

## Chapter 5

### Original Article

#### Root to leaf electrical signaling in avocado in response to light and soil water content

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## Summary

Phytomonitoring techniques for irrigation of avocado orchards indicate that plants respond very rapidly to fluctuations in soil water content. Root to leaf abscisic acid transport cannot fully explain the almost immediate response of stomata to either irrigation and/or sudden changes in climatic conditions. Therefore, we studied the existence of a fast conducting signal between roots and leaves, and the possible involvement of such a signal in the regulation of stomatal behavior. Two-year-old avocado trees were subjected to drying and re-watering cycles or changes in incident radiation (light or darkness). The difference in extracellular electrical potential between the leaf petiole and the base of stem ( $\Delta V_{L-S}$ ) was continuously recorded. Stomatal conductance ( $g_s$ ) was also recorded from the same leaves that were used for voltage difference measurements. A sudden change in soil water content induced by root drying and re-watering was accompanied by a slow significant change in the recorded  $\Delta V_{L-S}$  signal, which was fully developed at 52 and 32 min for root drying and re-watering, respectively. We found an inverse correlation ( $r = -0.56$ ) between the change of  $\Delta V_{L-S}$  and the stomatal conductance difference measured before and after each soil drying treatment. Plants that were girdled to disrupt the phloem and then irrigated tended to have lower  $\Delta V_{L-S}$  differences over time than non-girdled irrigated plants, suggesting that the electrical signal was transmitted in the phloem. The existence of a fast signal conducted from the root to the leaf that can be measured and correlated with stomatal control opens the possibility for developing a new phytomonitoring technique and/or artificially modifying plant responses by imposing agronomic

management strategies aimed at rapid stomatal adaptation to changes in soil water content.

**Key words:** Electrical surface potential; stress signal; variation potential; water stress.

**Abbreviations:**  $g_s$  = stomatal conductance; PPF = photosynthetic photon flux;  $\omega$  = gravimetric soil water content,  $\Delta V_{L-S}$  = voltage differences between the base of the stem and the leaf petiole.

## INTRODUCTION

Several possible routes exist for the communication of signals between the roots and the leaves of woody plants. Soil water deficits or excesses affect stomatal opening and closing but the mechanism of stomatal regulation in response to soil stress is not fully understood. Giorio *et al.* (1999) observed that the soil water content and the root water status directly affected stomatal conductance ( $g_s$ ), which diminishes considerably before any observable change in leaf water potential occurs. Stomatal conductance generally decreases as the soil water content falls below an adequate level to sustain normal plant water uptake (Khalil and Grace, 1992). In dry soils where  $g_s$  is low, it generally increases immediately after the beginning of an irrigation event (Grantz and Meinzer, 1990). Stomata open and close very rapidly, in scales from minutes to hours, in response to

changes in soil water conditions (Zavala, 2004; Gurovich and Gratacós, 2003; Meinzer, 2002; Kopyt *et al.*, 2001; Novak, 1997).

The effect of hydraulic and non-hydraulic signals between roots and shoots on stomatal closing during a period of water stress has been recently studied (Liu *et al.*, 2003; Comstock, 2002; Davies *et al.*, 2000; Thomas and Eamus, 1999). Significant variations in xylem hydraulic conductance have been measured in response to soil water deficits and hydraulic mechanisms have been proposed as signals arising from the roots that activate stomatal closure (Chazen and Neumann, 1994). In contrast, Davies and Zhang (1991) have reported the possible existence of non-hydraulic signals originating from the synthesis of phytohormones in the roots of some species. Partial closing of stomata in the absence of changes in leaf water potential has been observed in maize (*Zea mays* L.) (Zang and Davies, 1989; Blackman and Davies, 1985), melon (*Cucumis melo* L.) (Melkonian and Wolfe, 1993), grapevine (*Vitis vinifera* L.) (Dry and Loveys, 1999), chestnut trees (*Castanea sativa* Mill.) (Maurel *et al.*, 2004) and tomato (*Lycopersicon esculentum* Mill.) (Sobeih *et al.*, 2004).

Plants can adjust to water stress without detectable changes in leaf water status due to osmotic and/or stomatal adjustment (Davies and Zhang, 1991). Thus, the stomata may receive a signal indicating the water status of the soil independently from the leaf water status. It has been postulated that changes in the concentrations of ABA generated in the roots and transported to the leaves induce stomatal closure (Hartung *et al.* 2002; Sauter *et al.* 2001; Düring *et al.* 1997; Zhang *et al.* 1987). However, Düring *et al.* (1997) found that a decrease of  $g_s$  was

correlated with an accumulation of the ABA synthesized in leaves and not in the roots. Furthermore, much higher (100x) ABA concentrations than those found in the roots are necessary to decrease stomatal conductance (Munns and King, 1988). In *Pinus sylvestris* L. subjected to gradual soil desiccation, closing of stomata was observed before the arrival of ABA from the roots; thus, ABA translocation from root to leaves could be too slow to account for the stomatal closure in response to water stress, and closing of stomata was apparently not mediated by increases of ABA concentration in roots (Perks *et al.*, 2002). Thus, it is likely that in response to soil water deficit, ABA is neither the only nor the principal signal from the roots to the stomata (Munns and King, 1988). In addition, soil desiccation can induce an increase in the apoplastic pH of the leaf that causes rapid stomatal closure through the liberation of ABA to the symplast (Wilkinson and Davies, 1997; Slovik and Hartung, 1992). Nevertheless, it is not known how the pH of leaf apoplast decreases so rapidly after roots are water stressed.

These previous observations suggest that ABA is neither the only nor the principal signal traveling from the roots to the leaves which affects stomata opening and closing as a result of soil water stress. Therefore, other signaling mechanisms may exist. Another possible mechanism that has not been thoroughly investigated and may explain the response of stomata to soil water deficits, is the presence of fast conducting electrical signals generated in the roots and conducted through the vascular system to the leaf. Stimulation of roots in *Salix viminalis* L. by the application of nutrients, hormones or changes in pH, caused changes in the electric potential difference recorded between the roots and the leaves. These

changes were followed by a modification of leaf respiration and photosynthetic rates within three min after treatments were applied, indicating that the changes in the electrical signals might reflect or be a mechanism of communication between the roots and the leaves (Fromm and Eschrich, 1993). Similarly, osmotic stress suddenly applied to sunflower roots generated an electrical potential difference between the roots and the leaves that accompanied the decreases in  $g_s$  (Fromm and Eschrich, 1993). The role of the changes of electrical potential in the regulation of leaf gas exchange has also been studied in maize subjected to drought cycles (Fromm and Fei, 1998). Additionally, the bioelectric potential in jute plants (*Corchorus capsularis* L.) was related to soil and air temperature, as well as photosynthetic photon flux (Datta and Palit, 2004). A recent study of *Mimosa pudica* L. (Kaiser and Grams, 2006) showed that stomatal closure can be a response to heat-induced electrical signals. It has been postulated that electric signals could be transmitted in plants through sieve tubes of the phloem, serving as a communication pathway between roots and shoots under water stress (Fromm and Fei, 1998). These data indicate that several plant stressors may generate fast changes in the medium that can be recorded as changes in the extracellular potential difference between the roots and the leaves.

Avocado (*Persea americana*), an important commercial horticultural crop in Chile, is very sensitive to water stress and waterlogging. A phytomonitoring technique, to define the plant water status and to calibrate the irrigation program, has been used recently in Chile for irrigation scheduling of avocado orchards (Gurovich *et al.*, 2006). This technique indicates that plants respond very rapidly to fluctuations in

soil water content. The main goal of this study was to determine the existence of a root to shoot extracellular potential difference (variation potentials or slow wave potentials, see Stahlberg *et al.*, 2006; Stankovic *et al.*, 1998) in avocado plants and the possible role of this signal in regulating stomatal behavior affected by changes in soil water content and other environmental conditions.

## MATERIALS AND METHODS

### Plant material

Two-year-old 'Hass' avocado (*Persea americana* Mill.) trees on clonal Duke 7 rootstock were used in this study. The plants were grown in a commercial nursery in a medium composed of peat, perlite, compost and sand and fertilized according to standard nursery practices. The plants ranged in height from 1.2 to 1.4 m with a variable number of leaves (22 - 45) per plant.

### Measurement of extracellular surface potential difference between the roots and the leaves

Extracellular surface potentials were measured in avocado plants. Surface contact electrodes (Fromm and Fei, 1998) were placed on the stem 20 cm above the soil surface and in the petiole of a leaf located in the lower third of the plant canopy. The electrodes consisted of a thick cotton thread absorbed with KCl 0.1 M dipped in a 2.0 mL Eppendorf tube containing KCl 0.1 M. Ag/AgCl electrodes (0.4 mm in diameter) were immersed in the Eppendorf tubes and were connected to an amplifier with an input impedance of  $10^{11}$  ohm and DC-1 kHz bandwidth (M-707

Microprobe System; World Precision Instruments, Sarasota, Florida, USA), and the output was recorded with a Power Lab analog-digital acquisition system at 2 Hz (AD Instruments, Castle Hill, Australia) (Fig. 1). Prior to plant measurements, the electrodes were placed in KCl 0.1 M and calibrated to 0 mV to compensate the junction potential. To record  $\Delta V_{L-S}$  the electrode located on the leaf petiole acted as the recording electrode while the electrode located on the stem served as the reference.

### **Control Plants: baseline condition**

Extracellular surface potentials were measured for about 80 min in 8 avocado plants (replications) under stable environmental conditions to determine the voltage differences between the base of the stem and the leaf petiole ( $\Delta V_{L-S}$ ) in the absence of environmental alterations (control or baseline).

Treatments: darkness, light, root drying and root wetting

The same plants were exposed to 4 different treatments: 1) exposure to darkness ( $0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for 20 min, 2) exposure to artificial light ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for 20 min, 3) desiccation of the roots by exposure of the root system to a directed air current at ambient temperature ( $20^\circ\text{C}$ ) for about 80 min and 4) re-wetting of desiccated plant roots by adding 500 cc of distilled water to the soil and monitoring the  $\Delta V_{L-S}$  response for 120 min. These treatments were chosen because they all were expected to influence stomatal behavior, but the light and dark treatments should have impacted the stomata directly at the leaf level,



whereas the soil wetting and drying treatments would require communication (a signal) from the roots to the leaves to affect stomatal behavior. Also, times for signal registration were chosen according the expected time of response of stomata to modification in light or water availability in the soil.

Air temperature during the measurement period was between 22.5 and 23.3°C and leaf temperature ranged from 22.4 to 23.7°C. The photosynthetic photon flux (PPF) (Quantum sensor QSS-01 light meter, Lehle Seeds, Round Rock, Texas, USA) directly above the abaxial leaf surface was about 85  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , which is above the light compensation point of this species which is 30  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  (Whiley, 1994).

#### Measurement of stomatal conductance

Stomatal conductance (gs) was measured with a steady state porometer (Li-Cor 1600, Lincoln, Nebraska, USA) as described by Prive and Janes (2003) and Raviv *et al.* (2001). Stomatal conductance was measured on the same leaf on which the electrode was placed. Stomatal conductance was measured on each of the 8 replications before and after the voltage was recorded for each treatment (darkness, luminosity, root drying and root wetting).

#### Measurement of soil moisture

Plants in each treatment were placed on a digital balance (Mettler Toledo, Hispanic Precision, Model Wildcat, Columbus, Ohio, USA), and the total weight of the plant, soil and pot was determined before and after each treatment. Plants were then

excised at the soil surface, and pots and soil were reweighed. Gravimetric soil water content ( $\omega$ ) was then determined with the formula:

$$\omega = ((\text{wet soil weight} - \text{dry soil weight}) / \text{dry soil weight}) * 100$$

### Girdled plants

To detect if changes in  $\Delta V_{L-S}$  in response to changes in soil water content were transmitted through the phloem or xylem, seven avocado plants were not irrigated for 4 days and then girdled to disrupt the flow of phloem sap. After girdling, plants were irrigated with 500 cc of distilled water and  $\Delta V_{L-S}$  was measured.

### Data Analysis

Data were expressed as means. The effects of treatment (Darkness, Light, Drying and Wetting) on the maximum  $\Delta V_{L-S}$  – initial  $\Delta V_{L-S}$  ( $\Delta V_{L-S}$  difference) were analyzed by a one-way ANOVA and post-hoc comparison of means using Tukey's studentized range test.  $\Delta V_{L-S}$  differences arising after irrigation between girdled and non-girdled plants were analyzed by repeated measures two-way ANOVA and mean  $\Delta V_{L-S}$  differences at specific points in time were analyzed by a Bonferroni test. The correlation between  $\Delta V_{L-S}$  and  $\Delta g_s$  was analyzed by linear regression analysis. Statistical analyses were performed with the SAS statistical package (SAS Institute, Cary, North Carolina, USA).

## RESULTS

Control plants: baseline condition

Although, control plants showed different initial  $\Delta V_{L-S}$  values, they remained fairly constant during the experiments. The differences in baseline  $\Delta V_{L-S}$  values were probably due to differences in size, and numbers of branches and leaves among plants (height ranged from 1.2 to 1.4 m and the number of leaves ranged from 22 - 45 per plant). Therefore, small changes in resistance due to the size and number of branches may affect the measured potential. Notwithstanding, there was little modification in  $\Delta V_{L-S}$  in control plants (n= 8) kept in stable environmental conditions for about 80 min. These values were relatively constant until the end of the measurement period (Table 1 and Fig. 2), with a mean initial  $\Delta V_{L-S}$  value of 27.3 mV and a mean end  $\Delta V_{L-S}$  value of 21.1 mV; thus the mean  $\Delta V_{L-S}$  difference was - 6.2 mV.

Plants subjected to cycles of light modification and water availability

The recorded  $\Delta V_{L-S}$  was largely unaffected by most treatments (Table 1). Placing plants in the dark resulted in an increase in  $\Delta V_{L-S}$  of 47.3 mV after 13.3 min, whereas illuminating plants previously in the dark resulted in only a decrease of  $\Delta V_{L-S}$  in 0.5 mV after 13.3 min. However, the changes in maximum  $\Delta V_{L-S}$  difference were not significantly different from those of the control treatment (One way ANOVA,  $P \leq 0.05$ ) (Table 1). Figure 3 A, B shows the  $\Delta V_{L-S}$  recorded during darkness and light treatments, respectively in 8 plants.

Only the root desiccation treatment produced a statistically significant change in  $\Delta V_{L-S}$  (Maximum Voltage Difference) compared to the control plants. Drying the soil just -1.3%  $\omega$  from field capacity (1/3 atm) resulted in a significant reduction (One

way ANOVA,  $P \leq 0.05$ ) in the  $\Delta V_{L-S}$  (-214.3 mV) after 52 min (Table 1). In contrast, adding water to the dry soil resulted in an increase of 85.3 mV in the  $\Delta V_{L-S}$  (Maximum Voltage Difference) after 32 min of re-watering, but this value was not significantly different (One way ANOVA,  $P > 0.05$ ) from the control group. Figure 4 A and B show the  $\Delta V_{L-S}$  recorded during soil wetting and soil drying treatments, respectively in 8 plants. Figure 5 shows a summary of the average curves of  $\Delta V_{L-S}$  recordings for all treatments.

All the treatments produced changes in stomatal conductance (Table 2), which were often directly related with stomatal opening and closing (Buckley *et al.*, 2003; Tinoco-Ojanguren and Pearcy, 1992; Cowan, 1972). We found an inverse linear correlation ( $r = -0.56$ ), between the change of  $\Delta V_{L-S}$  and stomatal conductance difference measured before and after each treatment ( $\Delta g_s$ ) in the soil drying treatment (Fig. 6).

#### Girdled plants

When  $\Delta V_{L-S}$  changes recorded from girdled plants were compared to non-girdled plants after both treatments were irrigated, there was a significant time and time x treatment interaction for the  $\Delta V_{L-S}$  difference (repeated measures two-way ANOVA,  $P \leq 0.05$ ). The  $\Delta V_{L-S}$  change was higher in the non-girdled plants than in the girdled plants throughout the 60 min measurement period. However, due to high between-plant variability, voltage differences were only statistically significant ( $P \leq 0.05$ , Bonferroni test after a two-way ANOVA) at 50 and 60 min of measurement (Fig. 7).

## DISCUSSION

The observed changes in  $\Delta V_{L-S}$  between roots and leaves were concomitant with modifications in stomatal conductance. Changes in stomatal conductance are often directly related to stomatal opening and closing (Buckley *et al.*, 2003; Tinoco-Ojanguren and Pearcy, 1992; Cowan, 1972). Thus, our results suggest a link between the recorded electrical signal and stomatal behavior. Previous experiments with *Salix viminalis* L. (Fromm and Eschrich, 1993) and *Zea mays* (Fromm and Fei, 1998) showed that electrical potentials are generated in response to different stimuli in the roots (application of nutrients, hormones, changes in pH or drought) affecting leaf gas exchange. However, in previous studies action potentials were measured, whereas our study focused on variation potentials or slow wave potentials (Stahlberg *et al.*, 2006; Stankovic *et al.*, 1998). An action potential is defined as a transient change in the transmembrane difference of potential, which is triggered by a depolarizing stimulus at a given threshold, where the response becomes independent of the strength of the stimulus. On the other hand, a variation potential or slow electric potential referred to as a streaming potential (Stankovic *et al.*, 1998), which is conducted electrotonically, decreases exponentially in magnitude as the distance from the point of generation increases.

Environmental changes generate bioelectric potentials in plants (Datta and Palit, 2004). In this study with avocado the shape, magnitude and duration of  $\Delta V_{L-S}$  was dependent on the stimulus. In control plants that received no sudden environmental stimulation, changes in  $\Delta V_{L-S}$  were small and not statistically significant. However, drying the roots resulted in a large and significant decrease in

$\Delta V_{L-S}$ . Similarly changes in electrical signals were reported by Fromm and Fei (1998) after subjecting *Zea mays* plants to drought. Present results showed that in avocado, a soil drying of -1.3 %  $\omega$  resulted in a change in  $\Delta V_{L-S}$  that was observed within 28 to 56 min after forced-air soil drying was initiated, with an average Maximum  $\Delta V_{L-S}$  Difference of -214.28 mV, which corresponds to a 96.3% variation of the initial value. Thus, as a result of plant stress induced by decreased soil water content, there was a significant modification of the variation potentials or slow wave potentials that appeared to be conducted at a speed of 2.4 cm min<sup>-1</sup> or 144 cm h<sup>-1</sup>. The modification of  $\Delta V_{L-S}$  was correlated with reduced stomatal conductance, suggesting that there may be a cause and effect relationship between these two processes.

In contrast to the significant change  $\Delta V_{L-S}$  associated with soil drying, changing the light environment (from dark to light or light to dark) resulted in only a slight and non-significant change in  $\Delta V_{L-S}$ . Changes in light intensity directly affect leaf and stomatal opening and closing and would not be expected to involve an inducible signal from the roots to the stomata. However, as a result of changes in the root zone, such as soil drying, a transmissible signal generated at the root and transported to the leaf would be expected to stimulate stomatal opening and closing. As expected, root drying or wetting resulted in a much greater voltage difference between the trunk and the leaf than changing the light environment. The response to desiccation was a decrease in  $\Delta V_{L-S}$ , whereas a response to wetting was an increase in  $\Delta V_{L-S}$ . Therefore, the magnitude and the duration of the recorded changes in  $\Delta V_{L-S}$  were different for each condition.

Phytomonitoring techniques used for irrigation scheduling for fruit trees (Gurovich *et al.*, 2006; Gurovich and Gratacós, 2003; Kopyt *et al.*, 2001, Novak 1997) indicate that plant responses to changes in soil water availability are very fast processes. The transport of ABA from roots to shoots cannot explain the fast physiological response of the stomata (sometimes less than 15 min) to irrigation or to sudden increases or reductions of evapotranspiration. The high speed of the physiological changes in the leaves as a result of soil water changes suggests that communication from the root to the leaves is via hydraulic or electrical signals, such as a variation potential.

The sieve tubes of the phloem have been postulated to be a route of transmission of electrical signals between roots and shoots of several plant species (Koziolek *et al.*, 2003; Fromm and Fei, 1998; Fromm and Eschrich, 1993; Fromm, 1991). After desiccation of the soil, rewatering of *Zea mays* plants resulted in a voltage increase, which paralleled the increase in leaf gas exchange and water vapor exchange (Fromm and Fei, 1998). Moreover, a recent study of *Mimosa pudica* (Kaiser and Grams, 2006) showed that stomatal closure is elicited by heat-induced electrical signals, suggesting a possible involvement of electrical signals in long-distance signaling for coordinating leaf gas exchange. The significant difference in  $\Delta V_{L-S}$  after irrigation between non-girdled plants and plants that were girdled to disrupt the phloem indicated that the phloem is an important conduit for electrical signal transmission from the root to the shoot in avocado plants as was reported previously for other species (Fromm 1991; Fromm and Eschrich 1993; Fromm and Fei 1998; and Koziolek *et al.* 2003). Girdled avocado plants tended to have lower

$\Delta V_{L-S}$  differences than non-girdled plants, with statistically significant differences at 50 and 60 minutes from the beginning of the treatments. This suggests that the change in the electrical signal in response to a change in the soil moisture is occurring in the phloem.

Stahlberg *et al.* (2006) proposed that a variation potential can be induced by increasing xylem pressure or turgor. The ionic mechanism is yet uncertain, but it was postulated that it could be through an  $H^+$ -pump or ionic channels, such as a calcium channel or a transient shutdown of stretch-sensitive ATP-ases at the plasma membrane (Stahlberg *et al.*, 2006). Variation potential (or slow wave potential) characteristics (Stahlberg *et al.*, 2006; Stankovic *et al.*, 1998) match the shape, time and nature of the electrical signals recorded during this experiments. We originally hypothesized that the electrical signal in avocado plants was transmitted through the xylem because sap flow in avocado has been measured at speeds of 30 to 35  $cm\ h^{-1}$  (Cantuarias, 1995), comparable to speeds at which we could see changes in the voltage. However, the comparison of changes in voltage differences between girdled and non-girdled avocado plants after irrigation indicated that the electrical signal was transmitted through the phloem.

In summary, our results suggest that changes in  $\Delta V_{L-S}$  may play a role in root to shoot communication when avocado plants are water stressed. However, care must be taken when interpreting wave potential data in plants, because changes in extracellular voltage may be the result of true changes in the circulating extracellular current or modifications in extracellular resistance, which can be affected by water and/or by the ion content of the medium. Therefore, it is



necessary to separate true electrical signaling phenomena from conductivity changes arising from water and/or ion handling by the plant tissue. However, even if the change in electrical potential was generated by a shift in the water or ionic balance in the roots and xylem resulting from soil drying, it does not negate the fact that changes in the extracellular electrical potential may be the mechanism for root to leaf communication resulting in stomatal closure in response to soil drying. Also, the existence of a fast root to leaf signal that can be readily measured and related to stomatal control opens possibilities for developing a phytomonitoring technique for measuring plant response to soil water content or to artificially modifying plant responses by imposing agronomic management strategies aimed at rapid stomatal adaptation to changes in soil water content.

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Table 1. Effect of dark, light, soil drying and soil wetting on the root to leaf voltage difference in avocado plants. Each value of voltage difference represents the mean (n = 8 plants). Maximal different  $\Delta V_{L-S}$  is related to the initial value of the voltage curve for each replication within 80 min. The change in the maximum  $\Delta V_{L-S}$  difference was calculated by subtracting the initial  $\Delta V_{L-S}$  from the maximal different  $\Delta V_{L-S}$ . Different letters (a, b) indicate significant differences ( $P \leq 0.05$ ) among treatments (one-way ANOVA and Tukey's studentized range test).

Treatments	Initial $\Delta V_{L-S}$ (mV)	Maximal different $\Delta V_{L-S}$ (mV)	Time to maximal voltage difference (min)	Maximum $\Delta V_{L-S}$ difference (mV)
Control	27.3	21.1	58.0	-6.2 a
Dark	262.2	309.5	13.3	47.3 a
Light	328.9	329.7	13.3	0.5 a
Drying	436.8	222.5	52.0	- 214.3 b
Wetting	77.4	162.6	32.0	85.3 a

Table 2. Stomatal conductance difference after treatments were imposed. Positive numbers for the light and soil wetting treatments indicate stomatal opening, whereas negative numbers for the darkness and soil drying treatments indicate stomatal closure. Values represent means  $\pm$  SE (n = 8).

Treatments	$\Delta g_s$ (cm s <sup>-1</sup> )
Darkness	-0.023 $\pm$ 0.02
Light	0.022 $\pm$ 0.04
Soil Drying	-0.021 $\pm$ 0.03
Soil Wetting	0.017 $\pm$ 0.005

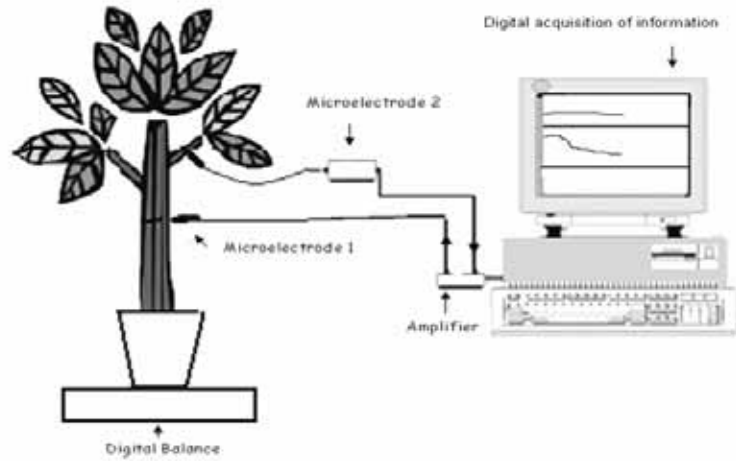


Figure 1. Schematic diagram of the setup for the digital acquisition of the recorded extracellular voltage difference between the leaf petiole and the base of the stem ( $\Delta V_{L-S}$ ).



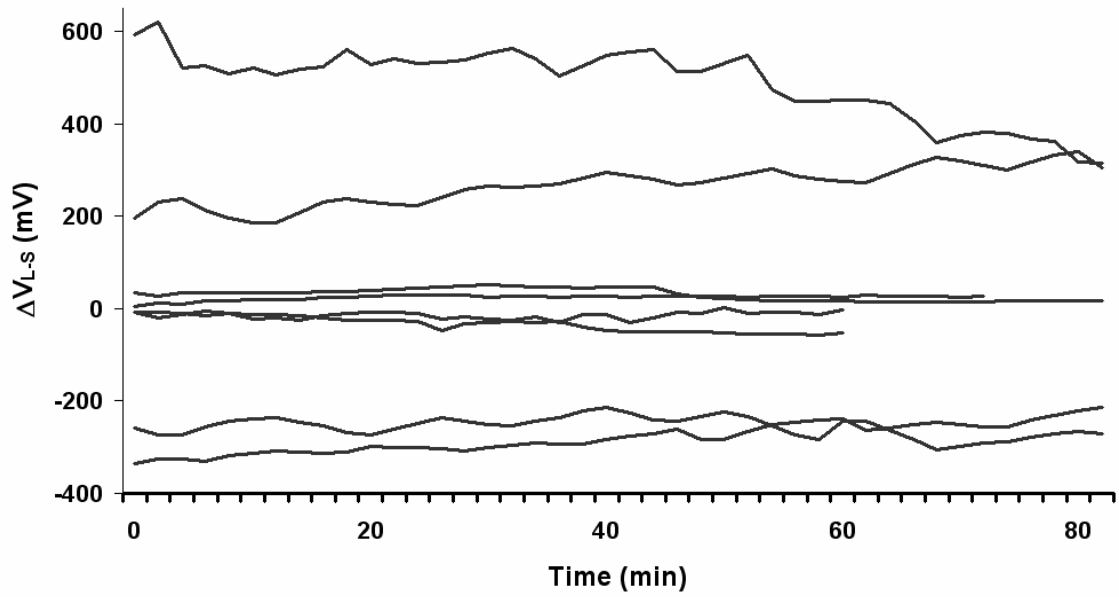


Figure 2. Voltage difference recorded between the leaf petiole and the base of the stem ( $\Delta V_{L-s}$ ) in 8 control plants during 80 min. Data were collected at 2 Hz and plotted at 2 min intervals.

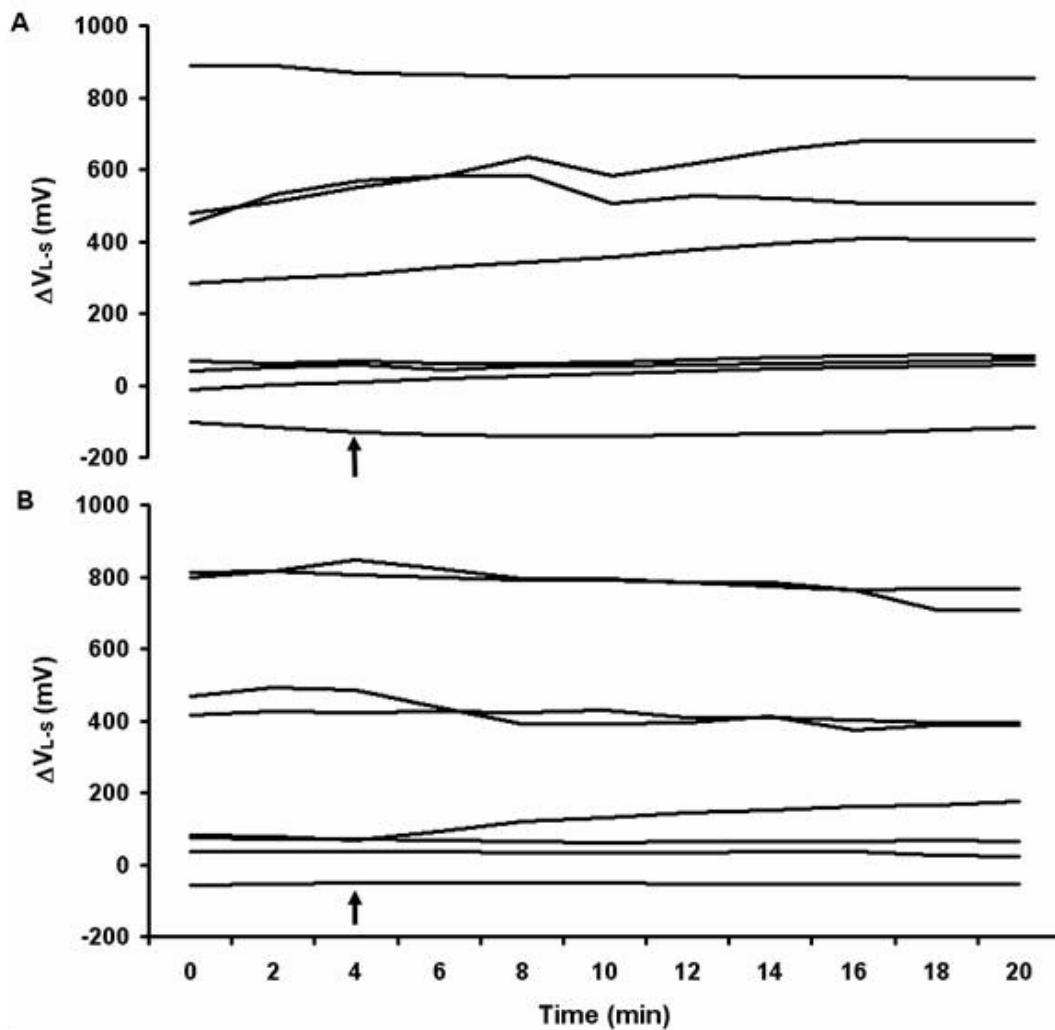


Figure 3. Voltage difference recorded between the leaf petiole and the base of the stem ( $\Delta V_{L-s}$ ) in 8 plants in darkness ( $0 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for 20 min (Fig. 3 A), and in 8 plants in artificial light ( $85 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ) for 20 min (Fig. 3 B). Data were collected at 2 Hz and plotted at 2 min intervals. The arrows indicate the beginning of each treatment.

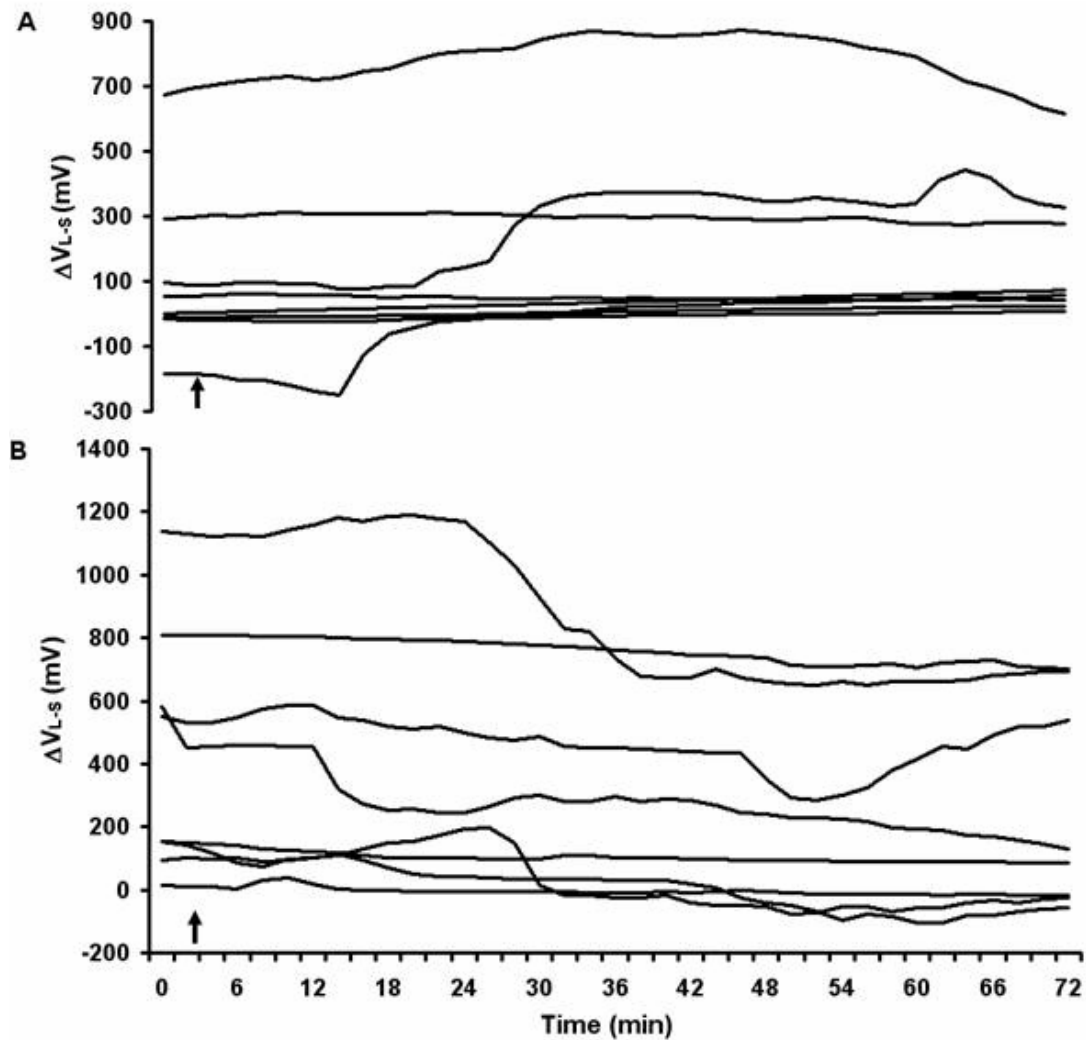


Figure. 4: Voltage difference recorded between the leaf petiole and the base of the stem ( $\Delta V_{L-S}$ ) in 8 plants after irrigation (Irrigation) for 72 min (Fig. 4 A) and in 8 plants subjected to root desiccation (Drying) for 72 min (Fig. 4 B). Data were collected at 2 Hz and plotted at 2 min intervals. The arrows indicate the beginning of each treatment.

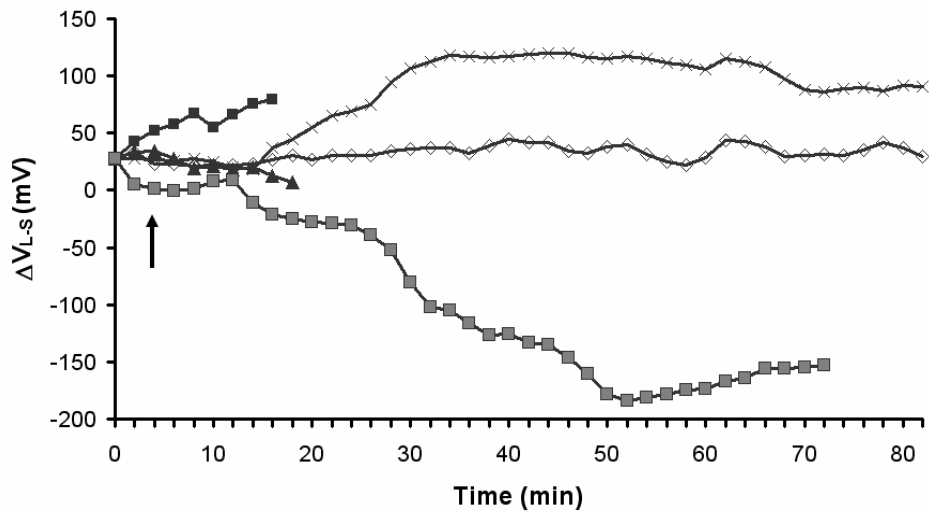


Figure 5. Effects of different treatments on the voltage difference recorded between the leaf petiole and the base of the stem ( $\Delta V_{L-S}$ ). Each data point represents the mean value ( $n=8$ ).  $\diamond$  Control,  $\blacksquare$  Darkness,  $\blacktriangle$  Light,  $\times$  Irrigation,  $\blacksquare$  Drying. The arrow indicates the beginning of each treatment. Data were collected at 2 Hz and plotted at 2 min intervals.

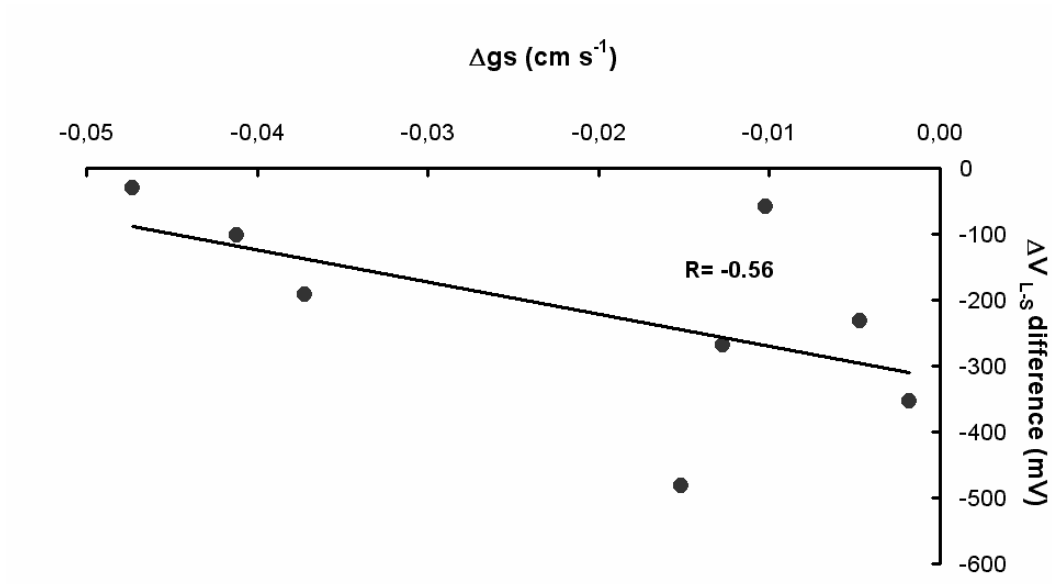


Figure 6. Linear correlation between  $\Delta V_{L-S}$  maximum difference ( $\Delta mV$ ) and stomatal conductance difference ( $\Delta g_s$ ) in the soil drying treatment.

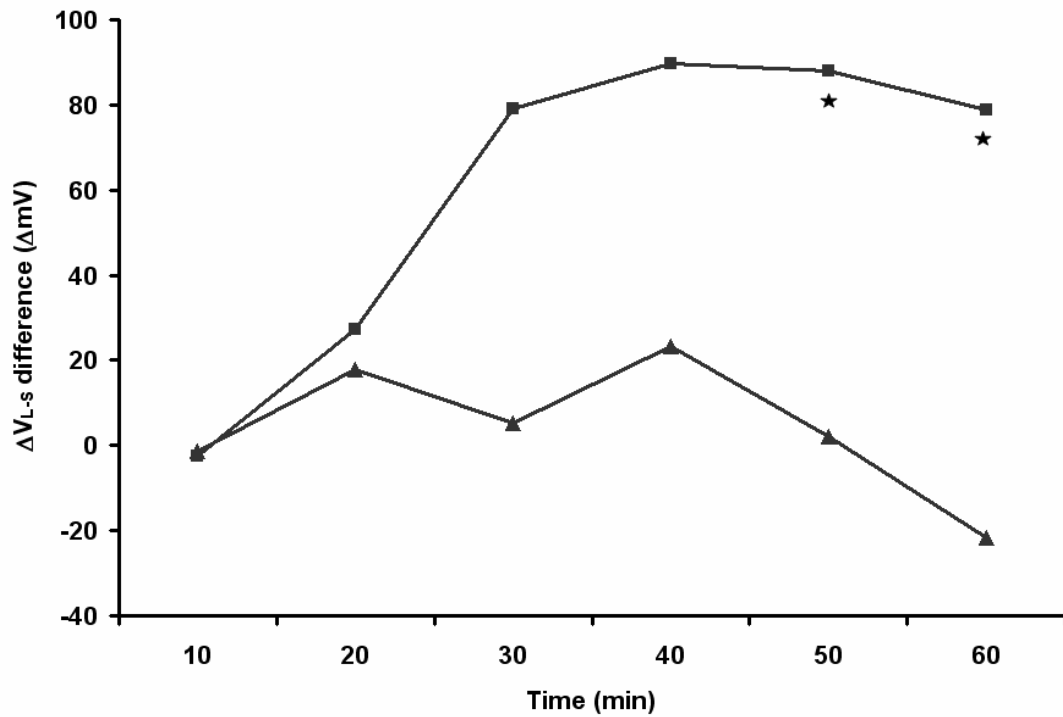


Figure 7. Comparison of  $\Delta V_{L-S}$  differences in irrigated girdled plants and irrigated non-girdled plants. Values represent the  $\Delta V_{L-S}$  differences ( $\Delta mV$ ) average (irrigated girdled treatment  $n = 7$ , irrigated non-girdled treatment  $n = 8$ ) at times 10, 20, 30, 40, 50 and 60 (min) from the beginning of the treatment. Asterisks indicate a significant difference between girdled and non-girdled treatments according to a Bonferroni Test after a 2-way ANOVA ( $P \leq 0.05$ ) at each time. —■— Irrigated non-girdled, —▲— Irrigated girdled.