PLANT SIGNALING & BEHAVIOR
2018, VOL. 13, NO. 6, e1486145 (6 pages)
https://doi.org/10.1080/15592324.2018.1486145

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Microwave radiation alters burn injury-evoked electric potential in *Nicotiana benthamiana*

M. D. H. J. Senavirathna and T. Asaeda

Department of Environmental Science, Saitama University, Saitama, Japan

ABSTRACT

The dielectric effect enforced on charged ions and dipolar molecules by the oscillating electric field of microwaves may influence electric signaling in plants. In the present study, the exposure of *Nicotiana benthamiana* plants to continuous wave 2.45 GHz microwave radiation with 1.9 – 2.1 W m^{-2} power density significantly reduced the amplitude of leaf burning-induced variation potential along the plant stem. The change in amplitude of the variation potential occurred mainly because of a significant reduction of the depolarization rate. This effect was not observed during the post-microwave exposure period. The unique characteristics observed in the variation potentials were also observed under microwave exposure, suggesting unaffected information delivery to distant locations or unaffected transport of specific chemicals generated by the injury.

ARTICLE HISTORY
Received 13 April 2018
Accepted 28 May 2018

KEYWORDS
Abiotic stress; wounding; electromagnetic radiation; non-thermal effect; plant signaling

In the natural environment, plants are continuously exposed to radiofrequency electromagnetic radiation (EMR) from various communication systems. The main EMR category which plants are currently exposed to, is microwave radiation. The prominent microwave range in present day environment is 0.9 – 2.5 GHz, within which, the higher frequencies are preferred by service providers to facilitate the increasing traffic and bandwidth demands.[1] Therefore, frequencies of 2.0 GHz and greater are becoming predominant in the environment over the lower frequencies.

Plants maintain communication pathways throughout their body to mediate its growth, development, and reproduction. These pathways are mainly categorized as chemical, hydraulic, and electrical. Chemical pathways are mainly represented by peroxidases and jasmonates; while, hydraulic pathways are dependent on the water activity/water potential gradients from roots to leaves.[2-4] Besides chemical and hydraulic pathways, electric signals play neuron-like functions in the plant body, which facilitate rapid communication, particularly between distant body organs.[5]

Electric signals are important especially for localized stress and injury signaling, such as heat, chilling, wounding, pest attack, etc. Usually, these signals are generated at the site of injury and propagate as waves of electric potentials (EP) towards the target destination through vascular tissues.[6] These signals bring to their destination encoded information regarding the stress;[7] further, they are important to generate specific reactions to a particular stress, such as release of chemical compounds, increase or activation of gene expression, inhibition of certain physiological activities, etc.[8,9]. Usually, EPs are categorized into two types, action potentials (APs) and variable potentials (VPs). Both, APs and VPs propagate through the charged ions and compounds involved in each case. Action potentials propagate by inward and outward flow of K^+, Ca^{2+}, and Cl^- ions; whereas, VPs propagate by transient shut-down of P-type H^+-ATPase mediated influx of Ca^{2+}, anion and cation channel activation.[3,6,10]

Electromagnetic radiation consists in oscillating electric and magnetic fields propagating perpendicular to each other. This is a common phenomenon to all types of EMR, including microwaves. Numerous studies have shown the effect of EMR on plants; clearly, both the electric and magnetic fields of the EMR affect plants.[11,12] However, due to the dielectric activity enforced on charged ions and dipolar molecules, the effects of the electric field can be intense. The dielectric activity causes charged ions and dipolar molecules to align along the direction of the electric field and to vibrate proportionately to the oscillation frequency (the wave frequency) of the electric field. In our previous study, the differential responses of plants to microwaves, depending on the wave polarization, evidenced that plants can respond differently to electric field polarity.[13]

As the electric field of microwaves can affect plants and alter their electric properties, there is a possibility to affect long-distance electric signaling of plants (i.e., variation potentials). The charged ions involved in electric signal propagation could be disturbed by the dielectric activity imposed by the microwaves. Therefore, the present research was conducted to investigate how a burn injury-induced electric potential would be affected by microwave exposure. Thus, burn injuries were inflicted on leaves of *Nicotiana benthamiana* wild-type plants and the resulting VP along the stem was analyzed for any possible influence associated with microwave exposure.

Nicotiana benthamiana seeds were planted in peat pellets (36-mm diameter, Jiffy 7 Peat Pellets) and grown under an 18/8 h photoperiod, under 90 – 95 μmol m^{-2} s^{-1} of photosynthetically

CONTACT M. D. H. J. Senavirathna jayasanka@hotmail.com Department of Environmental Science, Saitama University, 255, Shimo-okubo, Saitama 338-8570, Japan.

Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/kpsb.

active radiation provided by LED straight tube lights with color rendering index 80 (Model LT-NLD85L-HN, OHM Electric INC, Japan). Plants were grown to maturity; thus, they had at least six fully unfolded leaves and were about 15 cm high, prior to their use for experimentation. The growth medium was moistened only with water for seven days, after which, it was watered with a 0.01% commercial concentrated growth solution (Hyponex, Osaka, Japan) every other day. After 14 days, plants were watered with 0.05% Hyponex solution; to provide continuous moisture, plants growing in peat bags were kept in a tray containing 0.05% Hyponex solution to about 3 mm in depth. This promoted vigorous plant growth, so that they grew greener and relatively large leaf surfaces (Figure 1). All plants were grown in a growth chamber at constant 23 – 25°C.

All the experiments were conducted in an anechoic chamber layered with EMR absorbing, ferried containing foams, as explained previously,[14] after the following modifications: the internal light source was upgraded to six LED straight tube lights with color rendering index 80 (Model LT-NLD85L-HN, OHM Electric INC, Japan). The lights were set to an 18/8 h photoperiod. Photosynthetically active radiation density was set to 90 – 95 µmol m^{-2} s^{-1} at plant top by adjusting plant distance from the light source for each plant. A top mounted exhaust fan (Type ASEN104519, Panasonic Industrial devices SUNZ Co., Ltd, Aichi, Japan) was installed outside the chamber to remove heated air and to improve air circulation inside the cage. The cage was kept in a temperature-controlled room where the temperature inside the cage could be maintained at 23 – 25°C during the light period (all the studies were conducted only during the light period).

Figure 1. A representative image of *Nicotiana benthamiana* wildtype plant during electric potential recording. Letters a, b and c represent top hanging transmission microstrip antenna, reading glass electrode, and reference glass electrode respectively.

Burn injury was inflicted on the lower surface of leaves as a direct exposure to a flame produced by a general purpose, liquid-petroleum kitchen lighter. Each time, the burn injury was inflicted at the leaf tip. Therefore, the tip of the leaf suffered the worst damage; while, the surrounding tissues (around 35% of the leaf area) were exposed to extreme heat (Figure 2). Flame was applied for three seconds continuously on each leaf. Plants were exposed to background electromagnetic radiation for approximately 30 s during infliction of burn injury, as the door of the Faraday cage had to be open.

The experimental plants were exposed to microwave treatment from the top downwards. Microwave transmission was performed using a top hanging microstrip antenna (Figure 1). During the experiment, plants were exposed to 2.45 GHz continuous wave frequency microwaves with the power density at the top of the plant, at 1.9 – 2.1 W m^{-2}. The microwave generation and the power density measurement systems consisted in the same instrument setup previously reported[14] without modifications.

The EP along the plant stem was measured by inserting a Ag/AgCl gel-stabilized glass electrode (Unisense RD25, Unisense, Denmark) <20 µm in diameter into the plant stem, and another glass electrode (Unisense RD50) <50 µm in diameter, into the substrate. The reading electrode insertion depth was maintained at less than 1.5 mm horizontally through the stem. The reference electrode was inserted halfway through the culture medium (Figure 1). Electric potential was recorded into a computer through an mV meter with analog to digital converter (pH/mV-Meter, Unisense, Aarhus N, Denmark) using the recording software provided by the manufacturer (Sensor Trace BASIC 3.0, Unisense). Real time sampling was performed continuously at a sampling rate of 1 Hz, with 0.001 mV sensitivity.

Plant responses were registered before, during, and after microwave exposure (pre-microwave, on-microwave, and post-microwave, respectively). A healthy plant was selected from a stock and transferred into the experimental Faraday cage, 24 hours before initiation of the experiment. This is required to eliminate the mechanical shock due to transfer. A fresh plant was used in each case, which was discarded after the experiment. Insertion of microelectrodes into the plant stem caused EP excitation; therefore, requiring a period for stabilization. Thus, prior to treatment application, each plant was incubated for 90 to 120 min, until a steady EP was recorded continuously for 30 min.

Next, the topmost fully unfolded mature leaf (1st leaf) of the plant was exposed to burn injury. The burn injury-induced signal (depolarization and repolarization) usually requires 45 – 75 min, depending on plant. Initial burn injury-induced EP was recorded prior to microwave exposure (pre-microwave). Thirty minutes after pre-microwave EP recovery, microwave treatment was initiated and carried on for 30 – 35 more minutes, prior to the on-microwave recording of burn injury-induced EP. At that point, while microwave treatment continued, burn injury was inflicted to the 2nd topmost leaf. After the injury signal, microwave treatment was stopped, and the plant was incubated for another 30 – 35 min before inflicting post-microwave burn

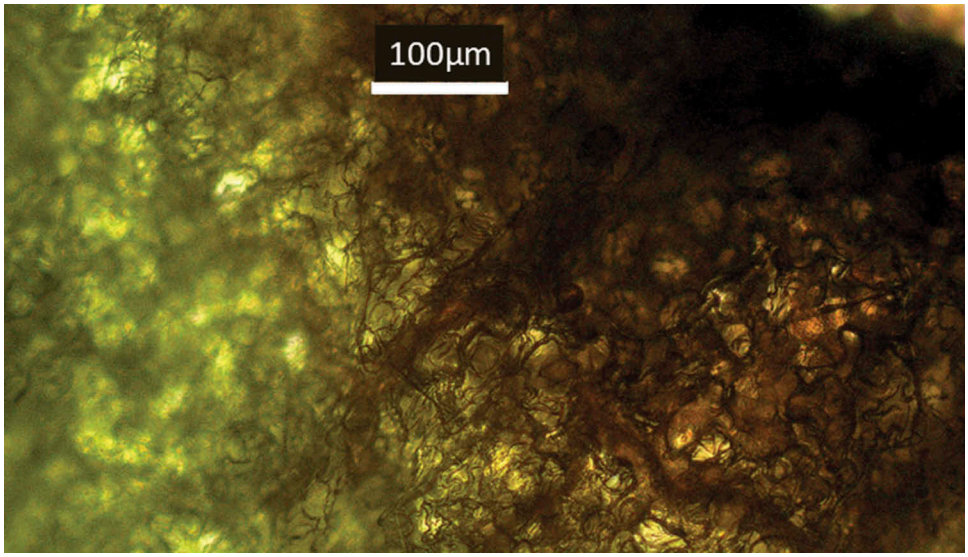

Figure 2. Microscopic images of a *Nicotiana benthamiana* leaf without injury and after burn injury. Leaf tip destroyed by flame exposure and fading brown color toward the unexposed area.

injury to the 3rd topmost leaf. After 15 – 20 more minutes from post-microwave EP recovery, EP recording was terminated.

The resting potential of each plant exhibited different mV values and did not remain steady in a single value but varied slightly over time (Figure 3(a)) and it was not influenced by the EMR exposure (Figure 4(a)). To make every measurement conveniently comparable, EP data of pre-microwave exposure, on-microwave exposure, and post-microwave exposure were normalized by bringing initial EP value of each measurement into zero (Ex. EP of on-microwave exposure duration was normalized by subtracting all EP values from the initial value at the start of signal propagation). Due to burn injury, EP depolarization occurs; therefore, maximum EP change recorded for each signal was compared between three durations (pre-microwave, on-microwave, and post-microwave). Further, the time from signal initiation to recovery and polarization rates were compared between three statuses. Although burn injury EP exhibited common characteristics (Figure 3(b)), variation in depolarized EP value and EP duration was noticed between plants. Therefore, when necessary, comparisons were made as percent change related to the initial burn injury EP, which is considered as the control signal for each plant. Statistical comparisons were performed with IBM SPSS statistics Version 25. The degree of polynomial distribution of depolarization and repolarization of EP were determined using inbuilt options of Microsoft Excel 2016. The depolarization and the repolarization phases were plotted separately, and best machine distribution was determined.

Burn injury inflicted at the tip of leaves caused complete cell destruction in the areas directly exposed to the flame. The surrounding area suffered cellular damage which caused sudden cell death. The rest of the lower epidermis showed intact cell walls; however, excessive heat may have caused protein denaturation (brownish, decolored entire cell). Vascular tissues were also destroyed near the leaf tip; whereas, vascular tissues of the surrounding area showed a cell discoloration gradually decreasing toward the unaffected area (Figure 2).

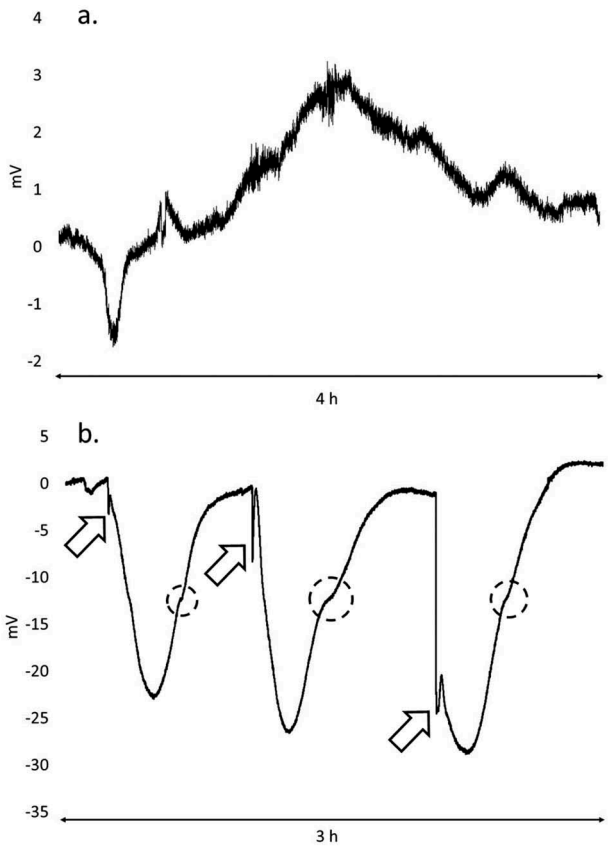

Figure 3. (a) Resting potential of a matured *Nicotiana benthamiana* plant without microwave exposure; (b) Three electric potential signals propagated after burning injury was inflicted without EMR exposure on first (left side signal), second (middle signal) and third leaf (right side signal) of the plant (fully emerged leaves were counted from the top downward). Arrows indicate EP spikes and dashed circles indicate observed slowing of signal repolarization rate. The example is representative of at least four similar experiments.

After insertion of the reading electrodes, plant EP increased gradually for 1.5 – 3 hours and became steady within an mV range unique to each plant. Stabilized EP (resting potential) exhibited a

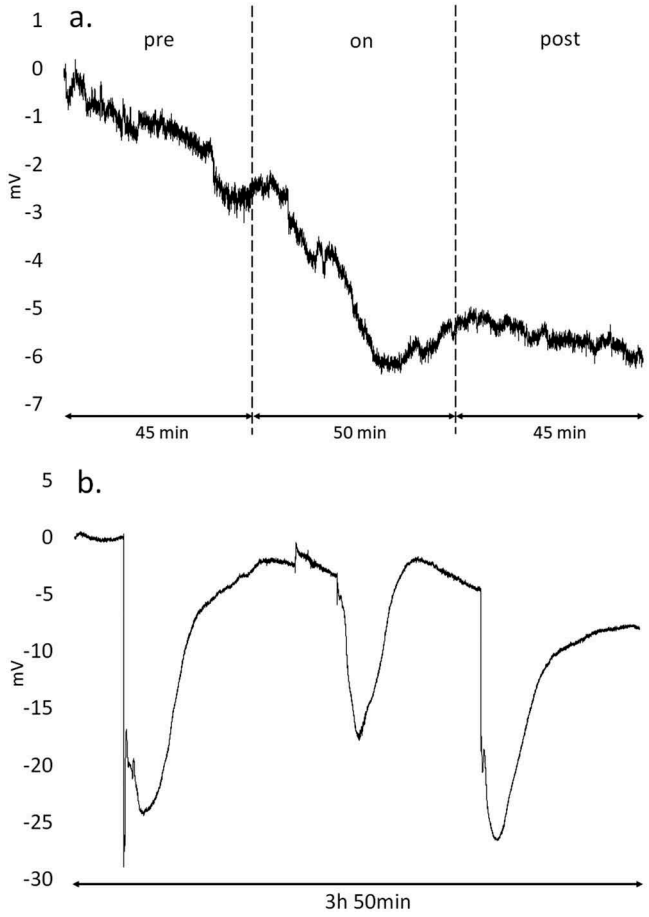

Figure 4. (a) Resting potential of a matured *Nicotiana benthamiana* plant during pre-microwave exposure (pre), on-microwave exposure (on) and post-microwave duration (post); (b) Three electric potential signals propagated after burning injury was inflicted during, pre-microwave exposure to first leaf (left side signal), on-microwae exposure to second leaf (middle signal) and post-microwave exposure to third leaf (right side signal) of the plant (fully emerged leaves were counted from the top downward). The example is representative of at least four similar experiments.

slight and slow fluctuation over time, which did not exceed ±7 mV (Figures 3(a) and 4(a)). Upon exposure to burn injury, leaf EP decreased (depolarized) rapidly at a linear rate until an EP spike showed, which is a common characteristic of each signal regardless of EMR exposure. The rate of rapid depolarization varied among signals from 0.17 to 1.04 mV s^{-1}. The EP spike occurred at a random point as the depolarization stopped and started to increase (repolarize) for a short time (varying between 30 and 150 s); this EP spike varied in magnitude from 2 to 12 mV among signals (Figures 3(b)and 4(b)). After this spike, EP started to decrease following a 2^{nd} degree polynomial distribution ($R^2 > 0.90$), again at a slow rate until reaching a minimum. After reaching this minimum value, EP started to recover. Recovery (repolarization phase) followed a 3^{rd} degree polynomial distribution ($R^2 > 0.95$) and took longer than the decreasing phase. During the repolarization phase, another common characteristic of the signal was observed, which consisted in a short-duration reduction in the repolarization rate, after which initial rate was quickly regained. However, this was not as prominent as the spike observed in the depolarization phase and not necessarily exhibit in each plant (Figure 3(b)). The recovered EP reached approximately the same magnitude as the initial resting potential.

The top three leaves of unexposed plants (control plants without microwave exposure) subjected to burn injury, resulted in three distinct EP signals with common characteristics. However, variations in amplitude were observed among signals. The mean percent difference between signal amplitude of the first two topmost leaves was 13.6%; while, the difference between the first and third leaves was 23% (Table 1). Further, it was observed that the difference between EP signal amplitudes increased in plants with high vigor, which is not discussed in the present study. When the time difference between EP depolarization and repolarization phases of the signals was considered, the averaged percent temporal difference remained within 65% to 70% for all signals (Table 2). The average difference in EP depolarization rate was 18.6% for first leaf and second leaf, while 2.7% for first and third leaf. Similarly, the average difference in EP repolarization rate was 23.5% between first and third leaf while 0.0% between first and third leaf (Table 3).

Microwave exposure caused a mean reduction of 43.3% in EP amplitude, compared to the amplitude of pre-microwave EP, which is a significant change (independent sample t-test $P < 0.05$). Again, post-microwave EP regained mean amplitude up to 17.5% of initial EP mean signal amplitude (Table 1). However, average percent time difference between EP depolarization and repolarization remained 68% – 77% for all three recordings (Table 2). The reduction in EP amplitude was mainly influenced by EP depolarization rate of the on-microwave exposure signal. The depolarization rate recorded averaged a 7.0% difference between pre-microwave and post-microwave durations; while, the on-microwave duration recorded average was 42.5% over the pre-microwave duration, which is a significant difference (t-test, $P < 0.01$). However, EP repolarization rate differed by 13.3% between pre-micro-

Table 1. Relative EP amplitude difference over initial EP in control experiments and microwave treatment experiments.

	mV		
	Leaf 1 (pre)	Leaf 2 (on)	Leaf 3 (post)
Control	28.8 ± 9.2	32.6 ± 12.6	22.1 ± 11.0
2.45 GHz	36.5 ± 6.3	20.7 ± 5.4	30.1 ± 8.2

The Leaf 1 (pre), Leaf 2 (on), and Leaf 3 (post) represent leaf one, two and three in control experiments and pre-microwave, on-microwave and post-microwave treatment stages, respectively. The 2.45 GHz represent 2.45 GHz microwaves exposed experiments, and the Control represents the experiment continued without the microwave exposure during the Leaf 2 (on) duration.

Table 2. Percent temporal difference between depolarization and repolarization of burn injury-induced electric potential.

	Leaf 1 (pre)	Leaf 2 (on)	Leaf 3 (post)
Control	64.6 ± 10.6	72.7 ± 7.9	68.0 ± 10.8
2.45 GHz	70.5 ± 15.6	68.0 ± 13.9	77.1 ± 0.9

Leaf 1 (pre), Leaf 2 (on), and Leaf 3 (post) represent leaf one, two and three in control experiments and pre-microwave, on-microwave and post-microwave treatment stages, respectively. The 2.45 GHz represent 2.45 GHz microwaves exposed experiments, and the Control represents the experiment continued without the microwave exposure during the Leaf 2 (on) duration.

Table 3. Mean depolarization rate (mV s^{-1}) in control experiments and microwave treatment experiment.

		mV s^{-1}		
		Leaf 1 (pre)	Leaf 2 (on)	Leaf 3 (post)
Control	Dip	0.035 ± 0.014	0.043 ± 0.011	0.036 ± 0.003
	Gain	0.013 ± 0.006	0.017 ± 0.010	0.013 ± 0.004
2.45 GHz	Dip	0.047 ± 0.003	0.027 ± 0.004*	0.051 ± 0.011
	Gain	0.013 ± 0.007	0.015 ± 0.003	0.014 ± 0.002

Leaf 1 (pre), Leaf 2 (on), and Leaf 3 (post) represent leaf one, two and three in control experiments and pre-microwave, on-microwave and post-microwave treatment stages, respectively. Dip and Gain represent depolarization and repolarization phases, respectively. The 2.45 GHz represent 2.45 GHz microwaves exposed experiments, and the Control represents the experiment continued without the microwave exposure during the Leaf 2 (on) duration. *indicates significant difference over pre-microwave exposure ($P < 0.05$).

wave and on-microwave status, and by 7.1% between pre-microwave and post-microwave status (Table 3).

Burning injury would trigger all three major signaling pathways in the plant; hydraulic, chemical, and electric.[15,16] When hydraulic signals are considered, a flame causes the destruction of cells in the area directly exposed, increased osmotic pressure by expansion of cell plasma in the surrounding area, and expansion of the water column in vascular tissues. This would cause a sudden increase of hydrostatic pressure in the whole water column. Therefore, there could be a flow of water generated from the damaged area of the leaf toward the intact area of the leaf, and from the shoots toward the roots. While vascular pressure increases it can alter the hydrostatic equilibrium of the shoot cells by drawing more water from the vascular tissues.[17] The changing hydrostatic pressure in cells would lead to reducing the ionic concentration of cytoplasm. Further, stretching of cellular membranes due to the influx of water may affect the mechanosensitive ion channels which affect membrane voltage and cytoplasmic ion concentration.[18] The VPs of plants are dependent on the vascular pressure surge along the stem, which propagates through the hydraulic wave generated at the injury site.[19] The burn injury-induced VP propagation speed can be reduced greatly with distance. In pea seedlings, VP propagated 40 – 50 cm min^{-1} at 5 cm from the wound site and was reduced to 5 – 15 cm min^{-1} at 20 cm away from it.[20] Similarly, in wheat seedlings the rate was 24 cm min^{-1} at 3 cm and 4.2 cm min^{-1} 15 cm away from the wound site.[21] However, VP propagation in the present study was 15 cm min^{-1} at 7 cm distance between wound site (zero point) and reading electrode (data not presented), suggesting species-specific propagation speed of VP. Also, the variation can result from plant size or age.[22] The intensity of the signal decreased with distance from the stimulus site,[6] which in the present study varied by 13.6% between the first two top leaves, and by 23% between the first and third leaves.

Microwave exposure caused a significant decline in EP magnitude, raising the question as to how the hydraulic pressure-propelled VP is affected by microwave exposure. Burn injury or wounding-induced VP can be generated by transportation of wounding substances and increased by the hydraulic wave propagation.[21,23] These chemicals can be transported through the vascular system and induce electron reactions.[3] The propagation of VP relates to the transient inactivation of H^+-ATPase and the inhibition of H^+-ATPase reduces the amplitude and the velocity of depolarization and repolarization of VPs.[3] Additionally, the depolarization and the repolarization events of VP can be influenced by activation and inactivation of cation and anion channels. The Katicheva et al.[24] confirmed the reduction of VP amplitude by lowered extracellular calcium cation (Ca^{2+}), decreased VP amplitude and depolarization rate by lowering extra-intra cellular chlorine anion (Cl^-) gradient and suppressed VP repolarization by blockage of potassium cation (K^+) efflux channels in wheat leaves. Therefore, one of the reasons for the alteration of the injury-induced VP upon microwave exposure can be the dielectric activity enforced on these dipolar (H_2O) and charged molecules. Our previous study confirmed the effect of microwaves on the rapid fluctuations observed in the resting potential, the standard deviation of which (SDEP) in *Myriophyllum aquaticum* plants, was affected by microwave exposure.[25] Additionally, SDEP responded differently to the polarity of the microwave which caused the vibration direction of charged ions and dipolar molecules to change.[13] Based on this evidence, we suggest that the reduction of VP amplitude resulted from the dielectric activity enforced on plants. The influence of microwaves can be higher on cations than it is on anions, as the propagation speed during the polarization phase was significantly reduced. However, further studies that include controlling anion, cation, H^+-ATPase and extra cellular ATP suggested by Roux et al[26] are necessary to determine whether the effect is solely due to charged ions or a combination of factors.

The VPs in plants exhibited common characteristics of the signal for a particular stress.[27] In this study VPs exhibited a distinct common EP spike despite microwave exposure. The reason for these characteristics can be the release of stress-specific chemicals and long-distance transport through the vascular tissues as necessary information to generate defense/adaptation mechanisms. On the other hand, release of chemicals to the vascular system as the response to a wound signal can be involved with specific characteristics. Therefore, we suggest that the exposure to microwaves did not affect or alter the burn injury-signaling mechanism. However, it is not known whether the reduced magnitude of the generated VP may not be enough to trigger an adaptive response mechanism by the plant. This should be further investigated with regard to injury-related gene expression[28,29] and release of stress-related chemicals in distant locations.[13,30] However, such research must be carefully conducted, as microwave exposure-induced wounding and stress-related gene expression and accumulation of stress-related substances have been reported in most studies.[11] Moreover, microwave frequency, power density, polarization and duration of exposure are factors which are known to determine the nature and the magnitude of the effects form microwave radiation. On the other hand, plant-related factors, such as species, growth stage, age and physical conditions are also very important. Therefore, further studies should consider these factors to better understand EMR effects on plant signaling phenomena.

Acknowledgments

The authors would like to thank Prof. Yuichi Kimura and Mr. Sakuyoshi Saito for their support in the preparation of the EMR exposure system (Saitama University, Japan). The knowledge on culture of *Nicotiana benthamiana* plants was obtained from Mr. Isura Nagahage (Saitama University, Japan). This work was supported by the annual budget allocations of the Saitama University, Japan.

Competing Interests

The authors declare that no competing interests exist.

References

1. Pont A, Pujolle G, Raghavan SV Communications: wireless in developing countries and networks of the future. Springer, 2010, IFIP Advances in information and communication technology, 978-3-642-15475-1. doi: 10.1007/978-3-642-15476-8.
2. Ueda H, Kikuta Y, Matsuda K. Plant communication mediated by individual or blended VOCs? Plant Signal Behav. 2012;7. doi:10.4161/psb.7.2.18765.
3. Vodeneev V, Akinchits E, Sukhov V. Variation potential in higher plants: mechanisms of generation and propagation. Plant Signal Behav. 2015;10. doi:10.1080/15592324.2015.1057365.
4. Christmann A, Weiler EW, Steudle E, Grill E. A hydraulic signal in root-to-shoot signalling of water shortage. Plant J. 2007;52.167–174. doi:10.1111/j.1365-313X.2007.03234.x.
5. Brenner ED, Stahlberg R, Mancuso S, Vivanco J, Baluska F, Van Volkenburgh E. Plant neurobiology: an integrated view of plant signaling. Trends Plant Sci. 2006;11.413–419. doi:10.1016/j.tplants.2006.06.009.
6. Fromm J, Lautner S. Electrical signals and their physiological significance in plants. Plant Cell Environ. 2007;30.249–257. doi:10.1111/j.1365-3040.2006.01614.x.
7. Zimmermann MR, Maischak H, Mithofer A, Boland W, Felle HH. System potentials, a novel electrical long-distance apoplastic signal in plants, Induced by wounding. Plant Physiol. 2009;149.1593–1600. doi:10.1104/pp.108.133884.
8. Herde O, Pena-Cortes H, Fuss H, Willmitzer L, Fisahn J. Effects of mechanical wounding, current application and heat treatment on chlorophyll fluorescence and pigment composition in tomato plants. Physiol Plant. 1999;105.179–184. doi:10.1034/j.1399-3054.1999.105126.x.
9. León J, Rojo E, Sánchez-Serrano JJ. Wound signalling in plants. J Exp Bot. 2001;52.1–9. doi:10.1093/jxb/52.354.1.
10. Stahlberg R, Cleland R, Van VE. Slow wave potentials–a propagating electrical signal unique to higher plants. In: Baluska F, Mancuso DV S, editor. Communication in plants, neuronal aspects of plant life. Berlin and Heidelberg: Springer; 2006. p. 291–308. doi:10.1007/978-3-540-28516-8_20.
11. Senavirathna MDHJ, Asaeda T. The significance of microwaves in the environment and its effect on plants. Environ Rev. 2014;22.220–228. doi:10.1139/er-2013-0061.
12. Vian A, Davies E, Gendraud M, Bonnet P. Plant responses to high frequency electromagnetic fields. Biomed Res Int. 2016;13. doi:10.1155/2016/1830262.
13. Senavirathna MDHJ, Asaeda T. Microwaves affect *Myriophyllum aquaticum* plants differently depending on the wave polarization. Biol Plant. 2017;61.378–384. doi:10.1007/s10535-016-0660-0.
14. Senavirathna MDHJ, Takashi A, Kimura Y. Short-duration exposure to radiofrequency electromagnetic radiation alters the chlorophyll fluorescence of duckweeds (*Lemna minor*). Electromagn Biol Med. 2014;33.327–334. doi:10.3109/15368378.2013.844705.
15. Stanković B, Davies E. Both action potentials and variation potentials induce proteinase inhibitor gene expression in tomato. FEBS Lett. 1996;390.275–279. doi:10.1016/0014-5793(96)00672-2.
16. Stanković B, Davies E. Intercellular communication in plants: electrical stimulation of proteinase inhibitor gene expression in tomato. Planta. 1997;202.402–406. doi:10.1007/s004250050143.
17. Malone M. Hydraulic signals. Philos Trans R Soc London Ser B Biol Sci. 1993;341.33–39. doi:10.1098/rstb.1993.0088.
18. Cosgrove DJ, Hedrich R. Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells of *Vicia faba* L. Planta. 1991;186.143–153. doi:10.1007/BF00201510.
19. Stahlberg R, Cosgrove D. Rapid alterations in growth rate and electrical potentials upon stem excision in pea seedlings. Planta. 1992;187.523–531. doi:10.1007/978-3-540-28516-8_20.
20. Stahlberg R, Cosgrove DJ. The propagation of slow wave potentials in pea epicotyls. Plant Physiol. 1997;113.209–217. doi:10.1104/pp.113.1.209.
21. Vodeneev V, Orlova A, Morozova E, Orlova L, Akinchits E, Orlova O, Sukhov V. The mechanism of propagation of variation potentials in wheat leaves. J Plant Physiol. 2012;169.949–954. doi:10.1016/j.jplph.2012.02.013.
22. Huber AE, Bauerle TL. Long-distance plant signaling pathways in response to multiple stressors: the gap in knowledge. J Exp Bot. 2016;67.2063–2079. doi:10.1093/jxb/erw099.
23. Malone M. Wound-induced hydraulic signals and stimulus transmission in *Mimosa pudica* L. New Phytol. 1994;128.49–56. doi:10.1111/j.1469-8137.1994.tb03985.x.
24. Katicheva L, Sukhov V, Akinchits E, Vodeneev V. Ionic nature of burn-induced variation potential in wheat leaves. Plant Cell Physiol. 2014;55.1511–1519. doi:10.1093/pcp/pcu082.
25. Senavirathna MDHJ, Asaeda T. Radio-frequency electromagnetic radiation alters the electric potential of *Myriophyllum aquaticum*. Biol Plant. 2014;58.355–362. doi:10.1007/s10535-013-0384-3.
26. Roux D, Faure C, Bonnet P, Girard S, Ledoigt G, Davies E, Gendraud M, Paladian F, Vian A. A possible role for extra-cellular ATP in plant responses to high frequency, low amplitude electromagnetic field. Plant Signal Behav. 2008;3.383–385. doi:10.1007/s00425-007-0664-2.
27. Mancuso S. Hydraulic and electrical transmission of wound-induced signals in Vitis vinifera. Aust J Plant Physiol. 1999;26.55. doi:10.1071/PP98098.
28. Vian A, Roux D, Girard S, Bonnet P, Paladian F, Davies E, Ledoigt G. Microwave Irradiation affects gene expression in plants. Plant Signal Behav. 2006;1.67–70. doi:10.4161/psb.1.2.2434.
29. Beaubois E, Girard S, Lallechere S, Davies E, Paladian F, Bonnet P, Ledoigt G, Vian A. Intercellular communication in plants: evidence for two rapidly transmitted systemic signals generated in response to electromagnetic field stimulation in tomato. Plant Cell Environ. 2007;30.834–844. doi:10.1111/j.1365-3040.2007.01669.x.
30. Tkalec M, Malarić K, Pevalek-Kozlina B. Exposure to radiofrequency radiation induces oxidative stress in duckweed *Lemna minor* L. Sci Total Environ. 2007;388.78–89. doi:10.1016/j.scitotenv.2007.07.052.