Fast acquisition of action potentials in *Arabidopsis thaliana*

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Abstract

We measured Action Potentials (APs) with extracellular wet recording electrodes at a sampling rate of 10 Hz (i.e. 10 Samples per second, 10 Ss). The touch stimulation of an adult Arabidopsis thaliana *(col accession) leaf elicited a transient (~10 sec) depolarization (~-50 mV) potential (AP), moving away from the excitation zone to the petiole at a speed of ~1.5 mm s^{-1}. These numbers are in average coherent with those reported in the literature. But some works have reported ultra fast APs in the ms duration range and with m s^{-1} propagation speed (i.e. thousands time shorter and with propagation speeds of thousands times faster). This kind of ultra fast APs can't be measured at 10 Ss sampling rate. We undertook here to sample our touch elicited APs in* Arabidopsis *at different sampling rates (i.e comparing 10 Ss and 10 000 Ss simultaneously and then at 100 000 Ss). It is clearly demonstrated that touch elicited plant AP cannot result from higher frequency aliasing and a 10 Ss sampling rate is sufficient for a signal with ~10 s duration. At 100 000 Ss sampling rate, it was possible to eventually observe some very fast electrical events. Their duration is from 10 µs (i.e. minimum sampling interval) to 200 µs. However, they occur simultaneously on 2 distant electrodes (Leaf – petiole) without any amplitude correlation, their median amplitude is not statistically different from the null value. There are no differences between different treatments on the leaf (i.e. none, water or H_2SO_4). It is very likely that these very fast electrical events don't have a biological origin. It is proposed that they are electrical interferences arising from external plant sources.*

Keywords: Arabidopsis, *plant Action Potential, mechanical stress, touch, aliasing, whole-plant electrophysiology*

Introduction

The history of electrophysiological signals in plants was significantly developed at the end of the 19th century when C. Darwin suggested the use of the carnivorous plant *Dionaea muscipula* to Burdon-Sanderson, an eminent animal physiologist. Burdon-Sanderson succeeded in recording the first true Action Potential (AP) in plants (Burdon-Sanderson 1873, 1888). Until recently, these experiments have been repeated with relatively similar results (AP duration [0.3-10 s]; propagation speed [60-200 mms^{-1}]: Di Palma et al. 1961; Hodick and Sievers 1988; Trebacz et al. 1996; Trebacz and Sievers 1998; Harrison RR. 2007; Pavlovič et al. 2011; Escalante-Peréz et al. 2011, and see ERC-2009 Carnivorom project[1]), as presented in table 1 of Volkov et al. (2008). These authors previously reported a fast AP duration [1-1.4 ms] with a propagation speed of 10 000 mm s^{-1} (Volkov et al. 2007) upon electrical excitation, but in subsequent studies, these authors demonstrated APs lasting 0.2 s (Volkov et al. 2013). For a more detailed review on *Dionaea muscipula* and other carnivorous plants see Król et al. (2011).

In animals, APs are transient changes in plasma membrane potential, conveying information in neurons and nerves. APs are at the core of the functioning of animal nervous system (e.g. our brain, Dale et al. 2004). In plants (Degli Agosti 2014; introduc-

[1] ERC-2009 Carnivorom. Molecular basis of carnivory. Excitability, movement, and endocrinology of plant traps. Biozentrum Universität Wurzburg. Project leader: Prof. Dr. RF Hedrich. http://www.bot1.biozentrum.uni-wuerzburg.de/forschung/hedrich/projekte/erc_2009_carnivorom/

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tory references), the participation of "electric" signals in distant signaling is an old and unevenly developed topic. Recent and other evidence (see review by Baxter et al. 2014) suggests important roles for these signals in different physiologically significant distant processes (see also Mousavi et al. 2013 and Sukhov et al. 2014).

However, electrophysiological signals in vascular plants are more versatile (see Pickard 1973), variable and diverse than those in animals. Moreover, the naming/definition and classification of these signals remain confusing (for additional information see recent reviews by Fromm and Lautner 2007; Król et al. 2010; Zimmerman and Mithöfer 2013). While APs share common characteristics in animals and plants, the latter have additional signals, such as variation potential (VP) and system potential (SP) (Zimmerman and Mithöfer 2013). Unfortunately, these signals can be mixed when extracellular methods are used. APs share common properties between plants and animals: (1) characteristic depolarization and repolarization phases, (2) the all-or-nothing law, i.e., associated with a threshold stimulation level, and (3) self-propagation and a refractory period, i.e., a minimum time interval separating two excitation signals before a full AP is again elicited; apparently the amplitude of these signals changes along the propagation pathway (e.g., in *Arabidopsis* the extracellular measured amplitudes increases from the leaf to the petiole) (Favre and Degli Agosti 2007, Degli Agosti 2014). Many of these characteristics (e.g., Favre and Degli Agosti 2007) have been obtained using extracellular electrodes on the whole plants, where a non-linear sum of many electrical cells signals can be achieved. Indeed, it is well documented in animal electrophysiology that the extracellular measurement method modifies strongly the shape and amplitude of single cell AP (e.g neuron, see Gold et al. 2006), but the duration and propagation speed of these signals is better maintained. The analysis of extracellular multicellular electrical potentials in animal tissues is still a challenging area of research (e.g., Buzsáki et al. 2012). To our knowledge, this subject has not yet been addressed in plant science. In different plants (Zimmerman and Mithöfer 2013), the AP duration is heterogeneous (median 60-90 s), with a median propagation speed of 2-5 mm s^{-1}. VP median duration is significantly longer (900-1050 s), and the amplitude of this signal decreases with increasing distance from the stimulated zone, although the median speed of propagation is similar to that of AP (0.9-1.3 mm s^{-1}). VPs are often superimposed with putative APs and oscillations of electrical potential (Favre et al. 2011, Mousavi et al. 2013). In contrast, the AP duration in animals is on the order of 1 ms, with a propagation speed of 10 ms^{-1} (Dale et al. 2004), thus several thousand times faster than that of plants.

Pyatygin (2008) reviewed this wide AP duration and propagation speed variation in plants and classified them as "slow", "fast" and "ultrafast" APs. Ultrafast APs have durations and propagation speeds similar or even shorter and faster than animal APs. They have been reported (durations and speeds of propagation indicated in brackets) in: *Glycine max*, i.e. [0.3 ms; 5.5-40 m s^{-1}]: Volkov (2000); [2 ms; 30 m s^{-1}]: Volkov et al. (2000); [0.3 ms; 40 m s^{-1}]: Shvetsova et al. (2001); [0.4-1 ms; 0.5-5 m s^{-1}]: Shvetsova et al. (2002); [0.2-20 ms; not detailed]: Volkov et al. (2004); [0.3 ms: n.d.]: Volkov et al. (2005); [5.6-106 ms; n.d.]: Lang and Volkov (2008); in *Sorghum bicolor*, i.e., [0.2 ms; 270 ms^{-1}]: Mishra et al. (2001), and in *Dionaea muscipula* [1.5 ms; 10 m s^{-1}]: Volkov et al. (2007a).

As a matter of fact, the detection of these events strongly depends on the equipment used. Jovanov and Volkov (2012) presented arguments on the basis of digital signal processing theory. These authors illustrated that an apparent slow peak could result from the inadequate sampling frequency (Ss = samples per seconds) of the analog to digital conversion acquisition card, whereas in fact faster peaks could be present in the signal. A continuous analog electric signal (i.e., an electrophysiological signal) could be displayed using a classical oscilloscope or a paper recorder, whereby the signal is correctly reproduced provided the bandpass of the measurement system is wider than the frequency range of the signal. Otherwise, the reduction and ultimate disappearance of its amplitude will occur. For digitally sampled signals, in addition to the bandpass property, the sampling frequency should also be considered. Indeed, the sampling frequency for a given (periodic) signal

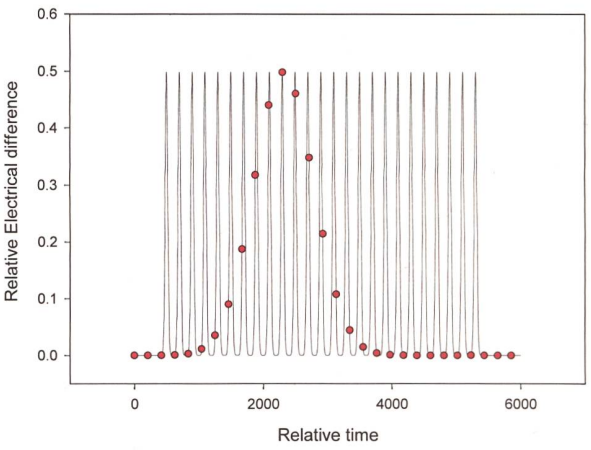

Fig. 1. Schematic illustration of aliasing when inadequately undersampling a fast signal. A burst of (artificial) fast event signals sampled at an insufficient frequency might generate a signal with an apparently longer duration (red dots).

should be more than twice the frequency of that signal (Nyquist frequency limit, see e.g. Proakis and Manolakis 1992). What happens when higher than Nyquist frequency (fast) signals are present? By virtue of their spectral properties, these signals will occur at lower frequencies than the original signals (aliasing). Thus fast high frequency signals will appear as low frequency signals (energy conservation). To avoid this problem, it is possible to add a low-pass analog filter prior to A/D conversion and/or to correctly sample the signals after ascertaining that higher frequencies are not present.

This phenomenon is illustrated in Fig. 1. When an artificial burst of high frequency peaks is sampled at an inadequate lower frequency-sampling rate, that burst will result in an apparently single slower peak. Notably, the high frequency burst should be time limited, otherwise undersampling would result in a slower oscillation, i.e., the slow peak will subsequently be repeated.

This aspect deserves attention, since we usually sample our signals at a relatively low frequency (10-50 Ss) in *A. thaliana* (Favre and Degli Agosti 2007, Degli Agosti 2014). Are these signals the apparent result of fast higher frequency burst signals? To answer this question, we generated plant APs with a touch treatment (Degli Agosti 2014) and simultaneously sampled our signals with 2 different acquisition systems: one at 10 and the other at 10 000 Ss. We also sampled touch-generated APs at 100 000 Ss to ascertain the absence of very fast signals. Moreover, we examined in detail measurements at 100 000 Ss, to detect whether fast electric events could be detected and carefully tested whether these events could have a biological origin through an analysis of the signal occurrence in untreated plants or using a small water drop and with a drop of H_2SO_4. This latter treatment has been reported to elicit ultrafast plant APs (Volkov 2000, Shvetsova et al. 2002).

Materials and Methods

Plant material

Arabidopsis thaliana (L.) Heynh Columbia (Col = Col-0) seeds were sown in potting compost under an 8 h:16 h Light:Dark (L:D) photoperiod for three weeks. The seedlings were individually transplanted into new pots (L×W×H=9cm×9cm×10 cm) and cultivated under an 8 h:16 h L:D photoperiod (Sylvania 36W Luxline-Plus, 75 µmole^{-2} s^{-1} PAR). Subsequently, 42- to 60-day old seedlings were transferred to a thermo- and hygro-regulated room (22±1 °C and 73±2% rH) under a 12 h:12 h L:D photoperiod (fluorescent tubes, Sylvania, 18W Standard, 115 µmole^{-2} s^{-1} PAR) for measurements in the experimental plant chamber.

Electrophysiological measurements

The method with wet contact electrodes, electrometer (impedance: 10^{15} Ω) and A/D D/A card have been previously described (Favre et al. 2001; Favre and Degli Agosti 2007; Favre et al. 2011). The measuring installation and touch stimulation zone for plants are shown in Fig. 2. The recorded electrical potential represents the difference between the measured (E1 for leaf and E2 for petiole) and reference electrode (Eref), with respect to the electrical earth. Wet electrodes were positioned via a homemade manipulator. All electrodes were Ag/AgCl types with chloridized wires immersed in an electrophysiological solution (1 mM KCl, 0.1 mM $CaCl_2$, 50 mM sorbitol, and 2 mM 2-(N-morpholino) ethanesulfonic acid/Tris, pH 6.8). To simultaneously compare different sampling acquisition rates (10 Ss with PC1 and 10 000 Ss with

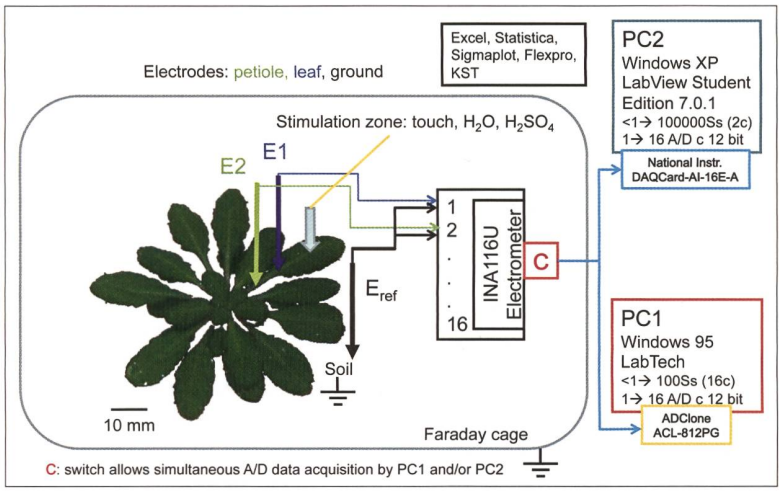

Fig. 2. Installation diagram for measuring the electrophysiological signals in Arabidopsis thaliana *(Col). The plants were maintained in a Faraday cage containing an electrometer (16 differential channel, INA116, 10^{15} Ω). The signal was acquired using an A/D board (for more details see: Favre et al. 2007, Favre and Degli Agosti 2011). A: First, a wet contact extracellular electrode is positioned at the end of the leaf (Leaf/E1) and a second electrode is positioned on the petiole (petiole/E2) with a cotton wool contact (diameter 3-4 mm). The orange arrow indicates the stimulation zone in the middle of the leaf and midvein center (touch, water or H_2SO_4).*

PC2 see Fig. 2), the signal from the electrometer was also sent to a second computer (Fig. 2 PC2) via a special connector (Fig. 2 symbol c). PC2 was equipped with an A/D DAQ-Card-AI-16E-A (12 bits, National Instruments) with a program driver specifically written (R.D.A) with a custom graphical user interface LabView software (student edition 7.01, National Instruments) (Fig. 3). With 2 channels, the system in PC2 can achieve acquisition rates up to 100 000 Ss.

Stimulations

The touch treatment was applied as previously described (Degli Agosti 2014). Briefly, a small brush (30 mm^2) touch was performed in the middle of the leaf (< 2 g during < 3s). The hand wrist of the manipulator was grounded to diminish electrical interaction, although these interferences remained visible. Ultrapure water and H_2SO_4, pH 3.0 (i.e., 0.5 mM H_2SO_4) were carefully deposited (10 µL) in the middle of the leaf on the main midrib during an acquisition period of ca 5 min.

Statistics and data treatment

Statistics and graphing were performed using the Statistics module of Sigmaplot (v 11.0 for windows, Systat software, Inc.) or/and Statistica (Statsoft Inc.). However, plotting and selecting data in a large dataset (> 500MB) can be difficult. ASCII data were imported using KST (both for Windows 7.0 and linux Ubuntu 14.04, http://kst-plot.kde.org/), a free and rapid software (Dell Optiplex 990, Microsoft Professional Windows 7, 3.3GHz, 8GB RAM) for total visualization (with details) and further examination at a very short time frame. The KST program facilitates the complete visualization of the data (5 min experiment duration: i.e., 6 x 10^7 samples both from leaf and petiole electrodes), with fast short event durations (≤ 10 µs) immediately visible through visual eye inspection.

Results

Touch

Touch treatment never visibly affected plant tissues, as examined by eye or magnifier after next treatment days. However, immediately after touch treatment, an AP is elicited in *Arabidopsis* leaves that spreads from the leaf to the petiole. The simultaneously sampled signals at 10 and 10 000 Ss with 2 different acquisition A/D cards in 2 different PCs are shown in Fig. 4. Both signals were obviously superimposed with the 2 sampling frequencies.

We next examined the electrical signals at a higher frequency sampling (i.e., 100 000 Ss), with no treatment or after treatment with 10 µL of water and 10 µL of H_2SO_4 for 300 s (i.e., 5 min). In Fig. 5, we show a typical recording using 2 electrodes (one electrode on the leaf and another distant electrode on the petiole), with no APs elicited after 10 µL of water treatment. The signals show a clear perturbation during deposition on the plant leaf within the Faraday cage, with a simultaneous perturbation at the petiole electrode. However, some very fasts events were visible at ~175 s and ~210 s. Power spectra (log scale) showed an almost flat spectrum, with low frequency (50 Hz) and

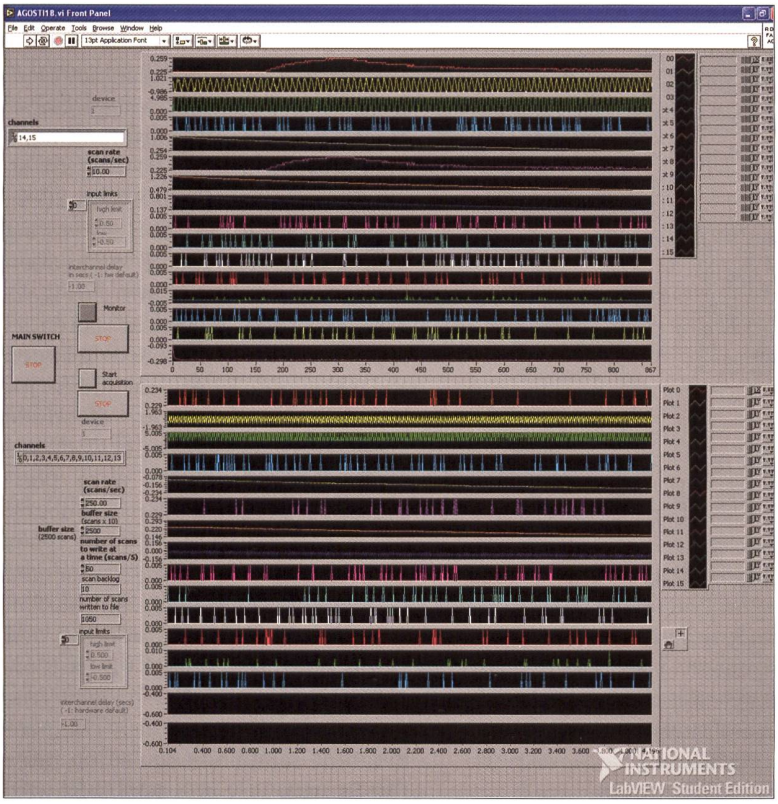

Fig. 3. Front Panel of the fast acquisition system driver in Labview. The 16 upper graphs show pre monitoring of the signals, whereas the lower 16 graphs show visualization during data-acquisition, in which an ASCII text file is first saved, and the data are added until the stop button is activated.

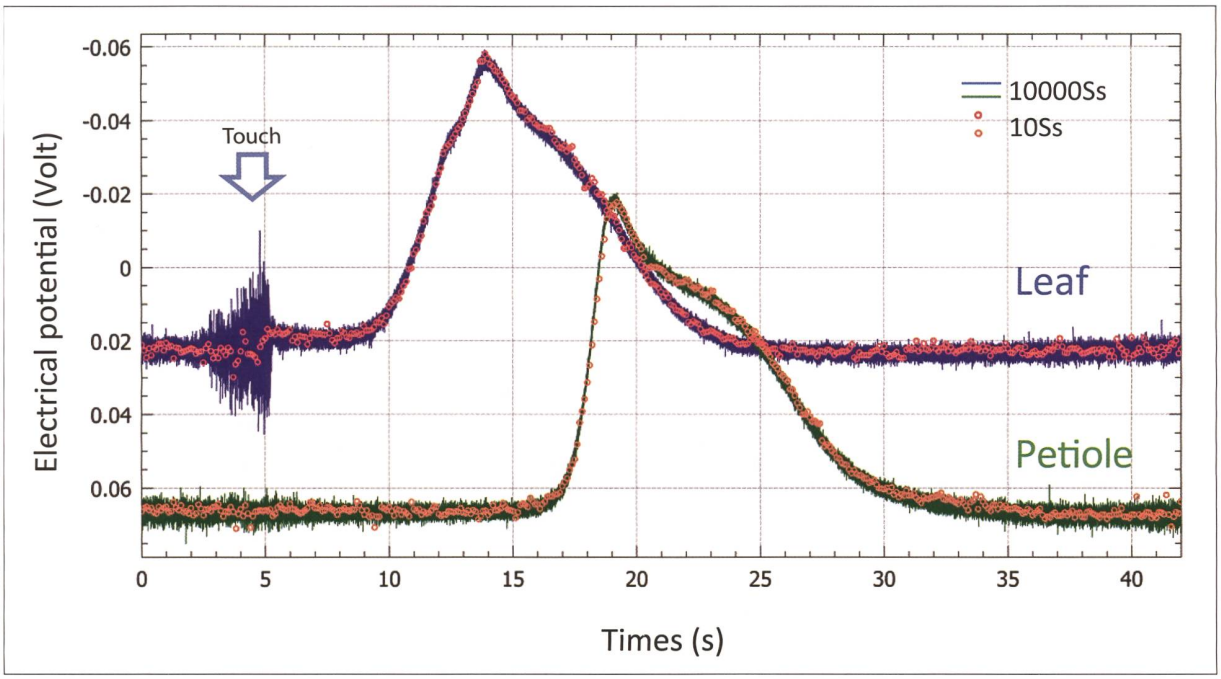

Fig. 4. Electrophysiological signals after a soft touch on the leaf (blue down arrow) with a brush simultaneously measured at 10 000 and 10 Ss. The signal on the leaf electrode appeared after ca. 5 s and was transmitted to the petiole after ca. 5 s. The duration was ~15s. The blue and green lines indicate the signals sampled at 10 000 Ss with Labview software using the first A/D card. The red and orange dots represent the signals sampled simultaneously at 10 Ss with the Labtech software using a second A/D card.

multiple integer spikes (typical from the interference of domestic power supply) from both leaf and petiole recording electrodes (Fig. 4B inserts upper right). The spectrogram (i.e. changes of spectra over time, Fig. 4C) shows enriched frequencies during deposition and at ~175 s, where a fast electrical event was observed.

It is clear that some (fast) signals could be detected on leaves and petioles. We examined these signals in more detail. Fig. 6 shows the results of a measurement of water deposition treatment with increasing time resolution. These fast events are clearly distinguishable from the baseline, as rapid signals that simultaneously occur on both petioles and leaves. In this example, we quantified the amplitude (i.e., ~+5 mV at the leaf and ~-3mV at the petiole), duration (20 µs at the leaf and 30 µs at the petiole, the sampling is of 10 µs) and time differences between the leaf and petiole occurrence (shift at maximum amplitudes i.e., here 0, that means simultaneous at the sampling frequency resolution of 10 µs) of these signals (Fig. 6D).

We repeated these experiments using either no treatment or the deposition of 10 µL of water and 10 µL of 0.5 mM H_2SO_4, with 8 experiments each, and examined all fast events recorded. Table 1 presents the detailed results, showing that no differences were observed between any treatment, water or 0.5 mM H_2SO_4, for both leaves and petioles. Furthermore, no differences were observed in the occurrence, duration or amplitude of the signals.

Importantly, statistical analyses showed that the overall amplitude on both the petiole and leaf was widely dispersed and not significantly different from 0. Moreover, the time shift (i.e., time difference at leaf peak and time at petiole peak) was also not significant, suggesting that the potential transmission speed, if any, is faster than the acquisition interval time (i.e., for 100 000 Ss) of 10 µs (i.e., a propagation speed > 1000 m s^{-1}). There was also no correlation between the amplitude measured at the leaf and petiole.

Fig. 7 shows the detailed distribution of the amplitude, duration and time shift between leaf and petiole. Regular distributions were visible at 0 mean for amplitude and time shift of these fast events between the leaf and petiole. Duration was obviously higher than 0 and spanned from 10 to 200 µs max.

Finally, we assessed whether our fast acquisition system could detect touch-generated ("slow") APs at a sampling rate of 100 000 Ss and examined whether it was attributable to a burst of ultra fast signals (see Fig. 1). Indeed, after treatment, an AP was elicited at the leaf, which moved to the distant (1.0 cm) petiole

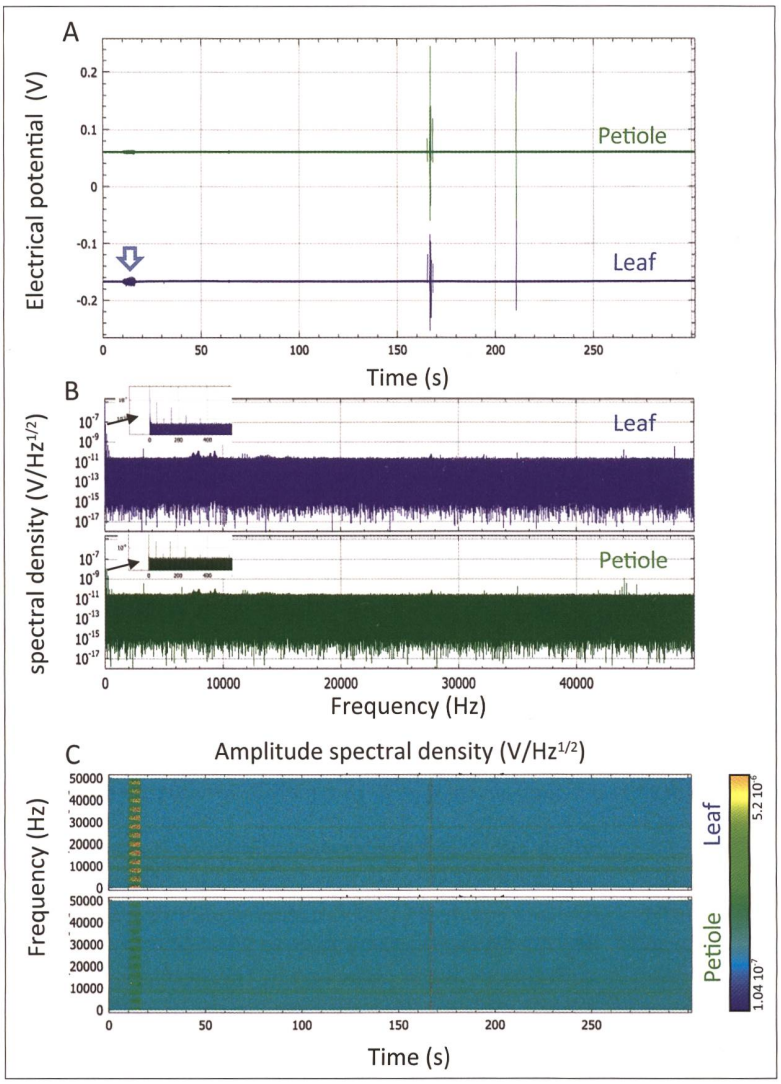

Fig. 5. Recordings at 100 000 Ss (i.e., interval between 2 points of 10 µs each). Fast electrical events (FEs) are shown in A (at ~165 s and 210 s). The leaf received a drop of 10 µL of water (blue down arrow). B Power spectra (note Y log scale) of both leaf (blue) and petiole (green) recordings. The inserts show peaks at 50 Hz and harmonics. C. Spectrogram (evolution of frequencies in time [span of 40 ms]). The stimulation introduced some artifacts with harmonics during the deposition of water (~5-8 s), and frequency rich perturbations were also visible at ca 165 s.

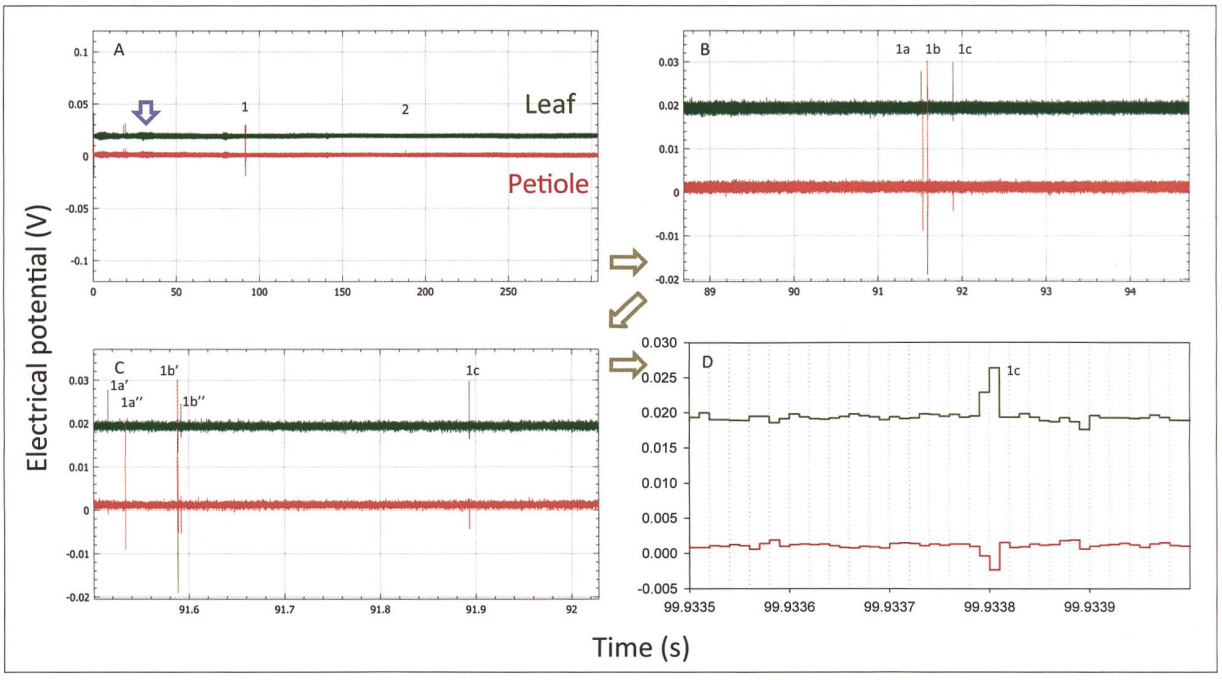

Fig. 6. Recording at 100 000 Ss. Fast events characterization. The results of treatment with a drop of 10 µL of water is shown, and time span was successively measured until individual peaks events could be characterized in amplitude, duration, and time shift between leaf and petiole peaks.

Table 1. Number of fast electrical events (FE) observed under different conditions, either (1) without treatment, or (2) after the addition of 10µL of ultrapure water (H_2O) or (3) 10µL of H_2SO_4 (0.5 10^{-3}M). Each experiment was repeated 8 times, with electrodes positioned on the leaf and petiole (ca 1.5 cm distant), respectively. Recordings were obtained at 100,000 Ss during ca. 300 s. When a signal was detected at the leaf, a corresponding signal was also detected at the petiole.

Group	(1) Untreated	(2) H_2O	(3) H_2SO_4	Pooled (1-3)	Pooled leaves and petioles
Nb. of measurements (leaf + petiole)	8+8	8+8	8+8	24+24	—
Total samples (x10^{-6})	480.36	455.058	494.24	1429.78	—
Total data size (GB)	4.396174	4.732711	4.56213	13.691015	—
Nb. of series with FE (leaf + petiole)	5+5	5+5	4+4	14+14	—
Total FE (leaf + petiole)	28+28	28+28	39+39	95+95	—
Median nb. of FE/Leaf or Petiole	3[a] (0; 6.5)	1.5[a] (0; 7)	0.5[a] (0; 11)	1 (0; 7.5)	—
Median FE amplitude leaf (mV)	13.3[b] (-6.1; 37.0)	-1.95[b] (-16.5; 137.0)	5.1[b] (-69.8; 60.5)	4.62[h, i] (-17.6; 34.4)	Median FE amplitudes (mV)
Median FE amplitude petiole (mV)	3.66[c] (-8.42; 13.3)	-4.4[c] (-13.3; 3.6)	17.6[c] (-30.3; 77.6)	3.54[h, i] (-129.0; 24.4)	3.66[i] (-1.65; 32.0)
Median FE duration leaf (µs)	30[d] (20; 40)	20[d] (10; 30)	20[d] (10; 37.5)	20 (10; 30)	Median FE duration (µs)
Median FE duration petiole (µs)	30[e, f] (20; 40)	20[e, f] (10; 20)	20[e, f] (10; 30)	20 (10; 30)	20 (10; 30)
Median time shift leaf-petiole (µs)	0[g] (-10; 0)	0[g] (-7.5; 0)	0[g] (-10; 0)	0[k] (-10; 0)	—

In parenthesis, following the medians, are the 1st and last quartiles (25% ; 75%)

Testing median differences (all normality test failed: P<0.05):

[a] Kruskal-Wallis One-Way Analysis of Variance on Ranks: H = 0.0122 with 2 degrees of freedom. P = 0.994: Not significant.

[b] Kruskal-Wallis One-Way Analysis of Variance on Ranks: H = 2.477 with 2 degrees of freedom. P = 0.290: Not significant.

[c] Kruskal-Wallis One-Way Analysis of Variance on Ranks: H = 4.072 with 2 degrees of freedom. P = 0.131: Not significant.

[d] Kruskal-Wallis One-Way Analysis of Variance on Ranks: H = 5.656 with 2 degrees of freedom. P = 0.059: Not significant.

[e] Kruskal-Wallis One-Way Analysis of Variance on Ranks: H = 6.645 with 2 degrees of freedom. P = 0.036: Significant

[f] All Pairwise Multiple Comparison Procedures (Dunn's Method): none significant.

[g] Kruskal-Wallis One-Way Analysis of Variance on Ranks: H = 0.515 with 2 degrees of freedom. P = 0.773: Not significant.

Comparing grouped median amplitudes of leaves and petioles (normality test failed: P<0.05):

[h] Mann-Whitney Rank Sum Test: U = 4381.000 T = 8920.0 n(s)= 94 n(b)= 94. P = 0.922: Not significant.

[i] Spearman correlation amplitudes at leaves and petioles: R= 0.16932. Not significant.

Testing Amplitude <>0 (normality failed: P<0.05):

[j] Wilcoxon matched pairs test: Valid N=190, T= 7944.5, Z=1.486137. P=0.137244: Not significant.

Testing Time shift between leaves and petioles <>0 (normality failed: P<0.05):

[k] Wilcoxon matched pairs test: Valid N=46, T=448.0, Z=1.010597. P=0.312210: Not significant.

electrode in approximately 7 s, with a propagation speed of 1.42 mm s^{-1}. Amplitudes of ~-50 and -70 mV and durations of 14 and 12 s at leaf and petiole, respectively, were observed (Fig. 8). Definitively, no ultrafast burst signals are detected during the AP on the leaf and petiole (compare Figs. 1, 4 and 8).

Discussion

A brief soft touch in the middle of an adult *A. thaliana* leaf induces a depolarization electrical signal that moves away from the stimulation zone to the petiole (Figs. 3 and 8). This signal can successively be detected using appropriate electrodes on the leaf and petiole. We refer to these signals as genuine plant APs. They have the same characteristics (durations, polarity, speed of propagation) obtained in *A. thaliana* after electrical stimulation on the leaf (Favre and Degli Agosti 2007). It has recently been demonstrated that the biotic stimulation of the *A. thaliana* leaf using caterpillars generated electrophysiological signals (Wound Activated Surface Potential: WASP, see Mousavi et al. 2013, 2014). WASPs have more complicated time dynamics than the relatively simple peak presented here. Some of these signals resemble touch generated APs (see Mousavi et al. 2013, extended figure 1). Moreover, the propagation speed of APs is similar to that of other moving systemic signals (Baxter et al. 2014), including WASPs.

We observed that the APs evoked through touch did not result from the undersampling of other fast (transiently repetitive) signals. Sampling at 10, 10 000 or 100 000 Ss generated the same results (Fig 4 and 8) for touch generated APs.

However, at 100 000 Ss, the occurrence of fast electrical events was clearly distinguished from the baseline. These fast events simultaneously occurred at the leaf and petiole electrode (i.e., ≤ 10 µs time interval) at an extremely wide (and leaf-petiole uncorrelated) amplitude from -0.5 to 0.5 V, with an average of 0 V. In these experiments, the distance between these 2 electrodes was ca 1.5 cm, thus the speed of

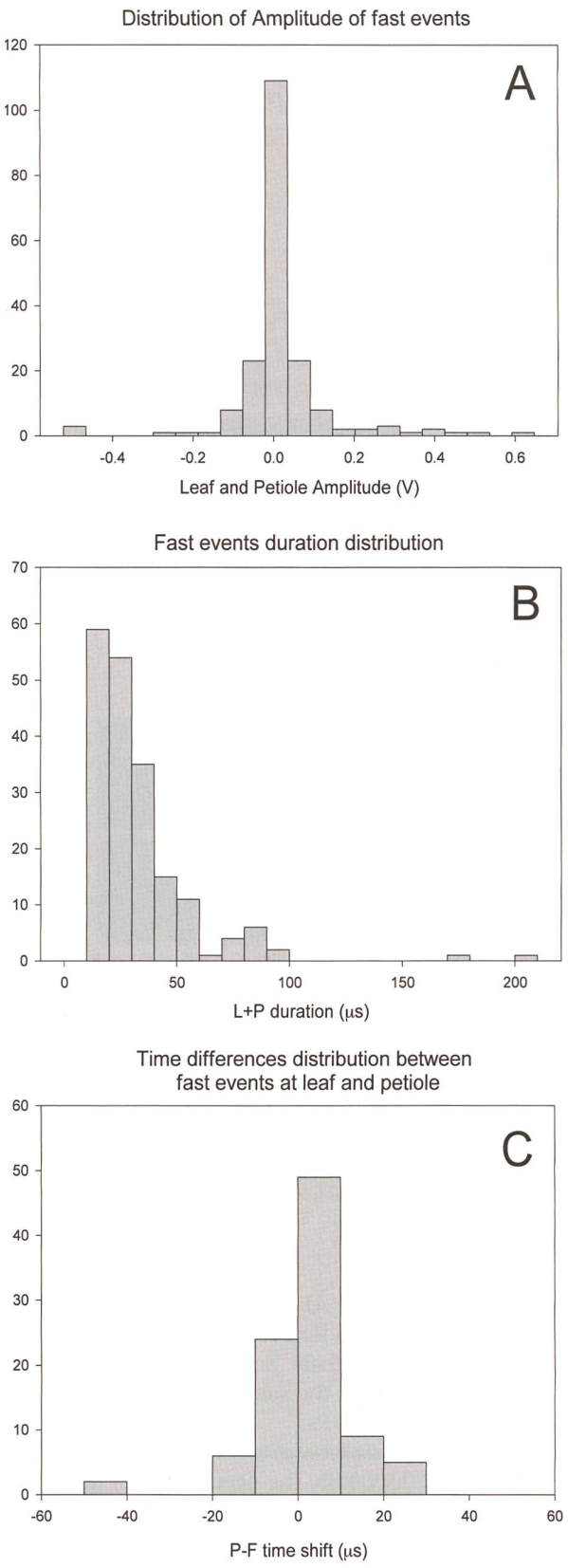

Fig. 7. Statistical distribution of overall fast event characteristics. A: Amplitudes pooled with a mean at 0. B: Durations from 10 to 210 µs. C: Mean time shift between maximum signals at the leaf and petiole electrode was 0.

transmission, occurring both acro and basipettaly, could be ≥ 1000 m s^{-1}. Notably, in animals, the fastest propagation speeds reported for APs are on the order of 10 m s^{-1} (Dale et al. 2004).

The biological origin of these fast electrical events can be challenged in *Arabidopsis*. Objects submitted to electromagnetic and/or electrostatic perturbations resemble antennas. Although the plants were maintained in a Faraday cage, some perturbations (i.e., fast intense discharge events) could reach these organisms. For example, when touching the leaf using a pencil, even when the wrist of the experimenter is grounded, perturbations were clearly visible (see Figs. 4, 8), even before contact with the hand of the experimenter on the leaf upon entering the Faraday cage. This observation suggests that interfering external signals could be introduced. Indeed, during this period, the signals were enriched at 50 Hz and harmonics, reflecting the alternative domestic power supply and not an electrophysiological mechanism inside the plant itself (Fig. 3 and 8). Other interferences (building high power switches, thermostats, electrical relays, machines, electric traffic sparks, etc.) at the origin of these fast events are thus very likely. Moreover, these events were not associated with the treatments on the plants, i.e., reference, water or 0.5 mM H_2SO_4.

We conclude that in *Arabidopsis*, concerning abiotic perturbations with KCl (Favre et al. 2001, 2011), electricity (Favre and Degli Agosti, 2007), rubbing (Favre 204), touch (Thouroude 2011, Degli Agosti 2014, and present paper) and biotic stresses (Mousavi et al. 2013), it is not necessary to use fast sampling rates. Such fast acquisition systems are data treatment, computer memory and hardware consuming.

Moreover, we show that our acquisition system (electrodes and electrometer) used in our preceding experiments isn't bandwidth limited. Clearly from the presented results the AP observed after the touch treatment is not an artifact resulting from other undersampled faster signals.

Previous studies, from Burdon-Sanderson (1888) to recent experiments, involving whole-plant electrophysiology, have reported very different signals in plants (e.g. Zimmerman and Mithöfer 2013). Yet, if we compare the progress achieved in animal and human electrophysiology knowledge, whole plant electrophysiology seems still in its infancy. In plants, the diversity in the characteristics and occurrence of electrical potentials signals is baffling, and a better classification using well described (e.g., Mousavi et al. 2014), quantitative and high throughput protocols is needed. Developing further research with

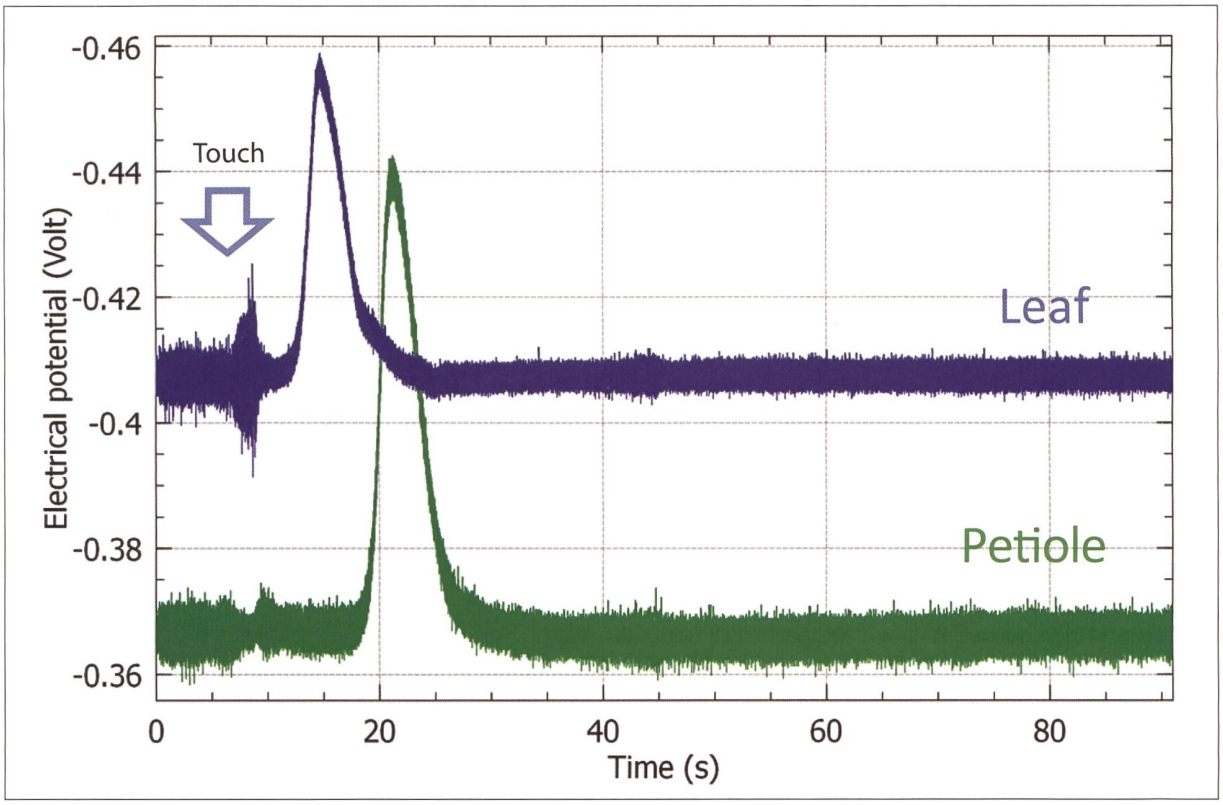

Fig. 8. Example of a genuine electrophysiological signal (plant AP) generated in Arabidopsis thaliana *after a soft touch on the leaf (blue down arrow) successively detected at the leaf and petiole electrodes. The signal was sampled at 100 000 Ss. Artifacts were introduced when the experimenter introduced his hand holding the brush in the Faraday cage and briefly touched the leaf (at time ~7-10 s).*

Arabidopsis is a unique opportunity to understand the true rule of APs and electrophysiological signals in plants.

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