Zeitschrift: Saussurea : journal de la Société botanique de Genève

Herausgeber: Société botanique de Genève

Band: 29 (1998)

Artikel: Computer-assisted measurement of variation potentials and electrical feedback excitations in Arabidopsis plants

Autor: Favre, Patrick / Guinnard, Olivier / Jouve, Laurent

DOI: https://doi.org/10.5169/seals-1099124

Computer-assisted measurement of variation potentials and electrical feedback excitations in Arabidopsis plants

PATRICK FAVRE, OLIVIER GUINNARD
LAURENT JOUVE, HUBERT GREPPIN
&
ROBERT DEGLI AGOSTI

RÉSUMÉ

FAVRE, P., O. GUINNARD, L. JOUVE, H. GREPPIN & R. DEGLI AGOSTI (1998). Mesures assistées par ordinateur du potentiel de variation et excitations électriques par rétroaction chez Arabidopsis. *Saussurea* 29: 65-75. En anglais, résumés en français et anglais.

Une installation permettant la mesure de potentiels extracellulaires est décrite. Celle-ci permet le suivi des propriétés bioélectriques et également la ré-injection de signaux électriques (ou d'autre nature) à la plante. L'ensemble du système est contrôlé par ordinateur avec une carte de digitalisation (carte A/D-D/A) et au moyen d'un logiciel de programmation d'instruments virtuels orienté-objet. En plus des caractéristiques de stabilité en conditions contrôlées, ce système a été testé avec une induction du potentiel par des transitions de lumière-obscurité chez *Arabidopsis.* Des réponses caractéristiques ont été obtenues (différences feuille-hampe florale). Après une transition obscurité-lumière, une diminution suivie par une augmentation du potentiel de l'ordre de quelques mV a été observée, alors que, avec quelques simplifications, le contraire était vrai lors de la transition lumière-obscurité.

ABSTRACT

FAVRE, P., O. GUINNARD, L. JOUVE, H. GREPPIN & R. DEGLI AGOSTI (1998). Computer-assisted measurement of variation potentials and electrical feedback excitations in Arabidopsis plants. *Saussurea* 29: 65-75. In English, French and English abstracts.

An installation to measure extracellular potentials in plants is presented. It allows to monitor electrical properties and also to re-inject electrical (and possibly other) signals to the plant. The whole system is controlled by a computer with an acquisition hardware (A/D-D/A) card and by an object-oriented virtual instruments programming software. Besides its stability in controlled conditions the system has been tested with classical light induced potential reactions in *Arabidopsis*. Characteristic responses have been observed (leaf-floral stem potential differences). After dark to light transition a decrease followed by an increase in potential in the mV range was observed, whereas, with some simplification the contrary holds true in the reverse light to dark transition.

Key-words: *Arabidopsis thaliana* – Extracellular potential – Electrical feedback.

CODEN: SAUSDH
ISSN: 0373-2525 29 65 (1998)

Introduction

Non-invasive methods which reflect whole intact plant functioning are important tools to understand how precise molecular and genetics manipulations/modifications will be expressed in the whole complex living network (phenotypic physiology). Among classical parameters which can be studied in this way are growth, plant movements/shape changes, photosynthetic/respiration activities by gaseous exchanges (CO_2/O_2) or/and by fluorescence (e.g. STRASSER & al., 1997), sap flow and extracellular potentials. Early measurements of variation (surface/extracellular) potentials in plants in response to light-dark and other stimuli have been performed by BURDON-SANDERSON in 1873 (cited by SIBAOKA, 1969) and HAAKE in 1892 (cited by BENTRUP, 1974). Since then, a lot of progress has been made mainly with the introduction of microelectrodes which can be inserted into specific cellular compartments. However, the extracellular method is still currently in use (GLEBICKI & al., 1989; ZAWADSKI & al., 1995). The interested reader will find useful reviews of the subject in PICKARD (1973), FINDLAY & HOPE (1976), SIMONS (1981), DAVIES (1987), THAIN & WILDON (1993), WAYNE (1994) and TREBACZ & al. (1997).

In contrast to human physiology, where the method of extracellular potential measurements has had huge practical impacts in medicine (e.g. electrocardiography, electroencephalography), in plants the situation is far less advanced due in part to historical reasons (see WAYNE, 1994). From the reviews cited and other references (STARRACH & MAYER, 1989; FRACHISSE-STOILSKOVIC & JULIEN, 1993), it appears

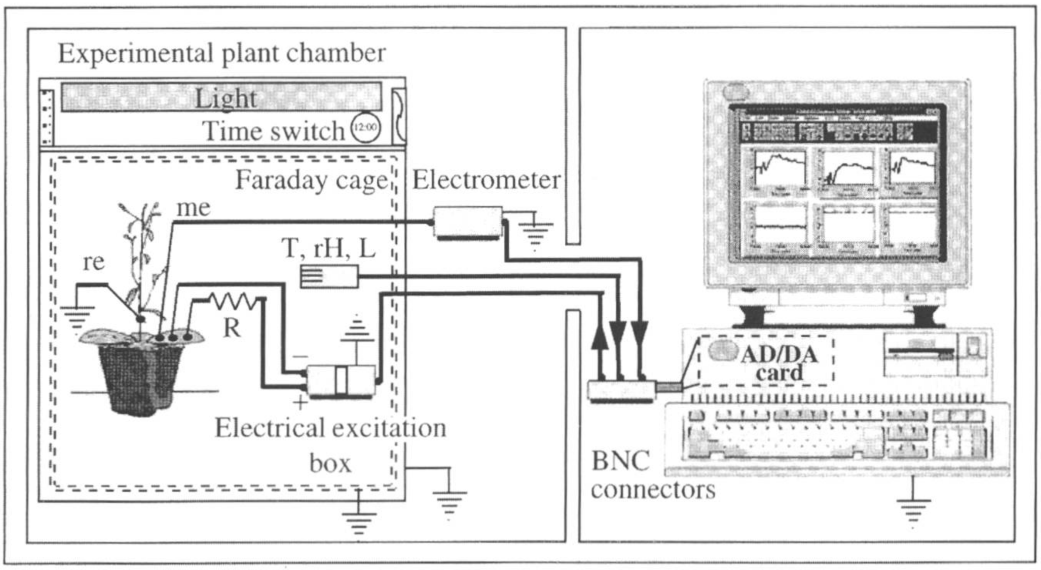

Fig. 1. – Description of the installation for the computer-assisted measurement of variation potential and electrical excitation in *Arabidopsis* plants. The whole installation is compartmented between two rooms. The first is thermo-and hygro-regulated and contains the experimental plant chamber with *A. thaliana*, the light, the sensors, the electrical excitation box and the Faraday cage. The light is provided with fluorescent tubes and controlled by a timer. In the second independent room the computer is installed to allow the real-time monitoring and control of the experiment with a specific software. The A/D part card of the computer receives the signal from the measuring electrode (me) with respect to the reference electrode (re) through the electrometer. Temperature (T), relative humidity (rH) and light (L) are also measured with specific sensors. The independent electrical excitation is achieved by an electrical excitation box whose signal is controlled by the computer via the D/A part. Excitation is current-limited by an 1 MΩ resistance (R).

that variation potentials represent an integrated response of passive and active ionic activities expressed in the extracellular (apoplastic) plant compartment reflecting various physiological processes depending on the stimuli considered (JULIEN & FRACHISSE, 1992; STAHLBERG & COSGROVE, 1996, 1997). These ionic activities are in a yet not fully understood way, linked directly or indirectly to other cellular and even organic compartments (e.g. stomatal and mesophyll cells: see SHABALA, 1997; or roots). In the case of a stimulation with light, variation potentials are elicited which have been mainly attributed to the functioning of the photosynthetic reaction indirectly reflected in the apoplast via the H^+-ATPase of the cellular membrane (WERNER, 1968; LUETTGE & PALLAGHY, 1969; RYBIN & al., 1972; RYBIN & OBOLONSKII, 1974; HARTMANN, 1975).

As a part of a program which address to whole intact plant physiology (organismic plant biology: Plant Physiomatics, GREPPIN & DEGLI AGOSTI, 1997), different non-invasive high resolution methods coupled to computer data acquisition and treatment are developed to investigate physiological parameters as growth (DEGLI AGOSTI & al., 1997), plant movements or shape changes (DEGLI AGOSTI & GREPPIN, 1998). We present here the developed system for measuring extracellular potentials of plants. Since there is a currently important area of active research at the molecular/genetics level in *Arabidopsis* we also have used this plant as a benchmark and potentially very interesting object for the Plant Physiomatics perspective. The system has been tested with classical light-dark transitions and also with a more sophisticated autoregulatory experiment involving an electrical negative feedback excitation triggered by the plant itself.

Materials and methods

Plant material and experimental conditions

Arabidopsis thaliana (L.) Heynh seedlings were grown in a potting compost under L:D (8:16) photoperiod for 3 weeks after sowing, then they were individually transplanted in a new pot and cultivated under L:D (12:12; Sylvania 36 W Luxline-Plus; 75 $\mu mol.m^{-2}.s^{-1}$ PAR) once initiation of inflorescence primordia occurs (3-4 weeks after transplantation). Then they were transferred in an experimental plant chamber within a thermo- and hygro-regulated room juxtaposed to a computer room (Fig. 1). During measurements light was provided by fluorescent lamps (Sylvania 18 W Standard) with an intensity of 45 $\mu mol.m^{-2}.s^{-1}$ PAR.

Light-dark transition experiments were performed with 6 weeks old *A. thaliana* Columbia ecotype, the light-on/off occurred at 8.00 am and 8.00 pm respectively. For electrical excitations *A. thaliana* Landsberg *erecta* ecotype was used at 7 weeks old. Moreover, as these experiments were conducted only to test the whole installation, the light on/off were arbitrarily performed during the day span. In every situation measurements were started after 1 or 2 days of adaptive conditions.

Experimental plant system

The whole construction set-up is presented in Fig. 1. The plant parts are connected to the electrometer, the ground (reference) and the electrical excitation box with surface electrodes (FERRIS, 1974). A short flexible copper wire is soldered to a chlorinated silver electrode (∅ 0.4 mm) to minimize mechanical constraints. The contact between Ag/AgCl electrode and the plant is established with the KCl gel strip of an electrocar-

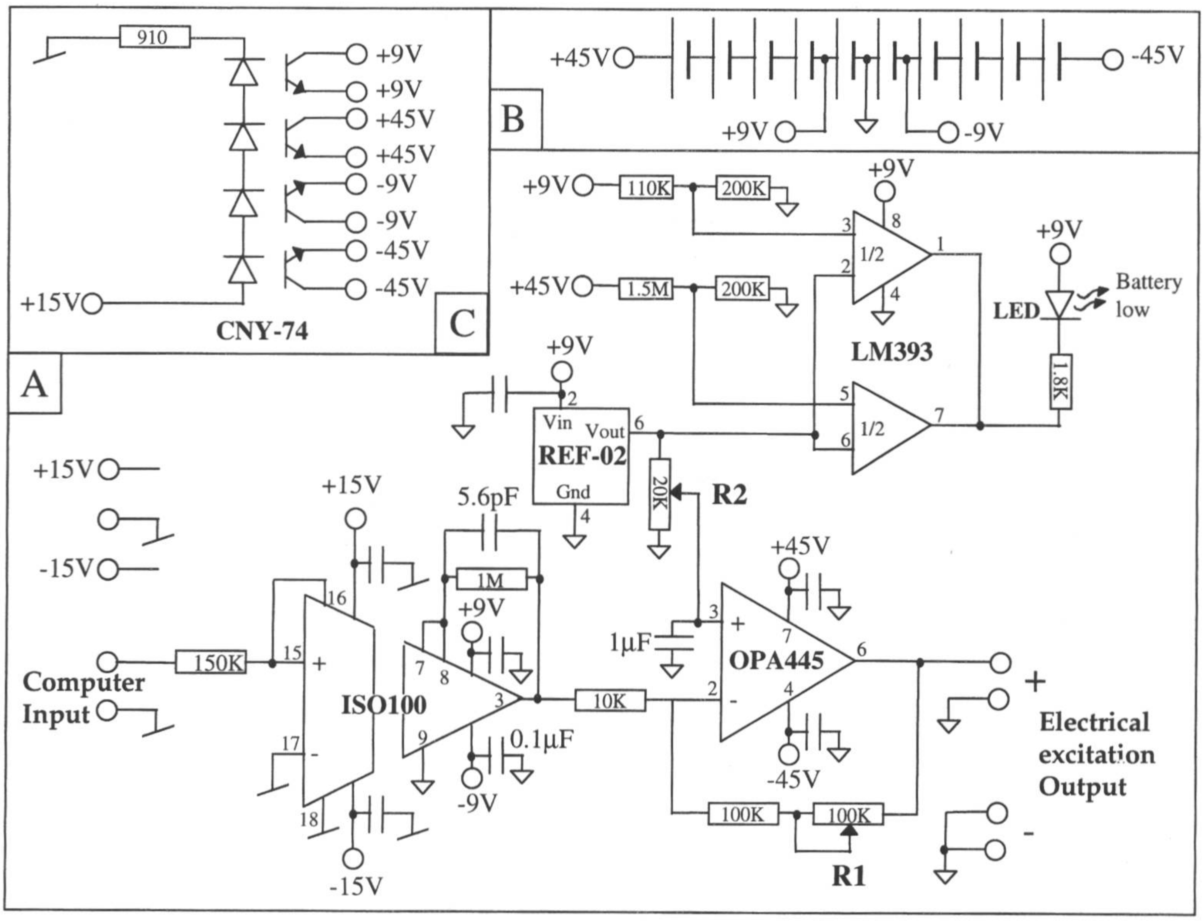

Fig. 2. – **A:** General electronics schematic of the electrical excitation box. Main integrated circuits are: the ISO 100 which is the isolation opto-coupler component where information is transmitted by light; the OPA445 which is an amplifier and the REF-02 which is a stable precise reference voltage. All these integrated circuits are from Burr & Brown Corp. (USA). The LM393 (National Semiconductor, USA) detects low power level of batteries and if necessary switches on a red light (LED). The 100 KΩ resistance (R1) with a black arrow controls the voltage gain and the 20 KΩ one (R2) controls the offset voltage. Default capacity is 0.1 μF. The input voltage coming from the computer via the D/A card is transformed into an independent excitation output voltage according to: $V_{out} = V_{in} \times 14 - 35$. Power supply (DC ± 15 V) is obtained by a standard 220V AC-DC transformer. **B:** The independent alimentation consists of ten 9 V batteries for the power supply of all integrated circuits following the ISO-100 (DC ± 9 V) and for the independent electrical excitation (DC ± 45V). **C:** Electronic opto-switches (CNY74-4, Siemens, Germany) for the independent alimentation (cf B) controlled by the standard power supply on/off.

diogram patch (electrocardiogram, Senstry Medical Products, USA). The reference electrode is on the lower floral stem part and the measuring electrode is on the distal leaf part.

Electrometers (custom built, 10^8 Ω input impedance) transmit the signal to an A/D input card (12 bits, 16 A/D channels, 2 D/A unipolar channel, ACL-812PG, ADClone Inc., Schait, CH) which is plugged into the ISA bus slot of the computer (PC-486DX2, 66 MHz, 16 Mb RAM).

The studied system was equipped with 6 electrometers allowing the direct potential measurement of either one plant at 6 different sites, or 3 independent plants at one

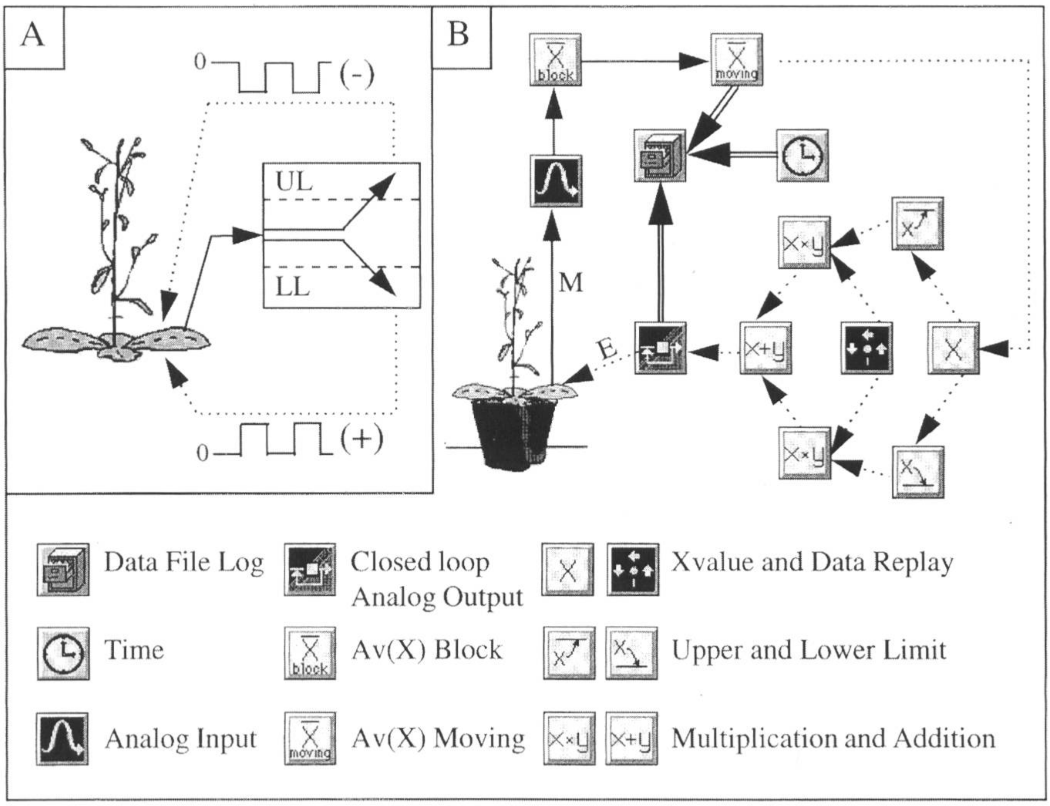

Fig. 3. – **A:** Schematic representation of the negative electrical feedback experiment. Black line arrows represent the biopotential measurements; the dashed line arrows are the electrical pulsed excitations (+8 V, –8 V) triggered when the upper (UL) or the lower (LL) thresholds are respectively reached. When the measurements remain inside the UL-LL range, no electrical excitation pulse occurs (0 V). **B:** Corresponding computer flow diagram of the Labtech Notebook build-time blocks. The continuous lines indicate pathway of the acquisition blocks process (M) and the dashed lines indicate pathway of the excitation blocks process (E). The dedicated block configurations are: Analog Input (sampling rate = s.r.: 150 Hz, range: ±0.313 V), Av(X) Block (s.r.: 0.33 Hz, number of points: 3), Av(X) Moving (s.r.: 0.33 Hz, number of points: 10), Time (s.r.: 0.33 Hz), Data File Log (log name), Xvalue (s.r.: 0.33 Hz, offset: - base line value), Upper Limit (s.r.: 0.33 Hz, scale: 1, limit: 2 mV), Lower Limit (sampling rate: 0.33 Hz, scale: -1, limit: -2 mV), Data Replay (s.r.: 1 Hz, name of the input file containing successively 30 rows of 0.6 value and 30 rows of 0 value), Multiplication (s.r.: 0.33 Hz), Addition (s.r.: 0.33 Hz, offset: 2.5), Closed Loop Analog Output (s.r.: 0.33 Hz, range: +5 V) with PID Control (P:1, I: 0.0001, D: 0.05, setpoint: 0.01).

site (differential mode). New electrometers (input impedance: 10^{15} Ω, INA116 U, Burr-Brown, USA) are actually implemented which will allow the potential measurement of up to 16 independent plants (differential mode).

Temperature and relative humidity conditions are monitored with a dual sensor (HT-732-M-00, Pewatron, CH), as is the light on/off change by a photoelectric sensor (photoresistor). The signals are also sent to the A/D card in the PC (Fig. 1).

For electrical excitations, one D/A channel is used to control the voltage between two excitation electrodes. These are 20 mm distant and situated on the distal part of the leaf, whereas the measuring proximal electrode is 2 mm away of the negative output electrode (Fig. 1). Over 35 volts of positive or negative independent voltage can be imposed between them with a computer-assisted control. For this purpose, a custom built electrical excitation box has been designed and constructed to provide the independent voltage to the plant (Fig. 2). Care has been taken to connect all the grounds (computer, electrometer, electrical excitation box and experimental chamber supplies) together with the Faraday cage and the reference electrode to a common and stable main ground.

Configuration of the user interface software

The application has been developed with the LabTech Notebook software (v. 8.02 for Microsoft Windows, Laboratory Technologies Corp., USA), a build-time interface and a real-time viewer, which allows to monitor and control the ADClone A/D-D/A card on-line with an object-oriented configuration program.

For measuring biopotential during the light-on/off experiments, Labtech build-time was configured to sample 3 points per second and store acquisition data in a text file to be exported. Then, 3 successive points were compressed (averaged) to one and further smoothed (moving mean) over 10 points with a spreadsheet software (e.g. Microsoft Excel). The presented experiments are the mean of at least 6 independent replicates.

In order to perform electrical excitation experiments, a more specific configuration with the multiple functional blocks of the Labtech software tools, has been elaborated (Fig. 3). An electrical pulsed excitation (1/60 Hz, amplitude 8 V) is given in response to the measurement of the light/dark-induced variation potentials in the following way: two triggering levels (2 mV above and 2 mV under a pre-measurement baseline) are defined. If the signal exceeds the upper level (UL) a negative electrical excitation is triggered, whereas a positive one is started once the lower level (LL) is reached (see Fig. 3A). The Labtech build-time specific configuration is detailed in Fig. 3B. These experiments should be considered as a benchmark for the whole installation system.

Results

The basic features (stability, noise, interference) of the installation were first tested without any plant. In this respect, the variation potential was monitored with the measuring electrode in contact through the KCl gel bridge with the reference electrode and light-dark transitions. Environmental conditions were also simultaneously monitored and are also presented in Fig. 4. During the light-on, as well as the light-off, there were no significant changes in the potential at a scale of our present experimental resolution. The background noise (peak-to-peak) is about 0.60 mV with the first custom built electrometers. Temperature and relative humidity were at the end of the 12 h dark period of 21.1°C and 76.1% respectively and of 23.3°C and 70.9% at the end of the 12 h light period. These changes were even smaller over the effective potential measurement period (Fig. 4).

The measurement of the potential variations between measuring and reference electrodes was done with *Arabidopsis* plants during light-on and light-off switches (Fig. 5). The incidences of this direct transition from light to dark and reciprocally lead to characteristic and significant fluctuations in the variation potential of the leaf with respect to the stem (Fig. 5). During the light-on the potential first decreased in 1-3 min by ~3 mV. It then increased again to peak at about 5 min with an amplitude of ~5 mV

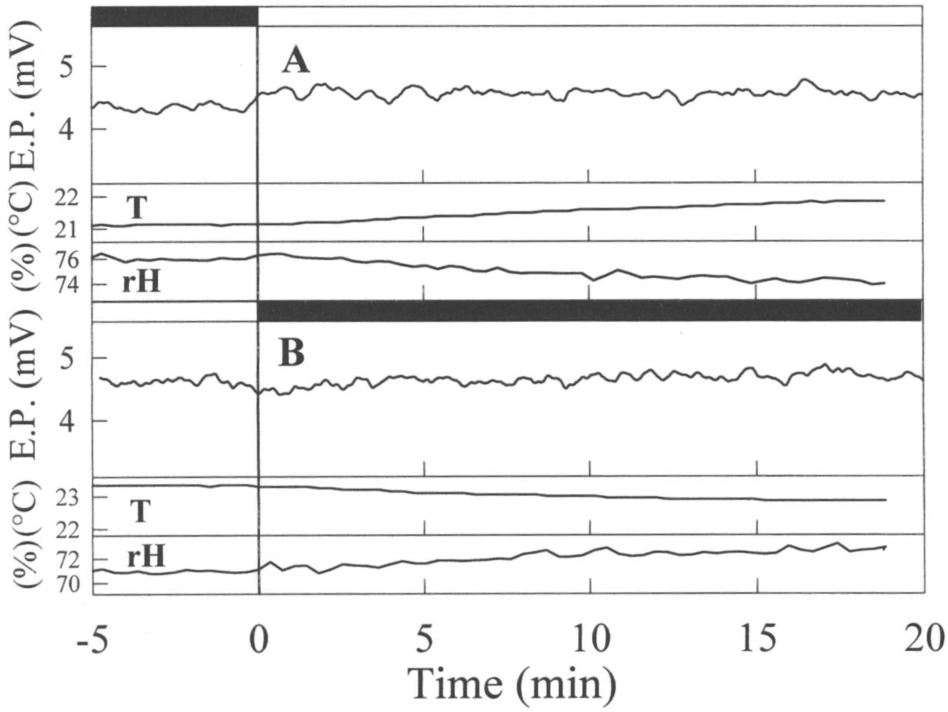

Fig. 4. – Variation potential (E.P.) between the measuring and reference Ag/AgCl electrodes connected with a KCl gel during (A) dark to light and (B) light to dark transitions. The corresponding environmental conditions (temperature : T, relative humidity: rH) are also represented. Only the 25 min around the light-dark transitions of the whole 12:12 L:D are shown.

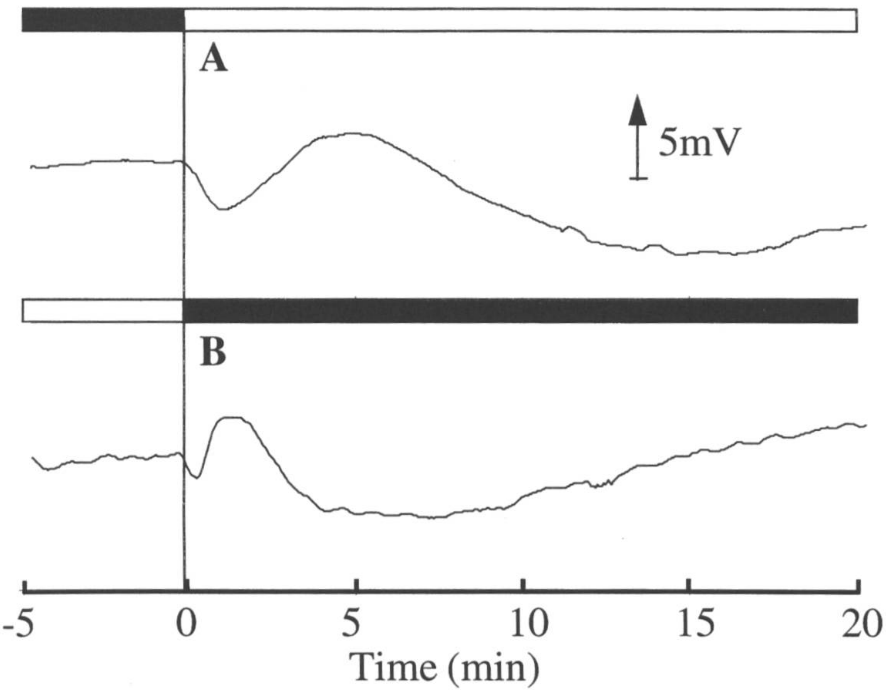

Fig. 5. – Variation potentials during light-dark transitions in *Arabidopsis thaliana*. **A:** during a dark to light switch (the results presented are the mean of 9 independent experiments). **B:** during a light to dark transition (n = 6 independent experiments).

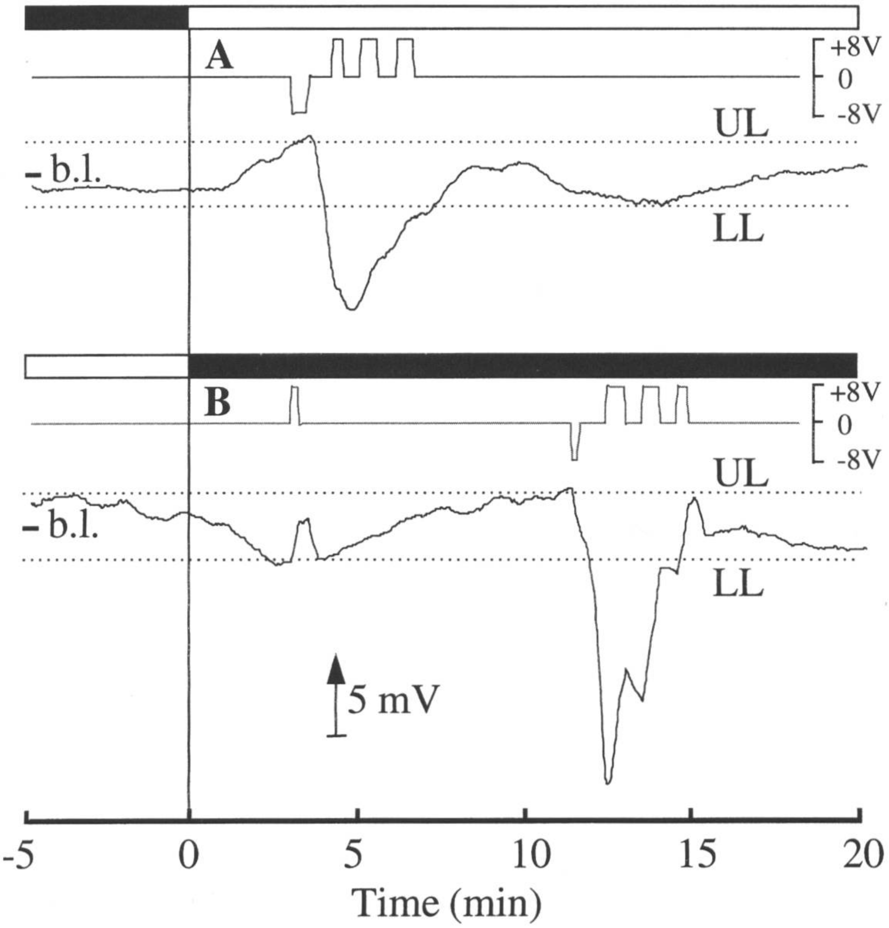

Fig. 6. – Potential difference and local negative electrical feedback excitation on the leaf of *Arabidopsis thaliana* during the light-on (**A**) and the light-off (**B**). The experimental protocol is detailed in Fig. 3A. Dashed lines are the thresholds control values (Upper Limit: UL and Lower Limit: LL); lines with squares are the recordings of excitations (0 V, +8 V or –8 V). The baseline (b.l.) is a pre-experimental measured value which allow to determine the UL and LL limits.

(Fig. 5A). An opposite-like dynamics could be observed during the light-off transition (Fig. 5B): a small rapid decrease occurred first, then a peak was observed at about 1-2 min after the transition with an amplitude of ~5 mV. Then the potential went a more slower but greater decrease in the 5 following minutes. The results presented are the mean of several independent experiments. Some variability is observed between individual plants both in the dynamics and the amplitude of the responses (results not presented). The extent of this variability in these two parameters is in order from half to twice the mean presented value.

In order to further test the whole installation, a more elaborated experiment has been designed with a specific Labtech configuration. It consisted to reinject electrical excitation signals to the plant which were triggered by the measured variation potentials in response to light-on/off. This experiment can be considered as a negative autoregulatory feedback controlled by the plant itself, its principle has already been detailed in Fig. 3A and 3B. The measurements of the variation potential of a plant submitted to such a feedback are presented in Fig. 6. After the light-on the potential increased over the upper threshold limit (UL), thus triggering a negative excitation (Fig. 6A). The effect was to strongly and rapidly decrease the potential under the lower limit (LL). Then, several positive excitations were automatically started to restore a potential included between the limits. In the same manner, the light-off provoked a variation in the potential which overstepped the LL limit (Fig. 6B). More precisely, the potential first decreased less than the LL. Thus, a positive excitation was triggered and imposed to the plant inducing a small peak included inside the limits. Afterwards, the potential increased progressively to transgress the UL. This overstepping immediately carried away a succession of one negative and, with the transition of the LL, some positive excitations that finally restored a potential included between the limits. It is interesting to observe that the first UL transgression induced an electrical pulse which provoked important and very rapid decreases of potential in both situations (Fig. 6A and 6B), which were recovered later "in-limits" after several positive excitations. In some cases this negative electrical excitation feedback loop went in critical unstable or auto-oscillatory behaviour due to the highly non linear structure of the experiment (data not shown).

Conclusion

A basic installation to measure extracellular potentials in plants has been presented. It allows to monitor electrical properties and also to re-inject electrical (and possibly other) signals to the plant. The whole system is controlled by a computer with an acquisition hardware (A/D-D/A card) and by an object-oriented virtual instruments programming software. Besides its stability in controlled conditions the system has been tested with classical light induced potential reactions in *Arabidopsis*. Characteristic responses have been observed (leaf-floral stem potential difference). After dark to light transition a decrease followed by an increase in potential was observed in the mV range, whereas, with some simplification the contrary holds true in the reverse light to dark transition. These extracellular responses are known to possess some variability in both the dynamics and voltage range in different species (NOVAK & IVANKINA, 1975). However, the presented results closely match the overall emerging picture from the literature (NOVAK & IVANKINA 1975; WERNER, 1968; GIROLDINI, 1988; GREPPIN & al., 1996).

Extracellular potentials can be considered in a very oversimplified way as the inverse of intracellular ones obtained by intracellular electrodes during light treatments (NOVAK & IVANKINA, 1975). This appears even true for other environmental stimuli such as stress (RHODES & al., 1996). In this respect our results also correspond to intracellular potential elicited by light-dark transitions already observed in the literature (e.g. MONTAVON & GREPPIN, 1977).

To our knowledge *Arabidopsis* plants have not been investigated often for extracellular potential: we found only one citation (abstract) with this plant (PARKS & al., 1996). The present article is thus the first presentation of these variation potentials in this widely used plant. Besides historical reasons which may explain that plant bioelectricity is rather not developed in comparison to animal electrophysiology (WAYNE, 1994), it is clear that the lack of precise and well established molecular/biochemical/physiological links together with non standardised and highly variable responses may explain this situation. However, it is clear that such a method after thorough and coherent calibration/validation with respect to cellular or/and whole plant responses will constitute a very interesting method to obtain non-invasive information in whole intact plants.

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Addresses of the authors: P. F., L. J., H. G. & R. D. A.: Laboratoire de Biochimie et Physiologie Végétales, Place de l'Université 3, CH-1211 Genève 4, Suisse.

E-mail R. D. A.: Robert.DegliAgosti@bota.unige.ch

O. G.: GAP optique, Groupe de Physique Appliquée, Rue de l'Ecole-de-Médecine 20, CH-1211 Genève 4, Suisse.