

# Radio-frequency electromagnetic radiation alters the electric potential of *Myriophyllum aquaticum*

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## Abstract

Electric signaling pathways are important for rapid and long-distance communication within a plant. Changes in the electric potential (EP) inside plants have been observed during the propagation of electric signals. Increasing radio-frequency electromagnetic radiation (EMR) in the environment raise the question about possible effects of EMR on the EP of plants. In the present experiment, we investigated the effect of 2, 2.5, 3.5, and 5.5 GHz EMR with a maximum field intensity of 23 - 25 V m<sup>-1</sup> on the EP in emergent *Myriophyllum aquaticum* plants. The 2 and 5.5 GHz exposures caused significant (16 and 13 %) decreases in the standard deviation of rapid fluctuations observed in the EP. The greatest change was caused by 2.5 GHz EMR (23 % increment), although it was not statistically significant. A recovery of the EP was only after 2.5 GHz EMR exposure. The temperature of the plants was not changed by the EMR exposure. These findings confirm the frequency-dependent non-thermal effects of EMR on the EP of plants.

*Additional key words:* communication technology, electric field, microelectrodes, parrot feather, plant signaling.

## Introduction

Plants growing in their natural environment are exposed to both abiotic and biotic stimuli. To respond to or resist these stimuli, different signaling pathways operate to allow communication among plant organs (Mancuso and Mugnai 2006, Ilik *et al.* 2010). These signaling pathways can be subdivided into three main categories: chemical (Seo *et al.* 1997, León *et al.* 2001, Gechev *et al.* 2006), hydraulic (Comstock 2002, Christmann *et al.* 2007), and electric (Fromm and Lautner 2007, Oyarce and Gurovich 2010). The electric signaling pathway transmits long-distance signals at a relatively high rate (Fromm and Bauer 1994, Stankovic *et al.* 1997, Volkov and Ranatunga 2006). Usually, these signals are subclassified in terms of the action potential (AP) and the variation potential (VP) based on the mode and rate of transmission (Stahlberg and Cosgrove 1997, Fromm and Lautner 2007). In addition to these two categories, another electrical signal type called the system potential (SP) has been discussed by Zimmermann *et al.* (2009). Collectively, AP, VP, and SP signals propagate as change in the electric potential

(EP) inside plants. These signals can contain encoded information that is determined by the shape of the signal (Krol *et al.* 2006, Fromm and Lautner 2007). Therefore, if the EP of a plant is altered or manipulated due to external factors, it is possible to transmit incorrect information to the destination of the signal or to disturb the electric signaling system. The wounding response of tomato plants evoked by the external application of electrical stimuli (Stanković and Davies 1997) and the activation of the Venus flytrap by an electric charge (Volkov *et al.* 2008) are examples of the external manipulation of the plant electrical signaling system.

The use of mobile phones and wireless broadband access base stations has greatly increased the radiated power of radio-frequency electromagnetic radiation (EMR) in the environment (Hyland 2005). Frequencies from 800 MHz to 2.5 GHz are primarily used for communication networks, and frequencies above 2.5 GHz are increasingly used due to increased band width demands in technology. Even higher frequencies are

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*Abbreviations:* AP - action potential; EMR - radio-frequency electromagnetic radiation; EP - electric potential; on-EMR - EMR exposure duration; post-EMR - post EMR exposure duration; pre-EMR - pre EMR exposure duration; SDEP - standard deviation of rapidly fluctuating electric potential; SP - system potential; VP - variation potential.

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anticipated for broadcasting networks as spectrum allocation tables list increasingly higher frequencies that are allocated to broadcasting purposes (Australian communications and media authority 2009, Electronic communication committee 2011, Federal communications commission 2012). When EMR propagates in space, it generates electric fields that depend upon the power density of the wave. Due to the sensitivity of plants to electrical stimuli, it is possible to manipulate the electric signaling or EP of plants with these EMR electric fields. If such an effect exists, it will affect the capacity of plants to adapt and respond to external stimuli. In contrast, if EMR exposure causes stress in plants (Tkalec *et al.* 2005, 2007, Roux *et al.* 2006, Sharma *et al.* 2009), this

stress can then be reflected by the EP due to the change in physiological status (Fromm and Lautner 2007). However, evidence for such effects in plants is lacking, and investigating the problem will broaden understanding the environmental impacts of the extensive use of EMR. Therefore, we conducted this study to investigate the existence of EMR effects on the EP of plants. For this purpose, we exposed emergent parrot feather plants (*Myriophyllum aquaticum*) to 2, 2.5, 3.5, and 5.5 GHz EMR with 23 - 25 V m<sup>-1</sup> field intensity, and studied the effect on the EP. To the best of our knowledge, this study represents the first attempt to investigate the effect of EMR on the EP of plants.

## Materials and methods

**Plant preparation:** Parrot feather (*Myriophyllum aquaticum* Verdc.) plants were grown in cubic glass tanks half-filled with river sand and a 35 % (v/v) Hoagland solution to a height of 5 cm above the sand. Healthy, vertical, emergent stems with roots in a submerged region were cut from a culture and individually planted in the glass tanks. The cuttings were allowed to further develop their roots and to grow to at least 10 cm above the top of the tank. The water level was maintained by adding distilled water every 2 - 3 d, and once a week, the water level was adjusted with the 35 % Hoagland solution. The plants were grown at temperature of 26 - 27 °C, irradiance of 55 to 60 μmol m<sup>-2</sup> s<sup>-1</sup> (photosynthetically active radiation), a 12-h photoperiod, and air humidity of 70 - 75 %. To allow acclimation after the mechanical shock to the plants, a tank with a plant greater than 10 cm in height was placed inside an EMR-shielded anechoic chamber (specially designed Faraday cage used in the present experiment to separate plants from environmental EMR and to prevent the internal reflection of the transmitted EMR) one day prior to the experiment. The chamber doors remained open until the beginning of the experiment. Inside the chambers, the environmental conditions were the same as mentioned above.

**EMR-shielded anechoic chamber:** It was necessary to prevent the exposure of the plants to environmental EMR

during the experiment because the environment contains a wide mixture of EMR emitted from terrestrial television stations, mobile phone networks, wireless broadband access, and internal WiFi links. The presence of these EMR sources affects the measurements obtained for an individual frequency. For these reasons, all of the experiments were performed in a specially designed anechoic chamber 100 (H) × 75 (W) × 75 (L) cm in size. The chamber was covered with a 3 mm<sup>2</sup> stainless steel mesh with a thickness of 0.8 mm (18 AWG). All of the vertical walls were internally covered with flat, 6 cm thick ferrite EMR-absorbing foam (*PFP F*, Riken, Tokyo, Japan), the bottoms of the chambers were covered with 20 cm pyramidal-type EMR-absorbing foam (*PFP 30*, Riken). The purpose of the application of EMR-absorbing foam was to prevent the internal reflection of EMR from forming standing waves which could affect the EMR treatment. The chambers were capable of reducing EMR penetration by more than 95 %.

**EMR exposure and EP measurements:** Plants were exposed to continuous-wave EMR at 2, 2.5, 3.5, and 5.5 GHz (the experiment repeated four times for each frequency). The maximum electromagnetic field at the top of the plant was maintained at 23 - 25 V m<sup>-1</sup> for every frequency. EMR was broadcast from above the plant, and the distance between the top of the plant and the

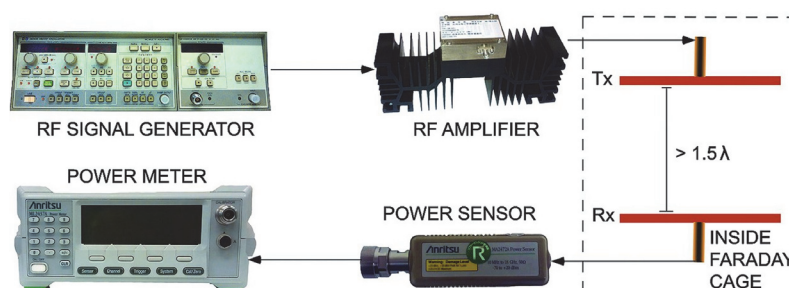


Fig. 1. The system used for the radio-frequency electromagnetic radiation (EMR) exposure and electromagnetic field intensity measurements. Tx - microstrip antenna for EMR transmission, Rx - receiving antenna. Experiments were always arranged to maintain a distance of more than 1.5 times the wavelength ( $\lambda$ ) between the Tx antenna and the Rx antenna/sample exposed.

transmitting antenna was maintained so that it exceeded 1.5 times the wavelength to ensure the absence of standing wave generation (stationary waves whose wave front does not move, formed by two waves traveling in opposite directions interfacing). The exposure to other environmental EMR was almost negligible as the experiments were conducted in an EMR-shielded anechoic chamber. The EMR was generated by a radio-frequency signal generator (an 8350 B oscillator with 83592B RF plugging, Hewlett Packard, Santa Rosa, CA, USA), and signal amplification was performed with a linear amplifier (ZVE-3W-83+, Mini-Circuits, New York, USA). The transmission (Tx) of the EMR was performed with microstrip antennas prepared for each frequency. The electric field strength was calculated by measuring the EMR power density at the top of the plant. A microstrip antenna of the same frequency connected to a power meter (ML 2472A, Anritsu, Kanagawa, Japan) via a power sensor (MA 2471A, Anritsu) was used to measure the EMR power density (Fig. 1).

The plants were subjected to EP measurements using 1 % (m/v) agarose gel stabilized Ag/AgCl, 3 M KCl glass microelectrodes (50  $\mu\text{m}$  diameter with less than 10  $\mu\text{m}$  diameter opening at the tip; REF-50, Unisense, Aarhus, Denmark). The electrodes were inserted into a stem 7 cm apart (Fig. 2). The first electrode, which was the reading electrode, was typically inserted 2 - 3 cm below the top of the stem, and the reference electrode was inserted 7 cm below the reading electrode. Both electrodes were inserted halfway through the stem to ensure electrode connectivity with vascular tissues (*M. aquaticum* does not consist of differentiated xylem and phloem). The electrodes were connected to a stand 7 cm apart vertically, and the plant stem was pushed firmly toward the electrode tip until the tip penetrated half of the diameter of the stem. A pointing light source (a pen light with a single white color light-emitting diode) was applied from the backside on to the plant stem to visualize the electrode path. The electrodes were connected to a sensitive ( $> 0.1 \mu\text{V}$ ) millivoltmeter, which contained an analog to digital converter (*pH/mV Meter*, Unisense) to convert the voltage signal to digital form and to record the data via the acquisition software (*Sensor Trace BASIC 3.0*, Unisense). The data were recorded continuously at a 1 Hz sampling rate. The data acquisition was initiated following a 1.5 h acclimatization period after the insertion of the microelectrodes. EP was recorded continuously for 4.5 h, including 1.5 h each of pre-EMR exposure duration (pre-EMR), EMR exposure duration (on-EMR), and post-EMR exposure (post-EMR). All the EP experiments were conducted under irradiance of  $55 - 60 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

**Electrode manipulation and control tests:** According to the manufacturer (Unisense), the microelectrodes used in the present experiment were resistant to electromagnetic noise, however, it is possible that EMR could have an effect. To investigate this possibility, we inserted the electrodes into a 10 mm external diameter (7 mm internal

diameter), 14 cm-long flexible vinyl tube containing a 3 % (m/v) agar gel in distilled water. The addition of agar increased the conductivity of the water. The tube was connected at the bottom to a copper cable grounded to the metal frame of the anechoic chamber to reduce the static energy charge on the tube (Fig. 3). To record the EP, the same procedure as used for the plants was performed. For this control test, 2.5 GHz EMR with maximum field intensity of  $30 \text{ V m}^{-1}$  at the top of the tube was used. The measurements were taken 3 times using three vinyl tubes.

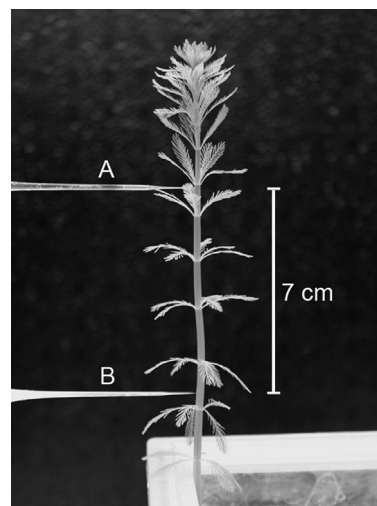


Fig. 2. Microelectrodes inserted into the *Myriophyllum aquaticum* stem. Reading (A) and reference (B) electrodes were inserted 7 cm apart. Both electrodes were inserted halfway through the stem.

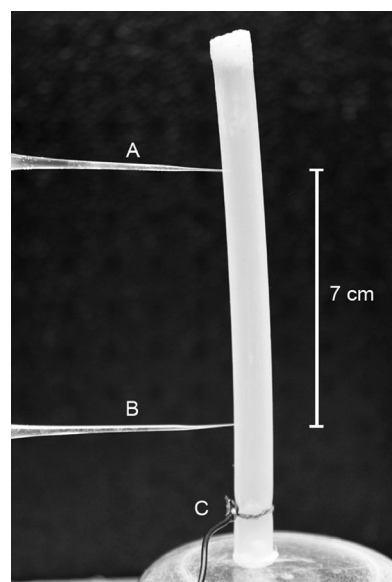


Fig. 3. Microelectrodes inserted into a vinyl tube filled with a 3 % agar in distilled water to measure the electric potential. Reading (A) and reference (B) electrodes were inserted 7 cm apart. The ground cable (C) is connected to the lower end of the vinyl tube.

To determine whether *M. aquaticum* plants naturally exhibited changes in EP properties over the experimental period, EP was continuously recorded for 4.5 h without EMR exposure using the same procedures described for the EMR exposure experiments inside the anechoic chamber. The data were then divided into three 1.5 h segments, and the variances were compared. This experiment was repeated four times.

**Infrared thermographic images** were used to observe the change in temperature on the apical area of *M. aquaticum* plants due to EMR exposure. Plants of the same size and prepared with the same procedure as those for the EP experiment were tested for temperature changes in response to 2 and 5.5 GHz EMR with  $30 \text{ V m}^{-1}$  maximum electric field intensity at the top region of the plants. Because the dielectric heating is maximal at the highest field intensity exposure, we observed the temperature at the top of the plants. Thermographic images were obtained using an infrared thermographic camera with a thermal sensitivity of  $0.08 \text{ }^{\circ}\text{C}$  (InfReC Thermo GEAR G120, NEC, Tokyo, Japan). An image was taken to determine the initial temperature immediately prior to the beginning of the EMR exposure. Upon completion of 1 h of the continuous EMR exposure, another temperature image was taken while the EMR exposure was ongoing.

**Data analysis:** The recorded electric potential along the 7 cm distance of the vascular tissues of the *M. aquaticum* plants changed (increased or decreased) slowly over time (Fig. 4A). The EP between the two electrodes varied within the range 10 - 31 mV, and the variation was different from plant to plant. However, in addition to this

slow change, a rapid fluctuation of the EP was observed over time. This fluctuation ranged within  $\pm 5 \text{ mV}$  between two adjacent data points. The standard deviation (SD) of the rapidly fluctuating EP (SDEP) remained steady when the plant was undisturbed, and when the plant condition was changed, the SDEP also changed (for example, a salt stress due to the addition of 200 mM NaCl reduced the SDEP by  $34 \pm 6 \%$ , data not presented). Therefore in the present experiment, we considered how the EMR was affected by the rapid EP fluctuation. To calculate the SDEP, the recorded EP data were first modified to reflect only the rapid fluctuation by calculating the differences between each adjacent data point ( $Y_i$ ), i.e., subtracting the previous value ( $X_{i-1}$ ) from the subsequent value ( $X_i$ ) and plotting it against time,  $Y_i = X_i - X_{i-1}$ , Fig. 4B). The standard deviation of the data was then calculated separately for the pre EMR exposure duration (pre-EMR), EMR exposure duration (on-EMR), and post EMR exposure duration (post-EMR).

The SDEPs calculated for the on-EMR and post-EMR of the plants were divided by the SDEP of the pre-EMR to normalize the data. Significance of differences in the SDEP was tested with a *t*-test for two independent samples using the Levene's test for equality of variance. To investigate the recovery of the SDEP in the post-EMR period, the 2, 2.5, and 5.5 GHz post-EMR exposure data were divided into three equivalent segments, and the SDEP was calculated separately for each. These three segments were tested with the *t*-test for two independent samples. All statistical analyses were performed using SPSS v. 16.0 (SPSS, Chicago, USA). The thermographic images were analyzed manually to examine the temperature difference.

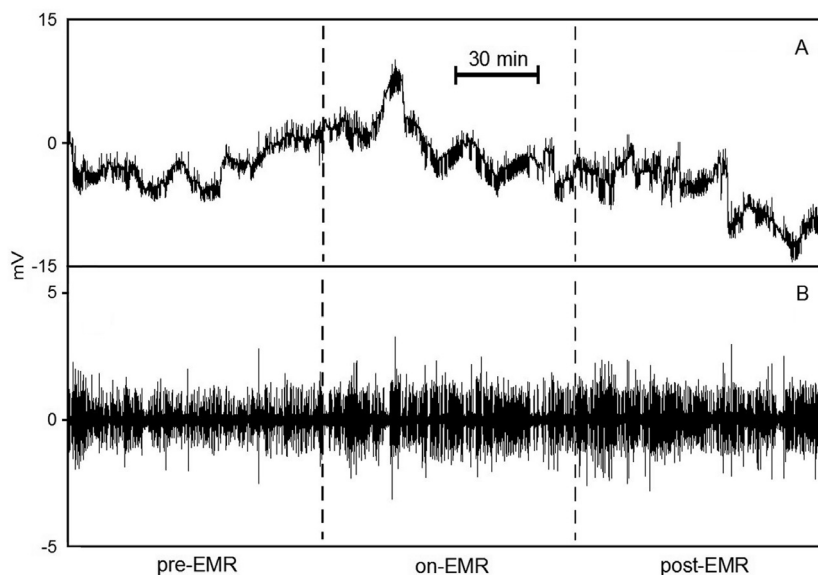


Fig. 4. Electric potential along a 7 cm distance of *Myriophyllum aquaticum* plant vascular tissues for the pre, during, and post radio-frequency electromagnetic radiation (2.5 GHz) exposures. A - Electric potential (EP) recorded during three exposure conditions at a sampling frequency of 1 Hz. B - Rapid fluctuation of EP plotted over time by subtracting the previous value from the subsequent value. Horizontal axis denotes time, and vertical axis represents EP.

## Results

During the 2 GHz EMR exposure, the SDEP decreased by 14 %. Following the exposure, the decrease persisted, stabilizing at a statistically significant ( $P < 0.05$ ) 16 % reduction. The greatest changes in the SDEP occurred during the 2.5 GHz EMR exposure, with SDEP increasing by 24 and 23 % in the on-EMR and post-EMR, respectively. The greatest change and the SD of the averaged SDEP were comparably higher during the 2.5 GHz EMR exposure than those at the other frequencies ( $\pm 0.45$  and  $\pm 0.26$  for the on-EMR and post-EMR, respectively). Therefore, the changes were not statistically significant. In the 3.5 GHz EMR exposure, the SDEP decreased by 10 %, however, for the post-EMR, the SDEP approached the pre-EMR levels, differing by only 4 % from the initial SDEP. The 5.5 GHz treatment produced a similar response to the 2 GHz treatment: the SDEP decreased by 16 % during the on-EMR and remained 13 % lower at post-EMR. Both of the changes

Table 1. Relative electric potential standard deviation of *Myriophyllum aquaticum* plants. Radio-frequency electromagnetic radiation exposure (on-EMR) and post-radio-frequency electromagnetic radiation exposure (post-EMR) periods relative to the pre-radio-frequency electromagnetic radiation exposure (pre-EMR) period. Means  $\pm$  SD. The standard deviation of the electric potential during EMR and post-EMR was tested against that of pre-EMR using independent samples ( $t$ -test, \* -  $P < 0.05$ );  $n = 4$  for plant experiments and  $n = 3$  for vinyl tube experiments)

Experiment	pre-EMR	on-EMR	post-EMR
2.0 GHz	1	$0.86 \pm 0.10$	$0.84 \pm 0.10^*$
2.5 GHz	1	$1.24 \pm 0.45$	$1.24 \pm 0.26$
3.5 GHz	1	$0.90 \pm 0.08^*$	$1.04 \pm 0.22$
5.5 GHz	1	$0.84 \pm 0.08^*$	$0.87 \pm 0.07^*$
Control	1	$1.02 \pm 0.05$	$1.00 \pm 0.06$
Vinyl tube	1	$0.84 \pm 0.07^*$	$1.02 \pm 0.01$

## Discussion

The electric field of EMR can increase the temperature of biological tissues by dielectric activity. Exposure to the oscillating electric field of EMR causes dipolar molecules and charged ions to vibrate (Jacob *et al.* 1995). The dipolar molecules rotate when trying to settle with the oscillating electric field, however, due to the high frequency, those molecules vibrate. In the case of charged ions, a phenomenon called ion drag occurs. Although there was no heat increment recorded in the present experiment, dielectric activity could be present at a low intensity, which could affect the physiology of plants.

Ion exchange between the symplast and apoplast spaces transmits signals through the phloem (Lautner *et al.* 2005) and can change the pressure potential of cells, causing fluctuations in the length of the plant (Shiina and

Table 2. Relative electric potential standard deviation of *M. aquaticum* plants in three equivalent segments of post-exposure duration to 2.0, 2.5, and 5.5 GHz radio-frequency electromagnetic radiation. Each value is the mean  $\pm$  SD,  $n = 4$ , \* - segments 1 and 3 are significantly different at  $P < 0.05$  using independent-sample  $t$ -test.

Experiment	Segment 1	Segment 2	Segment 3
2.0 GHz	1	$0.95 \pm 0.15$	$0.93 \pm 0.10$
2.5 GHz	1	$0.93 \pm 0.15$	$0.76 \pm 0.19^*$
5.5 GHz	1	$0.91 \pm 0.09$	$1.03 \pm 0.20$

were statistically significant (Table 1).

The SDEP values calculated for the three segments of the post-EMR data for 2 GHz were not significantly different and continued to exhibit decreased SDEP without much change. The 2.5 GHz treatment exhibited a significant decrease of 22 % in the SDEP of the third segment relative to that of the first segment. As in the 2 GHz treatment, the 5.5 GHz exposure did not show a recovery trend over the three segments because the SDEP values of the three segments were not significantly different (Table 2).

The SDEPs measured in the *M. aquaticum* plant stems over the three consecutive 1.5 h segments without EMR exposure remained almost linear, varying by less than 5 % across replications (Table 1). The EP of the vinyl tubes filled with agar also exhibited slight fluctuations. However, on-EMR reduced the SDEP by 16 %, which increased post-EMR to within 2 % of the pre-exposure value (Table 1).

The temperature of the *M. aquaticum* plants was not affected by the 2 or 5.5 GHz EMR with  $30 \text{ V m}^{-1}$  field intensity. The temperatures measured for the plants remained unchanged after 1 h of EMR exposure for both the frequencies and exhibited fluctuations with a maximum of only  $0.3^\circ\text{C}$  (Fig. 5).

Tazawa 1986, Fromm and Bauer 1994). Other pathways that alter the xylem water tension or ion concentration (Fromm and Fei 1998, Oyarce and Gurovich 2010) can also lead to fluctuations in the lengths of the plants (Tang and Boyer 2008). The presence of such signaling pathways is evident from the rapid fluctuations in the growth rate observed in rice seedlings (Kobayashi and Kadono 2010). These two mechanisms may cause the EP to fluctuate rapidly, maintaining a continuous signal along the stem until it is changed by an external stimulus. In the case of non-differentiated vascular tissues, *M. aquaticum* may maintain ion exchange along the vascular tissues. However, we observed a slightly fluctuating EP in the control experiment in which we connected electrodes to a vinyl tube filled with agar. This



fluctuation might be due to static charge fluctuation along the tube together with signal noise either between or within electrodes. The relatively low SDEP (less than  $\pm 0.15$ ) and unchanged SDEPs measured before and after the EMR exposure in the vinyl tube experiment indicates that the difference in the SDEPs in *M. aquaticum* pre-EMR and post-EMR was a result of EMR exposure. However, the reduced SDEP during the on-EMR compared to pre-EMR in the vinyl tube experiment indicates that the EMR affected the measurements of the electrodes. Therefore, the EP measured during the EMR exposure in the present study did not effectively explain the exact EP of *M. aquaticum* due to the influence of the EMR on the reading produced by the electrodes. Furthermore, the stable SDEP recorded during the non-EMR control experiment on *M. aquaticum* demonstrates that the SDEP did not vary over time if the plant conditions were unchanged.

The changes in the SDEP observed following EMR exposure demonstrate that the electrical signaling of *M. aquaticum* should have been affected by EMR exposure. The level and direction of the effect varied with EMR frequency. The decreases in the SDEP following 2 and 5.5 GHz exposures suggest that similar responses could occur at different frequencies. However, the 0.5 GHz difference between the 2 and 2.5 GHz treatments yielded

different SDEP responses. The photon energy difference represented by a 0.5 GHz difference is not negligible, as shown by the formula  $E = h \times f$  (where  $E$  is a photon energy,  $h$  is Planck's constant, and  $f$  is a frequency) used to calculate the photon energy of electromagnetic radiation (Challis 2005). In the present study, the treatment differences observed illustrate the frequency-dependent sensitivity of *M. aquaticum* to EMR. The frequency dependence of plant responses to EMR has also been observed in  $H_2O_2$  formation in *Lemna minor* following exposure to 400 and 900 MHz EMR (Tkalec *et al.* 2005, Tkalec *et al.* 2007). The results of the 2.5 GHz treatment (an increased SDEP upon EMR exposure and a recovery trend in SDEP during the post-EMR) is indicative of a phenomenon that is different from that of the other frequencies as the SDEP increased during the EMR exposure. It is unclear whether the increased SDEP during the EMR exposure reflected a potentially different sensitivity of the electrodes to this frequency or whether a high sensitivity of the plant to this frequency eliminated the decrease in the SDEP observed during exposure to the other frequencies. However, the control experiment with the vinyl tube conducted at 2.5 GHz found no evidence of a differential sensitivity of the electrodes to this frequency. We also observed that the SDEP varied among the plants even before the EMR exposure. Therefore, the

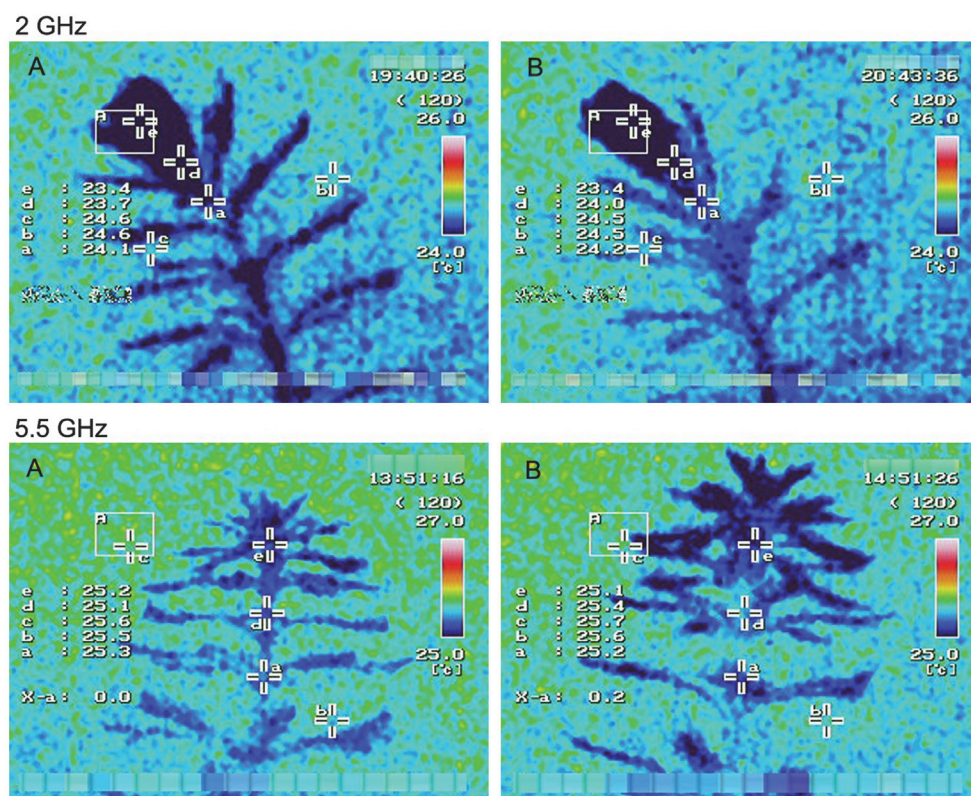


Fig. 5. Infrared thermographic images taken for temperature change measurements in *Myriophyllum aquaticum* due to a radio-frequency electromagnetic radiation (EMR) exposure. Plants were tested with 2 and 5.5 GHz EMR at a  $30 \text{ V m}^{-1}$  electric field intensity for 1 h. *A* - Before EMR exposure, *B* - after 1 h continuous exposure. Points denoted by a, d, and e are temperature measuring spots on plants.

sensitivity of individual plants to EMR can vary and produce effects of different magnitudes resulting from exposure to EMR.

Changes in the EP can occur due to changes in the factors that are important for the regulation of the electric potential, such as auxin content (Baluška *et al.* 2004),  $\text{Ca}^{2+}$ ,  $\text{K}^+$ , and  $\text{Cl}^-$  content in the vascular system (Bush 1995, Zimmermann *et al.* 1999), and water tension in the xylem (Fromm and Fei 1998, Oyarce and Gurovich 2010). An externally applied electric shock can alter phloem translocation and retard growth due to a decreased symplastic  $\text{K}^+$  and  $\text{Cl}^-$  content (Fromm and Bauer 1994). The dielectric activity of EMR can affect ion movements inside plants. Therefore, it is possible that the electric polarity existing in some plants (Clark 1937a,b, Volkov and Ranatunga 2006) can be affected by an EMR exposure, leading to changes in their electric properties. This hypothesis derives some support from observations of altered electric polarity in tobacco cells exposed to external electric stimuli (Mina and Goldsworthy 1991).

Another possible cause of changes in the EP could be dielectric heating (Jacob *et al.* 1995) the plant due to an EMR exposure, as heat was found to evoke electrical signals in tomato plants (Herde *et al.* 1999). However, the temperature was unchanged during a 1 h continuous exposure to 2 and 5.5 GHz EMR at a relatively higher field intensity than that used in the EP experiments. Furthermore, no significant temperature fluctuation was recorded in a protein solution and only a slightly

increasing trend (approximately 1 °C) in temperature was recorded in the 2 to 5.5 GHz EMR range for an equivalent power density (Coptý *et al.* 2006). Therefore, the altered EP in the *M. aquaticum* plants in the present study appears to have resulted from non-thermal effects of EMR.

Therefore, the present study provides the evidence of the effect of EMR on the EP in the *M. aquaticum* plants. The observed EMR frequency dependence of EP is consistent with the findings of previous experiments. The persistence of changes in EP even after the EMR exposure suggests complex underlying mechanisms, and further investigations are needed to identify them. In the natural environment, plants are continuously exposed to EMR. Therefore, plants in the natural environment may respond to EMR in different ways and may develop adaptations under continuous exposure. However, the interference of EMR with the microelectrodes, as identified in the present experiments, is a challenge for continuous EMR exposure research. Furthermore, the electric field generated by fluorescent radiations may have an influence on the electric properties of *M. aquaticum* plants, any such an effect was unaccounted for in the present observations since all the EP measurements were taken under fluorescent radiation throughout the study. Whether the response of *M. aquaticum* to EMR deviates due to the electric field generated by fluorescent radiation or due to other low frequency electric fields requires further study.

## References

- Baluška, F., Mancuso, S., Volkman, D., Barlow, P.: Root apices as plant command centres: the unique "brain-like" status of the root apex transition zone. - *Biología* **59**: 7-19, 2004.
- Bush, D.S.: Calcium regulation in plant-cells and its role in signaling. - *Annu. Rev. Plant Physiol. Plant mol. Biol.* **46**: 95-122, 1995.
- Challis, L.J.: Mechanisms for interaction between RF fields and biological tissue. - *Bioelectromagnetics* **S7**: S98-S106, 2005.
- Christmann, A., Weiler, E.W., Steudle, E., Grill, E.: A hydraulic signal in root-to-shoot signalling of water shortage. - *Plant J.* **52**: 167-174, 2007.
- Clark, W.G.: Electrical polarity and auxin transport. - *Plant Physiol* **12**: 409-440, 1937a.
- Clark, W.G.: Polar transport of auxin and electrical polarity in coleoptile of *Avena*. - *Plant Physiol.* **12**: 737-754, 1937b.
- Comstock, J.P.: Hydraulic and chemical signalling in the control of stomatal conductance and transpiration. - *J. exp. Bot.* **53**: 195-200, 2002.
- Coptý, A.B., Neve-Oz, Y., Barak, I., Golosovsky, M., Davidov, D.: Evidence for a specific microwave radiation effect on the green fluorescent protein. - *Biophys. J.* **91**: 1413-1423, 2006.
- Fromm, J., Bauer, T.: Action-potentials in maize sieve tubes change phloem translocation. - *J. exp. Bot.* **45**: 463-469, 1994.
- Fromm, J., Fei, H.M.: Electrical signaling and gas exchange in maize plants of drying soil. - *Plant Sci.* **132**: 203-213, 1998.
- Fromm, J., Lautner, S.: Electrical signals and their physiological significance in plants. - *Plant Cell Environ.* **30**: 249-257, 2007.
- Gechev, T.S., Van Breusegem, F., Stone, J.M., Denev, I., Laloi, C.: Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. - *Bioessays* **28**: 1091-1101, 2006.
- Herde, O., Cortes, H.P., Wasternack, C., Willmitzer, L., Fisahn, J.: Electric signaling and *Pin2* gene expression on different abiotic stimuli depend on a distinct threshold level of endogenous abscisic acid in several abscisic acid-deficient tomato mutants. - *Plant Physiol.* **119**: 213-218, 1999.
- Hyland, G.: How exposure to mobile phone base-station signals can adversely affect humans. - [http://www.tetrawatch.net/papers/hyland\\_2005](http://www.tetrawatch.net/papers/hyland_2005).
- Ilík, P., Hlaváčková, V., Krchňák, P., Nauš, J.: A low-noise multi-channel device for the monitoring of systemic electrical signal propagation in plants. - *Biol. Plant.* **54**: 185-190, 2010.
- Jacob, J., Chia, L.H.L., Boey, F.Y.C.: Thermal and non-thermal interaction of microwave radiation with materials. - *J. Materials Sci.* **30**: 5321-5327, 1995.
- Kobayashi, K., Kadono, H.: Expansion of the dynamic range of statistical interferometry and its application to extremely short- to long-term plant growth monitoring. - *Appl. Optics* **49**: 6333-6339, 2010.
- Krol, E., Dziubinska, H., Stolarz, M., Trebacz, K.: Effects of ion channel inhibitors on cold- and electrically-induced action potentials in *Dionaea muscipula*. - *Biol. Plant.* **50**: 411-416, 2006.

- Lautner, S., Grams, T.E., Matyssek, R., Fromm, J.: Characteristics of electrical signals in poplar and responses in photosynthesis. - *Plant Physiol.* **138**: 2200-2209, 2005.
- León, J., Rojo, E., Sánchez-Serrano, J.J.: Wound signalling in plants. - *J. exp. Bot.* **52**: 1-9, 2001.
- Mancuso, S., Mugnai, S.: Long-distance signal transmission in trees. - In: Baluška, F., Mancuso, S., Volkmann, D. (ed.): *Communication in Plants*. Pp. 333-349. Springer, Berlin - Heidelberg 2006.
- Mina, M.G., Goldsworthy, A.: Changes in the electrical polarity of tobacco cells following the application of weak external currents. - *Planta* **186**: 104-108, 1991.
- Oyarce, P., Gurovich, L.: Electrical signals in avocado trees: responses to light and water availability conditions. - *Plant Signal. Behav.* **5**: 34-41, 2010.
- Roux, D., Vian, A., Girard, S., Bonnet, P., Paladian, F., Davies, E., Ledoigt, G.: Electromagnetic fields (900 MHz) evoke consistent molecular responses in tomato plants. - *Physiol. Plant.* **128**: 283-288, 2006.
- Seo, S., Sano, H., Ohashi, Y.: Jasmonic acid in wound signal transduction pathways. - *Physiol. Plant.* **101**: 740-745, 1997.
- Sharma, V.P., Singh, H.P., Kohli, R.K., Batish, D.R.: Mobile phone radiation inhibits *Vigna radiata* (mung bean) root growth by inducing oxidative stress. - *Sci. Total Environ.* **407**: 5543-5547, 2009.
- Shiina, T., Tazawa, M.: Action-potential in *Luffa cylindrica* and its effects on elongation growth. - *Plant Cell Physiol.* **27**: 1081-1089, 1986.
- Stahlberg, R., Cosgrove, D.J.: The propagation of slow wave potentials in pea epicotyls. - *Plant Physiol.* **113**: 209-217, 1997.
- Stanković, B., Davies, E.: Intercellular communication in plants: electrical stimulation of proteinase inhibitor gene expression in tomato. - *Planta* **202**: 402-406, 1997.
- Stankovic, B., Zawadzki, T., Davies, E.: Characterization of the variation potential in sunflower. - *Plant Physiol.* **115**: 1083-1088, 1997.
- Tang, A.C., Boyer, J.S.: Xylem tension affects growth-induced water potential and daily elongation of maize leaves. - *J. exp. Bot.* **59**: 753-764, 2008.
- Tkalec, M., Malaric, K.I., Pevalek-Kozlina, B.: Influence of 400, 900, and 1900 MHz electromagnetic fields on *Lemna minor* growth and peroxidase activity. - *Bioelectromagnetics* **26**: 185-193, 2005.
- Tkalec, M., Malaric, K., Pevalek-Kozlina, B.: Exposure to radiofrequency radiation induces oxidative stress in duckweed *Lemna minor* L. - *Sci. Total Environ.* **388**: 78-89, 2007.
- Volkov, A., Ranatunga, D.: Plants as environmental biosensors. - *Plant Signal. Behav.* **1**: 105-115, 2006.
- Volkov, A.G., Carrell, H., Adesina, T., Markin, V.S., Jovanov, E.: Plant electrical memory. - *Plant Signal. Behav.* **3**: 490-492, 2008.
- Zimmermann, M. R., Maischak, H., Mithofer, A., Boland, W., Felle, H.H.: System potentials, a novel electrical long-distance apoplastic signal in plants, induced by wounding. - *Plant Physiol.* **149**: 1593-1600, 2009.
- Zimmermann, S., Ehrhardt, T., Plesch, G., Muller-Rober, B.: Ion channels in plant signaling. - *Cell. Mol. Life Sci.* **55**: 183-203, 1999.