correction have potential for quantifying soil moisture content indirectly from EP and (EP measurements in stems of woody perennial fruit crops.

Acknowledgments

We thank FONDECYT (Project number 11100384, CONICYT, Government of Chile) for funding this research. We also thank Mr. Jorge Pérez for assistance with data collection.

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