

Voltage-dependent action potentials in *Arabidopsis thaliana*

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Voltage-elicited action potentials (APs) have been reproducibly obtained in *Arabidopsis thaliana* ecotype col. Excitations pulses (voltage–duration: V–t) were given in the 0- to 18-V and 0- to 35-s ranges, respectively, by two galvanically isolated Pt/Ir small wires inserted through the main vein in the distal part of the leaf. Conventional liquid junction Ag/AgCl electrodes were placed at the zone between leaf/petiole (e1) and a second one on the petiole, near the central axis of the rosette (e2). A typical hyperbolic V–t relationship was obtained. The most excitable plants did have a chronaxy of 0.1 s and a rheobase of 2 V. Although the amplitude of the APs was highly variable (range 10–80 mV), it was related neither to the intensity nor to the duration of the stimulation pulse: the phenomenon is a typical all-or-none response. The APs were moving away from the excitation zone and could successively be detected at e1 and then at e2: their propagation speed was $1.15 \pm 0.26 \text{ mm s}^{-1}$. The absolute refractory period was approximately 20 min and the relative one approximately 80 min. The reproducibility of the voltage elicitation was in *A. thaliana* col ecotype 91%, with 83% of the APs propagating from the leaf to the petiole. In the Wassilewskija ecotype, 45% of the plants were responsive, with 78% of APs transmitted (propagation speed was $0.76 \pm 0.17 \text{ mm s}^{-1}$), whereas in the Lansberg erecta ecotype none of the plant tested elicited a voltage-dependent AP.

Introduction

Action potentials (APs) are rapid and transient local changes of the resting cellular membrane potential, which can propagate over long distances. They are a widespread phenomenon in living organisms. In animals, they constitute a well-known mechanism of information circulation and coding at the intercellular and whole organism level. Though they have been documented in various plants since a long time now (Burdon-Sanderson 1873), they are neither observed as easily nor studied as well in that latter kingdom (with the noticeable exception of some algae, see Wayne 1994). The interested reader is referred to reviews in the literature

for the general AP occurrence in plants (Davies 1987, Davies et al. 1997, Fromm and Lautner 2007, Pickard 1973, Simons 1981). This situation might be because their physiological function is somehow unclear in plants and/or because their observation, with the some already mentioned exceptions, might still appear a little elusive to experimenters. Nevertheless, clear functions have been shown in some cases (see also Fromm and Lautner 2007); in the carnivorous venus flytrap, *Dionea muscipula* (Di Palma et al. 1961, Jacobson 1965), time-coded APs trigger the rapid closing of the traps leading to the capture of little animals. In sensitive plants (*Mimosa pudica*), the touch-induced APs are transmitted to distant organs that

Abbreviations – AP, action potential; L:D, light:dark; RAP, repetitive action potential; RC, resistance and capacitance; V–t, voltage–duration.

loose their turgor leading to rapid movements (Sibaoka 1962, 1969, 1991). In tomato, flame or mechanical wounding can be transmitted by APs to distant plant organs where they could trigger a systemic response associated with specific gene expression (Wildon et al. 1992). Recently, other rules for APs (see Davies 2004) have also been suggested by simultaneous electrophysiological, photosynthetic and/or photosynthetic-related activity measurements: in *Mimosa pudica* (Hartmut and Grams 2006, Koziolok et al. 2004), *Populus* sp. (Lautner et al. 2005) and *Zea mays* (Grams et al. 2007) transient decreases in carbon assimilation processes follow the AP spreading in leaves. Other electrical phenomena, slow waves potentials (see Stahlberg and Cosgrove 1997), also called variation potentials (see Davies 2004, Stanković et al. 1998), have also been observed in plants and linked to various distant physiological responses. They will not be considered here because their characteristics are very different from genuine APs.

In animal electrophysiology, the use of electrical pulses to elicit APs is a standard and proven method. It allows reproducible and detailed quantification of both stimulations and organisms' responses. Comparatively to animals, very few investigations in plants have been fully performed with this method (see also Table 1). In this respect, Paszewski and Zawadzki (1973, 1974) have clearly shown that the electrical stimulation of *Lupinus* shoots produced APs with similarities to those occurring in nerves. It should be noted, however, that propagation velocities and other dynamical parameters are largely slower than in animals.

In the context of electrical excitations, the relevant parameters are as follows: the typical hyperbolic relation

between the strength intensity (voltage) and duration (time) of the pulse. This relation can be described by, first, the rheobase – the minimal strength of an electrical stimulus of indefinite duration that is able to elicit an AP – expressed in volts. It is commonly simplified by the concept of voltage threshold – the minimum voltage intensity, at a defined duration that triggers an AP. Second, the chronaxy, a dynamical parameter – the shortest duration of an effective electrical stimulus, at intensity equal to twice the rheobase – expressed in plants in seconds. The choice of an inferior value of either intensity or duration of the pulse applied to a plant will result in no AP response – the well known 'all-or-nothing' law also present in plant electrophysiology (Paszewski and Zawadzki 1973, 1974). Other important characteristics are the duration, amplitude, speed of propagation of the elicited APs and the refractory period – the time required for membrane recovery after the first AP, necessary before a subsequent AP, can be triggered. This later property, together with autoexcitation, is important as it will determine the first direction of displacement in conducting cells or tissues.

In *Arabidopsis*, AP elicitation by locally applying chemicals (KCl) (repetitive APs, i.e. RAPs, Favre et al. 2001), cold shocks (Krol et al. 2004, Lewis et al. 1997) and pulses of blue light (Lewis et al. 1997, Spalding 2000) have been described. Considering the wide interest in this model plant and the fact that a large set of voltage-gated ion channels have been identified in *Arabidopsis* (e.g. Krol and Trebacz 2000, Véry and Sentenac 2002), we further examined whether genuine APs could also be reproducibly elicited in this plant with classical electrical stimulation protocols as in animal nerves.

Table 1. Some characteristics of electrically induced APs in plants. Unknown values are marked by a question mark. ^aDziubinska et al. (1983), ^bZawadzki and Trebacz (1985), ^cPaszewski and Zawadzki (1974), ^dPaszewski and Zawadzki (1976).

Organism	Absolute/relative refractory period (min)	Propagation velocity (mm s ⁻¹)	References
<i>Chara corallina</i> (algae)	0.033–0.17/0.18–1.5	10–40	Johnson et al. (2002)
<i>Nitella translucens</i> (algae)	?	1.4, 5.6	Auger (1939)
<i>Conocephalum conicum</i> (liverwort)	1.5/3.5 ^a	0.9 ± 0.3 ^b	Dziubinska et al. (1983), Zawadzki and Trebacz (1985)
<i>Arabidopsis thaliana</i> col	20/80	1.15 ± 0.26	This study
<i>Arabidopsis thaliana</i> Ws	?	0.76 ± 0.17	This study
<i>Helianthus annuus</i>	10/20–300	Approximately 1.4	Zawadzki et al. (1991)
<i>Luffa cylindrica</i>	2/2–5	179–213	Shiina and Tazawa (1986)
<i>Lupinus angustifolius</i>	12/12–90 ^c	Approximately 1 ^d	Paszewski and Zawadzki (1974), Paszewski and Zawadzki (1976)
<i>Lycopersicon esculentum</i>	?	2 ± 0.2	Rhodes et al. (1996)
<i>Mimosa pudica</i>	0.5–3.3/?	3–50	Sibaoka (1969)
<i>Salix viminalis</i>	2/?	20	Fromm and Spanswick (1993)
<i>Sorghum bicolor</i>	?	270 000 ± 20 000	Mishra et al. (2001)
<i>Zea mays</i>	0.83/?	30–50	Fromm and Bauer (1994)

Materials and methods

Plant material

Arabidopsis thaliana (L.) Heynh cv. Columbia (col), Landsberg erecta (Ler) or Wassilewskija (Ws) seedlings were grown in a potting compost under light:dark (L:D 12:12 h, Sylvania 36 W Luxline-Plus, $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) photoperiod for 2 weeks after sowing. They were then individually transplanted into a new pot and cultivated in the same conditions except the photoperiod (L:D 8:16 h). When 42-days-old plants were transferred to a thermo- and hygro-regulated room ($22 \pm 1^\circ\text{C}$ and $73 \pm 2\%$ rH) under L:D (8:16 h, Sylvania 36 W Luxline-Plus and Gro-Lux, $48 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR) for 1–2 weeks. They were then installed in a climatized chamber within a Faraday's cage, under L:D (12:12 h, Sylvania, 18 W Luxline-Plus, $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ PAR), and stimulation electrodes were carefully positioned (see Fig. 1A). Experiments started ≥ 3 h after installation for a maximum of 4 days. Selected plants had approximately 27 leaves without floral structures and experiments were conducted on one leaf approximately 72 mm long (including petiole) and approximately 15–20 mm wide.

Whenever not specifically mentioned, the ecotype of plant used is of the col type.

Method of electrical measurement and stimulation

The characteristics of measuring and reference electrodes, electrometer (impedance: $10^{15} \Omega$) and A/D D/A card have been described in a previous paper (Favre et al. 2001). The plants, the installation and the electrical stimulation systems are shown in Fig. 1. The electrical potential recorded is the difference between a measuring (e1 or e2) and the reference electrode with respect to the electrical earth. Both electrodes are from the Ag/AgCl type with the chloridized wires immersed in an electrophysiological solution (1 mM KCl, 0.1 mM CaCl_2 , 50 mM sorbitol, 2 mM 2-(*N*-morpholino)ethanesulfonic acid/Tris, pH 6.8). The user interface has been developed with LABTECH NOTEBOOK software (v. 8.02, Laboratory Technologies Corp., Wilmington, MA). The original sampling rate was 20–40 Hz, and readings were averaged to result in 4–20 Hz acquired data.

The electrical stimulation was applied with two electrodes (Pt/Ir, \varnothing 0.102 mm, World Precision Instruments Inc., Sarasota, FL), inserted through the main leaf

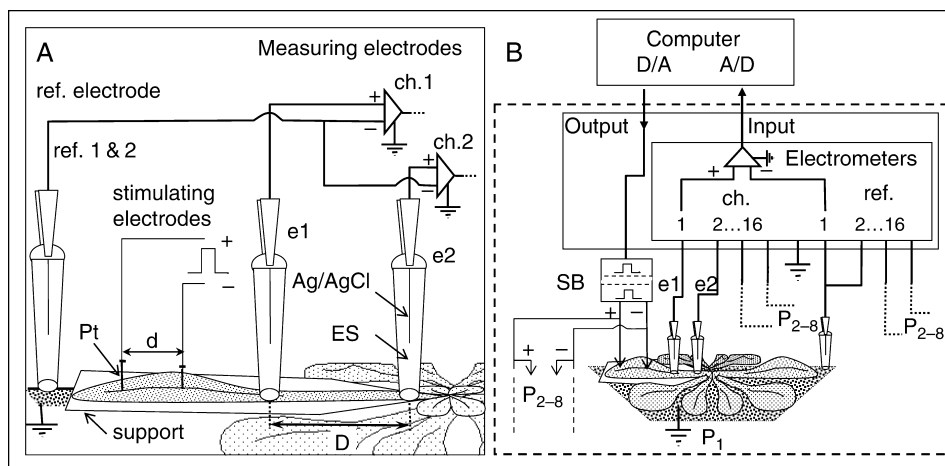


Fig. 1. Setup of the measuring and electrical stimulation system. (A) The examined leaf is on a plastic support. A first measuring electrode (e1) is located on the zone between the leaf and petiole (channel 1: ch. 1). The second is near the center of the rosette on the petiole (e2; channel 2: ch. 2). They are symmetrical to the reference electrode (ref.: reference; ref. 1 and 2), which is located in the potting compost of the plant. The distance (D) between e1 and e2 is determined for each studied leaf as indicated. The extracellular electrodes are made up with a piece of cotton forced at the small end of a sectioned plastic tube (contact surface with plant is approximately 7 mm^2); filled with the electrophysiological solution (ES) in which an Ag/AgCl wire is immersed. Stimulation electrodes are Pt/Ir wires (Pt; +: anode; -: cathode) inserted through the midrib of the leaf and separated by a distance of $d = 15 \text{ mm}$. (B) General setup. All the system is in a Faraday cage (discontinuous square), except for A/D D/A acquisition card plugged into the computer. Sixteen integrated instrument amplifier circuits allow measurement of the electrical potential between the measurement and reference electrodes with respect to the electrical earth (ground symbol). Each measuring and its reference electrode (ch. 1–16 and ref. 1–16) from plants 1 to 8 (P_{1-8}) are connected to their differential electrometer (e.g. ch. 1 and ref. 1), which sends the signal to the appropriate channel of the A/D card (input). Electrical stimulation consisted of sending defined voltage pulses from the D/A card (output) to the optocoupled stimulation box (SB), which converts it in a galvanically isolated desired voltage supplied by five serially connected 9-V power batteries. The stimulation was simultaneously applied on the leaves of eight plants [dotted line: connection with the others plants (P_{2-8}) as for the model plant (P_1)].

vein. The anode was spaced by 15 ± 0.6 mm to the cathode as indicated in Fig. 1. The optocoupled electrical stimulation box (galvanic isolation, Fig. 1) was slightly modified from that originally described by Favre et al. (1998) to send voltages only in the range of 0–20 V, whose timing, duration and voltage were computer controlled. For *A. thaliana* col ecotypes, numerous combinations of (voltage–duration) V–t were used (V: 0–18 V; t: 0.05–37 s). For the Ws and Ler ecotypes, the following V–t have been probed: at t of 0.5 s, V = 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 18 V; at V = 9 V, t = 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4.0, 4.5, 5 s; at V = 15 V, t = 0.25, 0.5, 0.75, 1, 1.25, 1.5 s; and at V = 18 V, t = 0.25, 0.5, 0.75 and 1 s. These 30 V–t combinations were applied on 24 and 8 different Ws and Ler plants, respectively. When a leaf was stimulated more than once, treatments were separated with a time interval of at least 2 h. An exception to this was when determining the refractory period. In the case of subthreshold stimulations (no AP elicited), an interval of a few minutes could be sufficient, but 1 h has been preferred. The current passing through the leaf was measured with a voltmeter (34401A multimeter, Hewlett Packard, Les Ulis, France) connected to the electrodes of

stimulation, and an amperometer (2000 multimeter, Keithley) serially connected to the stimulation cables.

Statistical analyses were done with STATISTICA (1998 edition, Statsoft (Europe), Hamburg, Germany).

Results

The application of a subthreshold voltage to the *Arabidopsis* (col ecotype) leaf created an immediate artifact of stimulation only (Fig. 2A, 3 V). However, an overthreshold stimuli (Fig. 2A, 6–18 V, 0.25 s) created an artifact of stimulation followed, 7.96 s (range: 5.3–12.7 s) later, by genuine APs. Their duration was 14 s (9.5–18.3 s), with an amplitude of 37.1 mV (25–65 mV). It is important to mention here that as recordings are extracellular, the amplitudes should be interpreted in terms of their absolute voltage excursions only. At first detected on the distal electrode (leaf: e1), these APs were later (mean: 22.2; 17.8–31.1 s) also detected on the proximal one (petiole: e2). They propagated from the stimulus area via the petiole to the central axis of the rosette with velocities ranging from 0.8 to 1.9 mm s⁻¹ (mean: 1.2 mm s⁻¹). Their duration was also of 15.3 s

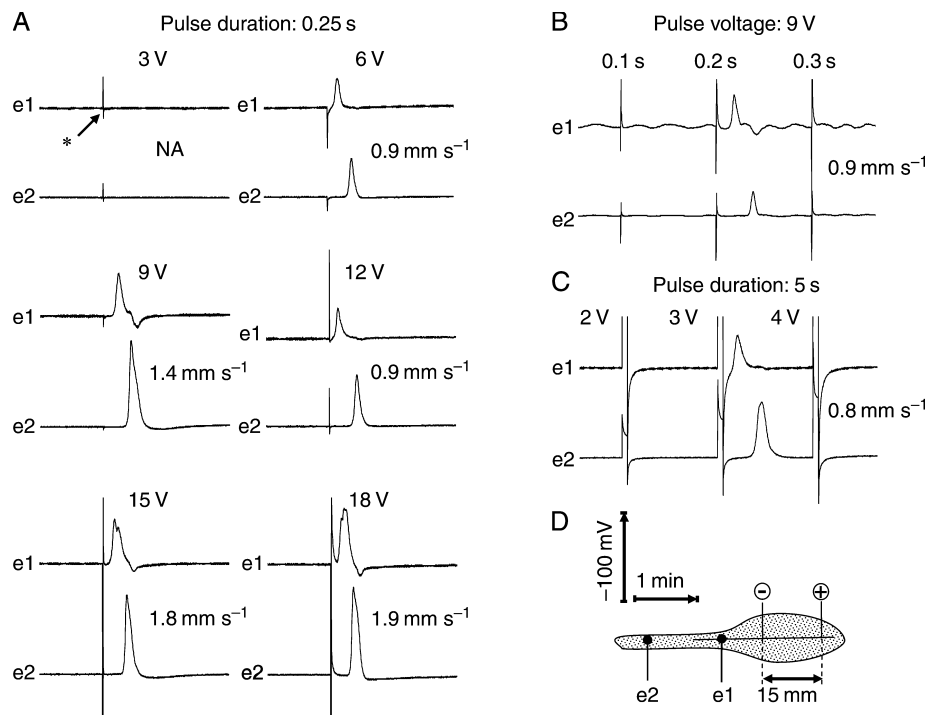


Fig. 2. Examples of typical bioelectrical responses obtained when an electrical pulse is applied to an *Arabidopsis thaliana* (col) leaf. Recordings are presented by pair, with the upper curve representing the distal part of the petiole/beginning of the leaf (e1), and the lower curve the proximal part of the petiole (e2). AP propagation velocity is indicated between the respective curves. (A) Bioelectrical responses recorded with a pulse of 0.25 s with different voltages. *Artifact of stimulation. NA: velocity cannot be calculated. (B) Successive stimulations with 9 V and increasing durations given with 1 min 30 s interval. (C) Successive stimulations of 5 s and increasing voltages. (D): V–t scales and diagram of excitation (+; –) and measuring (e1; e2) electrodes.

(10.0–20.1 s), but with higher amplitude (mean: 63.3; 24.3–98.0 mV). Fig. 2B shows that a short voltage pulse of 9 V during 0.1 s was not sufficient to trigger an AP, whereas 1 min 30 s later, a pulse of the same voltage but applied instead during 0.2 s triggered a propagated AP. The concept of the refractory period can also be illustrated with this experiment because no AP is triggered after 1 min 30 s when a stimulation of the same voltage during 0.3 s is applied (artifact only). The voltage threshold property is observed here (see Fig. 2C) between 2 and 3 V. Indeed, no AP was induced at 2 V (5 s), whereas on the same leaf, 3 V (5 s) triggered a propagated one. Thereafter, stimulating the leaf with 4 V (5 s) was unsuccessful because, again, of the refractory period.

The study of 72 plants (of which only 5 did not respond) submitted to pulses of different voltage intensities and durations is presented as a map of AP-elicited population response efficiency in Fig. 3A. A total of 211 APs were obtained, with 173 propagating from e1 to e2. The greater the strength of the stimulus, the more the proportion of plants responded with a genuine AP. With high V–t stimulations, the proportion attained a maximum of 80%, whereas it was $\leq 20\%$ at low V–t. With low voltages, we observed that some plants still reacted well to a 2.5-V pulse during 1.5 s. However, only one AP out of 16 plants

was detected at 2 V, 13.5 s (Fig. 3A). The rheobase/threshold voltage could thus be roughly estimated visually at approximately 2 V, corresponding in our situation, to a current intensity of $0.5 \pm 0.3 \mu\text{A}$, measured with eight independent plants.

To determine the chronaxy, other experiments were carried out on a total of 32 (including 9 non-responding) plants, the aim being to estimate the minimum electrical pulse duration to trigger an AP at a voltage higher, but near the approximately 2-V threshold and to zoom in the region of 0- to 1.2-s and 0- to 9-V stimulation. The empty dots (Fig. 3B) represent the threshold of excitation in individual plants. The strength–duration relation observed to trigger an AP in a single axon has been modeled with the equation described by Aidley (1971), which has also been applied to excitable plants by Zawadzki (1979). It considers the conductive tissue as a resistance and capacitance (RC) electrical equivalent circuit (see formula in Fig. 3B). We fitted this model to the points estimated to be at the frontier between elicited and non-elicited APs (see Fig. 3B double-circled empty points). The fitted parameters were V_{rh} (rheobase) = $2.54 \pm 0.30 \text{ V}$ and $RC = -0.14 \pm 0.03 \text{ s}^{-1}$ (r^2 was 0.69). The chronaxy $t_{\text{ch}} = -\ln(2) \times RC = 0.10 \text{ s}$. This is true for the most excitable plants. To obtain the chronaxy

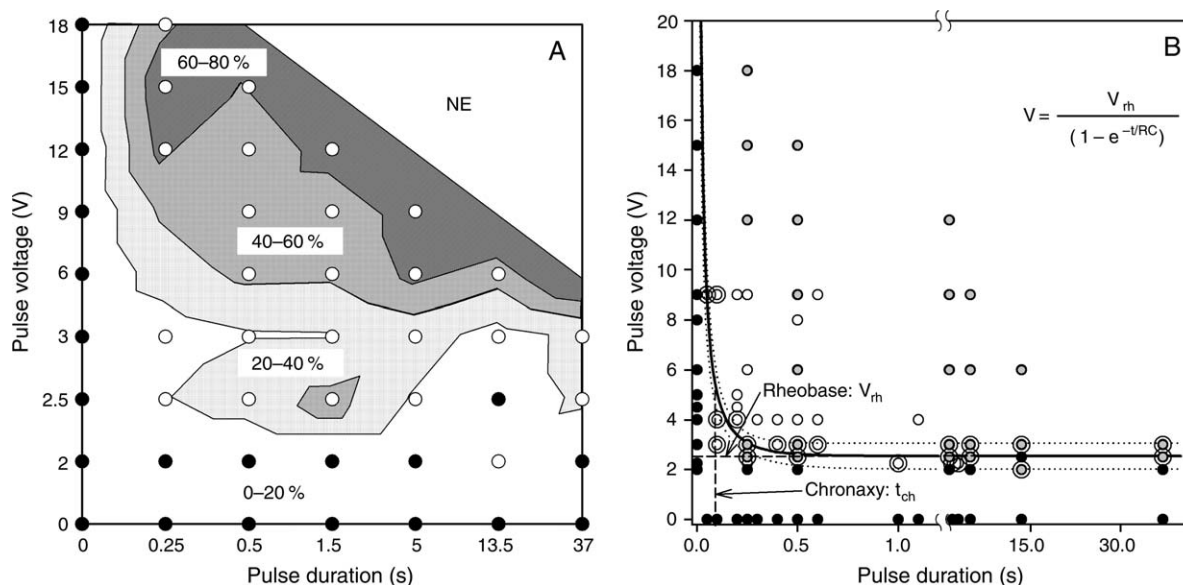


Fig. 3. Determination of the rheobase and the chronaxy in a population of *Arabidopsis thaliana* (col). (A) Map of AP responses efficiency (%) induced by electric stimulation on the leaf. Each circle (with exception of $V = 0 \text{ V}$ and $t = 0 \text{ s}$) represents 14–24 plants. The white circle means that at least one AP was triggered; the black one that no AP was detected. Numbers (per cent) define the range of response efficiency in the corresponding area (extrapolated values from point values). The efficiency (%) is the number of positive plant responses divided by the total number of plants experimented on at this point. The upper right area is not experimented (NE). Scales are not respected to enlarge the visualization of the response. (B) Determination of the chronaxy and rheobase in *A. thaliana*. The equation of Aidley (1971) shown in the figure was fitted to frontier measurements data (double-circled dots) selected between responding (empty or light grey dots) and non-responding plants (filled black dots) to different V–t pulses. Small dotted lines are the confidence interval ($\pm 90\%$) of the fitted model. Light grey points are data from (A).

and rheobase such as approximately 50% of plants in a population will respond ($V_{rh50\%}$ and $t_{ch50\%}$), it is possible, by visual inspection of the Fig. 3A, to roughly estimate $V_{rh50\%}$ approximately 5 V and $t_{ch50\%}$ approximately 0.25 s.

We examined the dependence of the AP amplitudes (Z), recorded on electrodes e1 and e2, to the stimulation voltage intensities (V) and durations (t) with the equation of a plane; $Z = a + b \times V + c \times t$, using the results of plants from Fig. 3A. The fitting to $n = 211$ APs recorded on e1 (leaf) yielded $a = 23.3$ mV (with a Student's t -test value of 10.75, highly significant: $P > 0.000001$); $b = -0.01$ ($t = -0.10$, non-significant: $P = 0.92$); $c = -0.17$ ($t = -0.84$, non-significant: $P > 0.40$). The fitting to $n = 173$ APs recorded on e2 (petiole) yielded $a = 53.5$ mV (with a Student's t -test value of 11.54, highly significant: $P > 0.000001$); $b = -0.48$ ($t = -1.71$, non-significant: $P > 0.09$); $c = -0.26$ ($t = -0.61$, non-significant: $P > 0.55$).

The refractory period was evaluated by giving double electrical time-separated stimulations. As a result of the variability of the individual plant thresholds (see Fig. 3B), experiments were realized as follows: a first stimulation during 0.5 s and 6 V or, if this was unsuccessful, with 9 V was applied. After the first AP was obtained, at varying time intervals, a second stimulation with the same $V-t$ characteristics was given. A total of 88 plants (9 non-responding) elicited 353 APs, of which 293 did propagate from e1 to e2. In each situation, the number of plants tested was from a minimum of 16 up to 40. Fig. 4 shows ratios of both e2 amplitude and propagation velocities of the second to the first AP, when the second AP could be elicited. Because this was not always the case, we also present in Fig. 4 the recovery efficiency (per cent number of 2nd AP elicited at given intervals). A time interval of 80–100 min is necessary to obtain an 80% recovery of the initial AP amplitude. Moreover, to determine the absolute refractory period (i.e. the minimum interval at which no 2nd AP could be elicited), we did a further experiment: after a first AP (0.5 s, 6–9 V) a second stimulation with higher $V-t$ values (5 s, 9 V) was given at intervals of 10, 20, 30 and 40 min after the first AP. The percentage of efficiency was, respectively, of 0, 3, 11 and 20%. This allows the determination of an absolute refractory period of approximately 20 min.

To better characterize the phenomenon we pooled the data of experiments shown in Figs. 3A and 4 because for Fig. 3B, measurements at e2 were missing. With this subset grouping, 160 *A. thaliana* col plants of which 14 did not respond (9%) have been analyzed. On the 564 APs elicited, 466 (83%) did propagate from e1 to e2. Their mean propagation speed from leaves to petioles was 1.15 ± 0.26 mm s⁻¹. The correlation between the

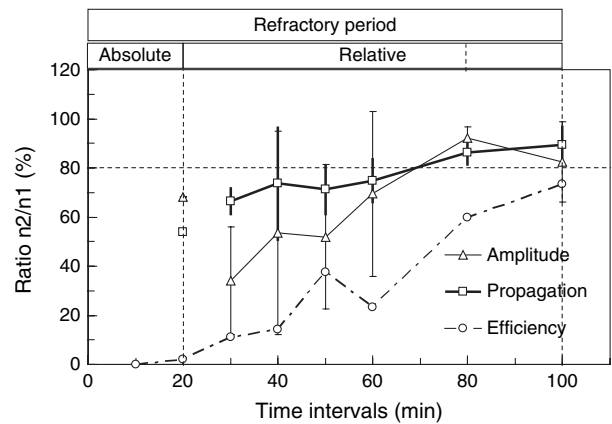


Fig. 4. Refractory period of electrically elicited APs in *Arabidopsis thaliana* (col). Data are represented as the ratios of AP amplitude (triangles), propagation speed (squares) and efficiency (circles) of second to first bioelectrical response ($n2/n1$) induced by two pulses with the same $V-t$ electrical characteristics, but given at variable separated time intervals. No AP was observed when intervals were shorter than 20 min, and only 1/49 was obtained at 20 min (unlinked dots on plot at 20 min). The absolute refractory period was 20 min. The relative refractory period was estimated at 80 min, which was defined as when the ratios (second to first response) of the parameters reached 80% (horizontal dotted line).

amplitudes at the leaf (e1), with their corresponding propagation velocities is shown in Fig. 5. The linear regression was highly significant (Pearson correlation coefficient test; $r = 0.50$, P approximately 0, $n = 466$), with intercept and slope, respectively, 0.87 ± 0.03 mm s⁻¹ and $9.9 \cdot 10^{-3} \pm 7.9 \cdot 10^{-4}$ mm s⁻¹ mV⁻¹.

For the Ws ecotypes, results differed markedly in the general occurrence of APs and their transmission. Indeed, in 24 plants, only 11 (45%) did respond with an AP. On

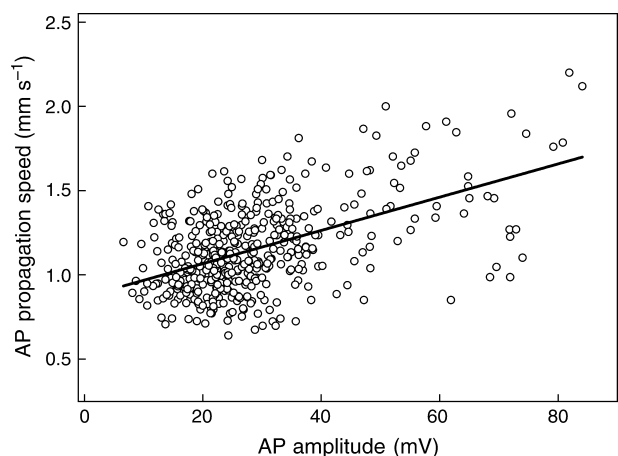


Fig. 5. Correlation plot of the AP propagation velocities vs AP amplitudes (leaf) with its linear significant regression slope.

the 23 APs thus obtained, 18 (78%) did not propagate from the leaf to the petiole. Their characteristics were similar to those of the *col* ecotype. Indeed, durations of the AP were 17.85 ± 5.40 s and 23.61 ± 5.42 s for the leaf and petiole, respectively. Similarly, the amplitudes were 23.46 ± 5.40 mV and 35.21 ± 19.94 mV. Finally, the transmission speed was of 0.76 ± 0.17 mm s⁻¹. Concerning *A. thaliana* Ler ecotypes, none of the plants exhibited any AP with the different V-t tested (see Materials and methods).

Discussion

When an electrical pulse is applied to an *A. thaliana* (*col*) leaf, a subsequent electrical response is elicited by the plant, which moves away from the treatment zone. We observed typically a depolarization followed by a repolarization returning toward the initial voltage (Fig. 2). The duration of that phenomenon is from 10 to 20 s, with amplitude ranging from 5 to approximately 100 mV (Figs. 2 and 5). These results are comparable with the very few electrophysiological measurements of AP made on *A. thaliana* by Spalding (2000) and Krol et al. (2004). Its propagation speed is approximately 1.2 mm s⁻¹, which compares also well with the majority of velocities in other plants (Table 1). Together with the other typical characteristics discussed later, strength-duration response (Fig. 3), chronaxy (Fig. 3B), threshold (Fig. 3), refractory period (Fig. 4) and all-or-none response, there is almost no doubt that we observed genuine voltage-dependent APs in *Arabidopsis* plants.

The electrical stimulation follows the strength-duration relation (Fig. 3B) giving clues on the dynamical scale of the underlying molecular components that should respond at least in the subsecond time scale. Relatively rapid signals could thus be potentially generated and transmitted, which could be somehow linked to regulatory mechanisms for whole plant and cell integration activities. The chronaxy value (time duration to trigger an AP at twice the rheobase) was between 0.1 and 0.25 s. Together with the low threshold value of 2 V, this shows the high electrical excitability of leaf cells in this *A. thaliana* accession.

The threshold value of approximately 2 V we found in *Arabidopsis* leaves (Fig. 3) is consistent with that observed in the stem of *Lupinus angustifolius* (Paszewski and Zawadzki 1973), *Helianthus annuus* (Zawadzki et al. 1991), *Salix viminalis* (Fromm and Spanswick 1993) and the leaves of *Zea mays* (Fromm and Bauer 1994). See also Table 1 for comparative plant AP properties.

Although the APs amplitude was highly variable, this parameter was clearly independent on the stimulation voltage and duration. Indeed, no correlation was found

between AP amplitude and neither voltage intensity nor its duration. This shows that either the AP is elicited or not, provided the stimulation is higher than the threshold. That illustrates the all-or-nothing property of voltage-dependent APs in *A. thaliana*. The amplitude variability could be in part attributed to the extracellular method of biopotential measurement. In fact, only the proportion of responding plants in the population increased with an increase in V-t pulse intensity (Fig. 3). This suggests that individual electrical threshold values of different plants (leaves/tissues?) might be different.

In order for APs to move in a unique direction away from the excitation zone, APs must be followed locally by a transient refractory period [i.e. lack of cell ability to immediately (re)elicit an AP]. This has been clearly observed in our experiments by giving two varying time-separated electrical pulses (Fig. 4). As in animal nerves (e.g. Carpenter 1984) and plants (Paszewski and Zawadzki 1976), the AP amplitude of *A. thaliana* during the relative refractory period had a lower value than the first induced APs (see Fig. 4). The return to the original state is conventionally determined when the characteristics of APs have reached 70–90% of their original value, which is for *Arabidopsis* plants after 80–100 min. To guarantee complete non-interference with the previous stimulation, a 2- to 3-h time lag would thus be necessary between two electrical stimulations. The *Arabidopsis* refractory period was in the range of that of *L. angustifolius* and *H. annuus* (3–5 h; Zawadzki et al. 1991) but was much longer than that of the liverwort *Conocephalum* and the algae *Chara* (see Table 1). Our results confirm the relative long refractory period generally found in higher plants. In a previous paper, we described the elicitation of RAPs after wounding and subsequent application of a KCl (>0.25 M) microdrop (Favre et al. 2001) with the *c24* *A. thaliana* ecotype. The range of amplitudes was from 10 to 100 mV, with durations of 20–60 s. Propagation speed was approximately 0.59 mm s⁻¹. The amplitudes of the RAPs were decreasing with respect of the first events (see Favre et al. 2001; Figs. 4–6). Moreover, the number of APs on a given interval of time was ≤ 3 to 5 AP h⁻¹, i.e. they are in average separated by >20 to 12 min (minimum). All these results are comparable with those presented here.

The APs propagation speed is significantly amplified along the propagation pathway, depending on the AP amplitude at the leaf/petiole site (Fig. 5). This phenomenon suggests the involvement of a positive feedback (chain reaction) sensitive to voltage depolarization along the conducting tissues. The autopropagated electrochemical waves could be longitudinally and transversally sustained and amplified by the all-or-nothing phenomenon to allow a chain reaction of cells triggering APs. This is possible with the membrane AP local elicitation

effect by the electrochemical field associated with the number of excitable cells, their sensitivity and their proximity from each other (e.g. triggering of nearby membrane or cell K^+ or/and Ca^{2+} voltage-gated channels). In this respect, this result can be interpreted as Sibaoka already did in 1969 that the propagation velocity is dependent on the number of excited cells, which in turn can induce APs in more neighboring excitable cells to propagate the electrochemical wave.

Higher plant AP mechanism implies successive events of ion fluxes with cation and anion channels activation/inactivation, which might be initiated by Ca^{2+} (see Pyatygin et al. 1999, Trebacz et al. 1994). To elicit a voltage-dependent AP that can propagate, the presence of responsive ionic voltage-gated channels is necessary (i.e. Ca^{2+} , Cl^- , K^+). Of particular interest to plant AP are the Cl^- channels. Indeed, these channels produce a bistable I/V relation similar to the Na^+ channels in animals and are thus the best candidates for making the plant membrane excitable (Gradmann et al. 1993, Thiel et al. 1992). Recent and significant progress has been made in the identification, isolation and characterization of voltage-dependent gated ion channels in *Arabidopsis*. The K^+ voltage dependent (Kv) group is clearly present (see Michard et al. 2005, and references cited therein). This is also true for Cl^- voltage (Hechenberger et al. 1996 and review by Barbier-Brygoo et al. 2000) and Ca^{2+} (AtTCP1; Furuichi et al. 2001, Peiter et al. 2005, Ping et al. 1992). Clearly, it is not a surprise that the application of an electrical stimulation can be successful. Actually, it is more difficult to explain why plants do not always respond. However, this is clearly the case, as our present work did show it. Indeed, in some col ecotypes (10%), we were never able to elicit responses. This was even more marked in the Ws plants (55%), and finally worse (although the limited number of plant may restrict a little our statement) in Ler (100%) where we were not able at all to obtain any voltage-dependent AP. In the present state of knowledge, we have no explanations for these observations, but it is clear that we might suggest the existence of at least a genetic basis that differs between these accession lines. It would thus be of general interest in plant electrophysiology to determine whether those small number of non-reactive col plants (and also Ws and Ler) are in a state of a physiological 'refractory'-like period or whether there exists some missing molecular link (e.g. restricted or silenced voltage-dependent protein channel expression) in that particular plants.

The advantage of studying APs in a model plant like *Arabidopsis* is that its genome sequence is known, many techniques are now used to assign genes to physiological functions and detect whether particular transcripts are present in a given ecotype or situation. In this respect, our

study on electrically induced APs by a non-invasive extracellular method unambiguously shows the existence of genuine APs in *Arabidopsis*. It may open some perspectives to understand at different integration levels the functions and mechanisms of membrane excitation in plants.

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