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

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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

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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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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"