Spatial and temporal distribution of magnetic field due to injury currents in Vicia faba plants

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1 Introduction

Different types of wounds on plants cause measureable changes in the biochemical and biophysical behaviour of plants. Among them are also electrophysiological changes. Studies using extracellular and intracellular electrodes have revealed that wounding of tissue causes in a variety of plants changes in the electrical membrane voltage e.g. [1, 2, 3, 4]. Typically, the electrical response consists of a rapid action potential-like depolarization followed by a slower long lasting depolarization usually termed the variation potential. The elementary basis of these transient voltage changes is not yet known. It has been speculated, that the fast transient depolarization is an action potential and is therefore propagated - just as in nerve cells - as a true long distance electrical signal [2]. The slow voltage transient on the other hand might be the consequence of chemical signals which are distributed via the xylem [5]. Wounding induced voltage changes are transmitted from the site of wounding along the plant with a velocity of less than 1 cm/s [1, 3] and reach the remote tissue before the systemic molecular responses are initiated in this tissue.

We can add to these statements: Electric potential difference (voltage) in conducting living tissue is connected with ionic currents, which can also be detected magnetically as follows from the Ampere's or Biot-Savart's law. As a pathway for propagation of the electrical signal, the low resistance electrical continuum of the sieve-tube element/companion cell complex, or of the entire vein including the apoplast is suggested [2].

To further elucidate the mechanism of electrically-based signaling, biomagnetic measurements of electrical activity in bean plants Vicia faba were conducted over the entire plant. The SQUID sensor is sensitive enough to detect very low quasi-static biomagnetic field. The currents causing the measured biomagnetic field should be closed within a large volume. This was achieved by immersing the whole plant in a suitable ionic solution. This method has been used in the past to monitor electrical activity in animal cells, which also respond to injury with electrical activity [6, 7]. The measurements aim to provide information on electrical propagation, injury-induced currents and source of currents in wound stimulated tissue.

2 Methods

Measurements were performed on 21-23 days old Vicia faba (cv Hangdown). Plants were grown in garden peat in a green house. Two types of wound were applied: (i) Serious wound - a large cut on the stem. (ii) Small wound induced by local burning: Approximately 2 cm^2 of a leaf were exposed to the flame of a burning candle.

Electric measurements of the membrane potential of vascular leaf cells were conducted with conventional intracellular electrodes on some plants and are described in details elsewhere [8].

Magnetic measurements were performed using 49 low noise first order SQUID gradiometers (70 mm baseline) covering a planar area of 210 mm diameter (Drung 1995), operated inside a conventional shielded room (Vakuumschmelze Ak 3b) in the Klinikum Benjamin Franklin, Berlin. The analog magnetic field signals were bandpass filtered from 0.16 Hz to 64 Hz and sampled at a rate of 250 Hz. Only the z-component of the magnetic field was measured. Since the expected injury currents are in the frequency range below 0.1 Hz, a mechanical modulation of the measured plant was introduced. The measured plant was installed on a hydraulically driven moving table [9] that was oscillating sinusoidally with a peak-to-peak amplitude 4.4 cm and a frequency 0.7 Hz. Part of measured plant was immersed in a square Plexiglas chamber (inner dimensions 17 cm $\times$ 17 cm $\times$ 2 cm) containing standard recording solution [1 mM KCl, 0.5 mM CaCl2, 0.5 mM NaCl and 0.5 mM Mes/KOH pH 6.1] (see Fig. 1). We also tested a high salt solution (100 mM NaCl, 2 mM KCl, 1.8 mM CaCl2) as recording solution with practically the same results. That was to be expected since the recording solution is used to spread the return current over a larger volume. Duration of each measurement was 20-25 min. An HP workstation was used for data acquisition.

The modulation of the distance between the source and detector transforms the dc magnetic field to the modulation frequency. The covariance method was applied for extracting the the dc-component of the modulated magnetic field data. This method is based on the calculation of covariance between the modulated magnetic field $B_k(t)$, recorded by the k-th channel and the modulation signal $m(t)$, which describes positions of the sample recorded by a potenciometer attached to the moving table. The dc-amplitude A_k for each channel is calculated from the the folowing equation [9]

$$A_k = \sqrt{\frac{2}{N}} \frac{\sum_{i=1}^{N} (B^k(t_i) - \bar{B^k})(m_i - \bar{m})}{\sqrt{\sum_{i=1}^{N} (m(t_i) - \bar{m})^2}} \ , \qquad (1)$$

where $\bar{B^k}$ and $\bar{m}$ are the mean values of B^k and m in the given time interval t_i ($i = 1, 2, ..., N$). The only requirement for using Eq. 1 is that the time interval used in this calculation is equal to the multiple of modulation period. We have chosen to calculate A_k within 7 periods of modulation, i.e. within 10 s, which means $N = 2500$. To reconstruct the time evolution of a dc response, we applied Eq.1 sequentialy in time steps of 1 s over the whole measurement time. The time evolution of A_k can be equivalently reconstructed with the short time Fourier transform method [10]. We applied this method in our earlier work [8] where it was shown by a careful simulation study as well as preliminary electric and magnetic measurements, that extremely slow events which accompany plant injury can be registered. Beside the amplitude at the modulation frequency, the Fourier transform reveals information about the measured signal at other frequencies, for example characteristics of low frequency noise and amplitudes at higher harmonics of the modulation frequency. However, only the amplitude at the modulation frequency reflects the amplitude of the modulated quasi-dc signal. In the present study we prefer to use the covariance method for calculation of A_k because Eq. 1 is mathematically simpler and therefore much faster than the short time Fourier transform method.

Two equivalent current source models - the current dipole (CD) and the linear current source(LS) [11] - were applied for modeling the cases where a clear dipolar isofield pattern was evident. Standard Levenberg-Marquardt least square method was used.

3 Results and Discussion

Altogether 8 plants were investigated. Several measurements were performed on each plant: some trial recordings without injury and one or two measurements with injury. The most illustrative examples are shown in Fig. 1 where the results of measurements are presented as a time evolution of the signal in all channels. The dotted silhouette of a given plant is superimposed on each of these graphs. Before making plots, additional boxcar averaging on 25 s intervals was applied for further smoothing. In Fig. 1a the time evolution of a plant response before introducing a large wound is shown in all 49 channels. Under these conditions, a small activity in the area above the plant's leaves can be noticed. Fig. 2a shows the characteristic isofield map for this case. There is no indication of symmetry between the positive and negative isofield patterns, which is typical for a dipolar isofield pattern. On the contrary, there is a dramatic change after cutting the plant's stem and removing the top part. A clear symmetric dipolar-like isofield pattern indicates an active localizable current source (Fig. 2b). From the time evolution of the signal in Figs. 1b and 3 an exponential decay of electrophysiological activity is clearly noticeable. Fig. 1c illustrates the measurement with a burning injury. After the burning stimulation on one of the younger leaves, both the signal (Figs. 1c, 4) and the isofield maps (Figs. 2c,d) reveal the onset of activity reminiscent of some slow depolarization starting approximately 4 min after the stimulus onset and increasing in the following 6 min. During the subsequent time all signals experience an amplitude reduction. Note that in this case, where one of the younger leaves was burnt, the plant's stem was oriented so that the y-axis pointed from the youngest leaf towards the roots (Fig.1c). In other experiments, where one of the older leaves was burnt, as well as in the experiment with cutting injury (Figs.1a,b), the plant's stem was oriented in the opposite direction. It is evident from the comparison of isofield maps in Figs. 2c,d that the center of excitation moved along the stem by about 3.5 cm in 5 min.

For the case of (Figs. 1c and 2c,d) where the isofield lines well resembled the dipolar pattern, both source model functions (CD and LS) were applied in the fitting procedure. Source parameters were calculated

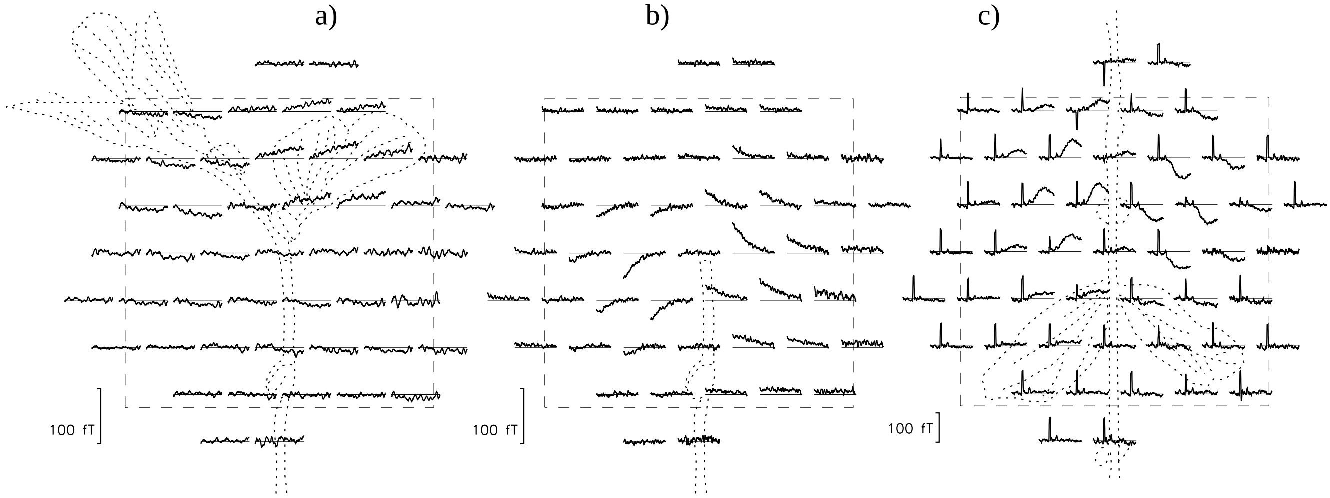

Figure 1: *The time evolution of the magnetic field in all channels: a) 25-10 min before cutting the plant 8, b) 1-16 min after cutting and c) stimulation by burning 5 min after the beginning of measurements on plant 5. A sketch of the plant is shown with dotted lines and the inner side of the chamber is indicated by a dashed line. The length of time axis is 15 min in the panels a) and b), and 20 min in c).*

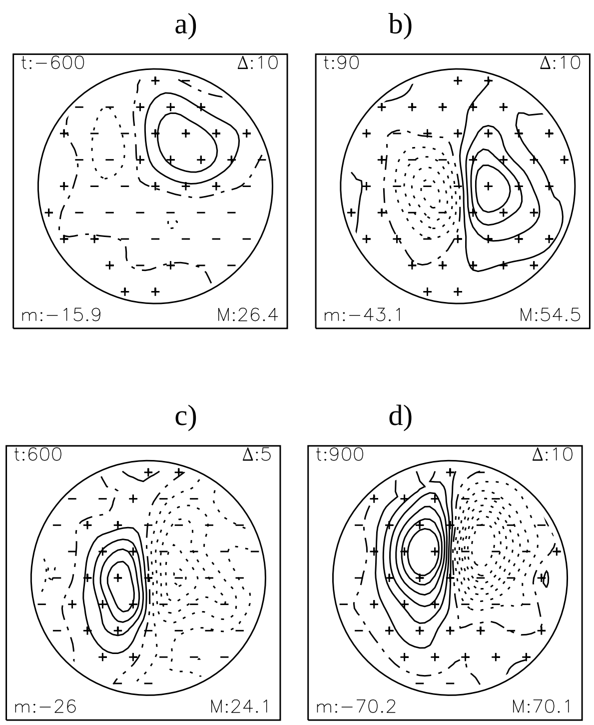

Figure 2: a) Isofield maps 10 min before (Fig. 1a) and b) 1.5 min after (Fig. 1b) the cutting injury. c) and d) two isofield maps 5 and 10 min after the injury by burning, respectively. Here t denotes time from the injury in s, m and M are minimal and maximal field values in fT, and Δ step between two isofield lines in fT. Solid, dash-dot-dash and dot line styles represent positive, zero and negative isofield lines, respectively. Positions of magnetic sensors are shown by + and - in accordance with the measured field sign.

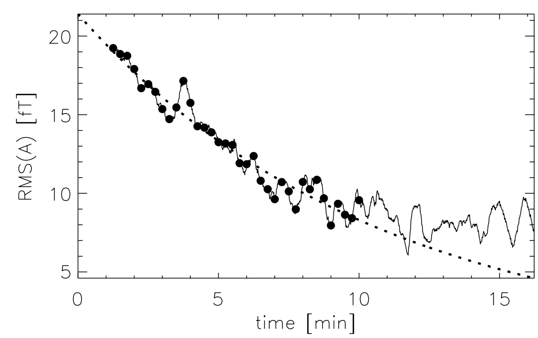

Figure 3: The solid line represents the time evolution of injury-related RMS magnetic field from Fig. 1b, full dots show RMS values of magnetic field each 15 s in the first 10 min after the injury. These values were fitted with the exponential decay function shown by the dotted line. The characteristic decay time was about 10 min.

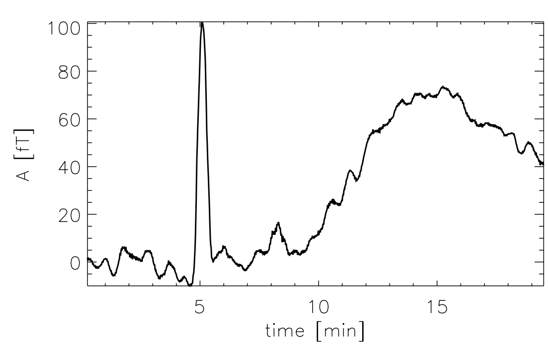

Figure 4: The time evolution of injury related magnetic fields in one of the channels from Fig. 1c (the 3rd channel in the 4th raw). Notice the artifact at time=5 min due to the burning stimulation procedure.

for each second in a given time interval after the stimulation. The LS gave slightly better results. That was to be expected because the elongated isofield pattern (cf. Fig. 2d) indicated the presence of a longer current distribution. In addition, we also obtained an estimate of the excitation length (2a).

Table 1 summarizes the results of the fitting procedure with LS model for all measurements where we observed a dipolar response to stimulation (plants number 1,5,6,7,8). Plants number 2,3,4 also produced a magnetic signal upon burning stimulation but with a non-dipolar magnetic field distribution. The depth of the source z was successfully localized within the acceptable region for all cases. The LS length 2a gives an estimate of the excitation length, which is between 6 and 10 cm for stimulation by burning and between 4 and 5 cm for cutting injury. The total current $\mathcal{J}$ show the maximal level of activity, equal to 0.1 to 0.24 μA. From this current one can estimate the average order of magnitude for the current density of 0.01μA/mm^2, where it is assumed that the beam cross-section is approximately 20 mm^2.

Localization results (x,y,z-coordinates of equivalent current source) show that there is no significant spreading of excitation in almost all cases. The only exceptions are the two measurements (5a, 5b), performed on the same plant within 16 hours, where we noticed a time limited excitation propagation with velocities between 0.5 and 2 cm/min. For all other cases it seems that the center of excitation is almost fixed, only the amplitude of response is changing with time.

In conclusion, we can say that all measured plants responded to stimulation by burning and that the phenomenon was magnetically detected. We could not confirm by magnetic measurements the long distance spreading of electric activity.

Table 1: *Some parameters calculated from the LS model, averaged over 1-3 min subinterval where the maximum level of activity is reached, for the cutting injury (i) and stimulation by burning (ii).*

Plant (type)	depth $[cm]$ $z \pm \sigma_z$	length $[cm]$ $2a \pm \sigma_{2a}$	current $[\mu A]$ $\mathcal{J} \pm \sigma_{\mathcal{J}}$
8(i)	-5.24±0.05	4.6±0.2	0.14±0.01
1(ii)	-5.24±0.10	9.4±0.5	0.15±0.02
5a(ii)	-5.72±0.07	7.2±0.1	0.24±0.01
5b(ii)	-5.57±0.05	6.2±0.3	0.18±0.02
6(ii)	-5.54±0.10	6.8±0.2	0.17±0.01
7(ii)	-5.48±0.15	8.1±1.2	0.10±0.03

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