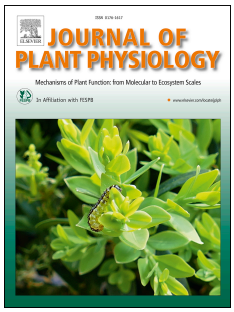
Non thermal 2.45 GHz electromagnetic exposure causes rapid changes in Arabidopsis thaliana metabolism

Alexis Porcher, Sébastien Girard, Pierre Bonnet, Raphaël Rouveure, Vincent Guérin, Françoise Paladian, Alain Vian

Journal Pre-proof



Non thermal 2.45 GHz electromagnetic exposure causes rapid changes in Arabidopsis thaliana metabolism

Alexis Porcher, Sébastien Girard, Pierre Bonnet, Raphaël Rouveure, Vincent Guérin, Françoise Paladian, Alain Vian

PII: S0176-1617(23)00093-7

DOI: https://doi.org/10.1016/j.jplph.2023.153999

Reference: JPLPH 153999

To appear in: *Journal of Plant Physiology*

Received Date: 12 December 2022

Revised Date: 13 April 2023

Accepted Date: 28 April 2023

Please cite this article as: Porcher, A., Girard, Sé., Bonnet, P., Rouveure, Raphaë., Guérin, V., Paladian, Franç., Vian, A., Non thermal 2.45 GHz electromagnetic exposure causes rapid changes in Arabidopsis thaliana metabolism, *Journal of Plant Physiology* (2023), doi: https://doi.org/10.1016/j.jplph.2023.153999.

Credit author statement

AP, SG, PB, RR, VG, FP and AV conceived the experiments. AP, SG and AV made the experiments. AP and AV write the manuscript draft. SG, PB, RR, VG and FP proof-read the manuscript. All authors have approved the manuscript.

Control plants

Horn antenna

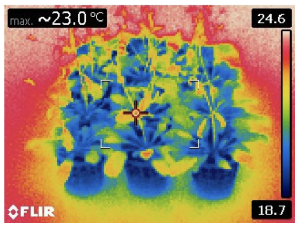
2.45 GHz
EMF radiation

Exposed plants

Luxtron
reflectometric probe

Leaf surface
temperature

Plant
thermal imaging



EMF non-thermal exposure vs control unexposed plants

0 15 30 60 min

C_{15} C_{30} C_{60}

qRT-PCR analysis
H_2O_2 quantification
Enzyme activities

E_{15} E_{30} E_{60}

EXPOSURE

0 15 30 60 min

Results

gene expression

Calmodulin
ZAT12
RBOH-F
APX1 ↗

RBOH-D
CAT →

biochemical markers

DHA
H_2O_2 ↗

ASA
MDA
GSH/GSSG →

Title : Non thermal 2.45 GHz electromagnetic exposure causes rapid changes in Arabidopsis thaliana metabolism.

Alexis Porcher[1†], Sébastien Girard[1], Pierre Bonnet[1], Raphaël Rouveure[2], Vincent Guérin[3], Françoise Paladian[1], Alain Vian[3]

(1) : Université Clermont Auvergne, Clermont Auvergne INP, CNRS, Institut Pascal, F-63000 Clermont-Ferrand, France
(2) : INRAE Clermont Clermont Auvergne University, INRAE, UR TSCF, F-63000 Clermont-Ferrand, France
(3) : Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

† present address : Crop Light group, Faculty of Biological and Environmental Sciences, Biocenter 3, Viikinkaari 1, 00790 Helsinki, Finland.

Correspondence to :

Professor Alain Vian, Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France

Phone: (33) 241 225 669

Email : alain.vian@univ-angers.fr

Abstract

Numerous studies report different types of responses following exposure of plants to high frequency electromagnetic fields (HF-EMF). While this phenomenon is related to tissue heating in animals, the situation is much less straightforward in plants where metabolic changes seem to occur without tissue temperature increase. We have set up an exposure system allowing reliable measurements of tissue heating (using a reflectometric probe and thermal imaging) after a long exposure (30 min) to an electromagnetic field of 2.45 GHz transmitted through a horn antenna (about 100 $V.m^{-1}$ at the plant level). We did not observe any heating of the tissues, but we detected rapid increases (60 min) in the accumulation of transcripts of stress-related genes (*TCH1* and *ZAT12* transcription factor) or involved in ROS metabolism (*RBOHF* and *APX1*). At the same time, the amounts of hydrogen peroxide and dehydroascorbic acid increased while glutathione (reduced and oxidized forms), ascorbic acid, and lipid peroxidation remained stable. Therefore, our results unambiguously show that molecular and biochemical responses occur rapidly (within 60min) in plants after exposure to an electromagnetic field, in absence of tissue heating.

Keywords : electromagnetic exposure, non-thermal, stress-marker genes, hydrogen peroxide

Abbreviations:

HF-EMF: High Frequency-Electromagnetic Field

SAR: Specific Absorption Rate

Introduction

In nature, plants are increasingly subjected to various kind of high frequency electromagnetic fields (HF-EMF) due to the densification of base station networks that comply with the exposure limits defined by the standards (ICNIRP, 2020; Israel et al., 2013). The question of whether high-frequency electromagnetic fields are likely to induce changes in plant metabolism and/or development remains however largely open, although many studies tend to show that they do (Kaur et al., 2021; Vian et al., 2016). Biological effects of electromagnetic field are generally interpreted in term of specific absorption rate (SAR) that relies on tissue heating after exposure to HF-EMF. No clear biological changes have been proven to occur in animal or human cells after EMF exposure in the absence of thermal effect (Habauzit et al., 2014; Roux et al., 2010). However, some studies clearly suggested that it could be different in plants in which various kind of biological responses have been detected after exposure to low amplitude, non-thermal EMF. Indeed, Halgamuge et al. (2015) have shown that soybean seedlings exposed to various levels of 900 MHz HF-EMF (SAR of 4.8 10^{-7} to 20 10^{-3} WKg^{-1}) for durations ranging from 2h to 5 days induced changes (mainly growth inhibition) of the plant root and hypocotyl. Similarly, 4h exposure to HF-EMF (1800 MHz, SAR of 1.69 10^{-1} Wkg^{-1}) affected root development, coleoptile length and total chlorophyll content in maize (Kumar et al., 2016). Short exposure of rose buds to low amplitude HF-EMF (900 MHz, SAR of 7.2 10^{-4} W kg^{-1}) in a mode stirred reverberation chamber (MSRC) did not change the length of the axis produced after bud outgrowth, but the post-formed axillary buds produced 45% shorter axis, suggesting that growth changes may occur in a delayed manner (Grémiaux et al., 2016). Recently, Upadhyaya et al. (2022) noted after 12-120h exposure to HF-EMF (1800 MHz , SAR of 3.16 10^{-2} W kg^{-1}) a decrease of seed germination, a reduced growth of plantlets, a reduction of leaf size and an increase in H_2O_2 production. Environmental sensing and signaling in plants are largely dependent on reactive oxygen species (ROS) as signaling molecules, particularly H_2O_2 which is the most stable and transportable ROS, involved in subcellular signaling as well as in cell-to-cell signaling (Mittler et al., 2022). Singh et al. (2012) observed reduced growth of mung bean seedlings hypocotyl and roots after 2h low amplitude HF-EMF exposure, along with a

4.5-fold elevation of H_2O_2 content and to concomitant increase of the activities of several antioxidant enzymes: superoxide dismutase (4-fold), catalase (3.3-fold), ascorbate peroxidase (2.5-fold), guaiacol peroxidase (2-folds) and glutathione reductase (5-fold). Similar finding was reported by Chandel et al. (2017) in *Allium cepa* roots after 4h exposure to low level HF-EMF (2100 MHz, SAR of 2.82 10^{-1} W kg^{-1}): H_2O_2 content was increased by 5-fold, while superoxide dismutase and catalase activities were up-regulated 2.4-fold. Gene expression was also affected after exposing plants to low level (non-thermal) HF-EMF: tomato plants exposed to 10 min at 900 MHz (5 Vm^{-1}) displayed rapid and transient changes in the expression of several stress-related genes, namely calmodulin, calcium-dependent protein kinase, *bZIP* transcription factor, proteinase inhibitor (*PIN2*) and Chloroplast mRNA Binding Protein (*CMBP*, Roux et al., 2006; Roux et al., 2008; Vian et al., 2006). These results were partially and independently replicated (Rammal et al., 2014), although these authors used a much less sophisticated exposure system and a different exposure condition (10 days, 1250 MHz, 6 Vm^{-1}). These changes occurred in a systemic way, since an exposure of a single leaf to HF-EMF triggered changes in gene expression not only locally (*i.e.* at the site where the exposure to HF-EMF was performed), but also at distance in the terminal leaf that was shielded from HF-EMF (Beaubois et al., 2007). More recently, Kundu et al. (2021a) showed that exposure of rice plants over a long period of time (6h per day for 32 days, 2.75 mW m^{-2}) resulted in a significant increase in calmodulin, calcium dependent protein kinase and phytochrome C genes expression. Furthermore, the same research group (Kundu et al., 2021b) demonstrated in 40 days-old ´Swarnaprabha´ rice plants that a single HF-EMF irradiation (2 h 30 min, 1837.50 MHz, 2.75 mWm^{-2}) increased the expression level of some stress and light-signaling related genes: calmodulin (2.5-fold), *bZIP* transcription factor (2.27-fold) and phytochrome B and C (3.98- and 5.87-fold, respectively). A mechanism that integrates the rapid responses of plants to exposure to very high frequency electromagnetic waves has recently been proposed (Kaur et al., 2021).

In the present work, we used *Arabidopsis thaliana* as a plant model to assess whether a short (30 min), non-thermal exposure to HF-EMF could trigger rapid changes in plant metabolism. We used a horn antenna that delivers an electromagnetic signal with a fixed angle of incidence and polarization to get similar exposure configuration to those used in the vast majority of the works, in preference to the mode-stirring reverberation chamber that we used in our previous work (Vian et al., 2006; Roux et al., 2006), which allows to create a homogeneous and isotropic electromagnetic environment, but which remains an equipment that is seldomly used for bioelectromagnetic studies. We paid particular attention to ensure that the exposure (30 min, 2450 MHz) did not induce thermal effects in the plants and investigate the possible resulting effects of this non-thermal exposure in terms of rapid (within one hour) changes in gene expression and biochemical pathways. Using these carefully controlled experimental conditions, we provide evidence that exposure to non-thermal HF-EMF was perceived by plants and induced metabolic changes in the rosette of *Arabidopsis thaliana*.

Materials and Methods

Plant material and culture

Arabidopsis (*Arabidopsis thaliana* ecotype Col-0) were grown in controlled environmental conditions in culture chambers (Binder KBW720) under 150-160 $\mu mol.m^{-2}.s^{-1}$ (provided by OSRAM Fluora L18W/77 fluorescent tubes), 21±1°C at a 16h light period in an adapted substrate for young plants in module trays (TraySubstrat U44-551, Klasmann-Deilmann, Bourgoin Jallieu, France). The plants were watered by sub-irrigation with a nutritive solution and grown for 4 weeks to obtain rosettes with a diameter of 4-5 cm.

Plant exposure to electromagnetic field and material sampling

The experiments were set-up in a shielded environment (metal-walled room) to avoid possible interaction with the external (environmental) electromagnetic background. A signal synthesizer (SMB100A, Rohde & Schwarz, Munich, Germany) and modulator (AFG3021B, Tektronix, Beaverton, USA) produced a signal, amplified to its nominal amplitude by a high frequency amplifier (ASO104-30/17, Milmega, Ryde, United Kingdom) and frequency modulated by a 100 kHz square signal. Thus, the amplitude of the signal remained constant while the 100 kHz frequency modulation broadens the spectrum of the radiated signal that was emitted as a high-frequency electromagnetic field through a horn antenna (Model 3115, ETS Lindgren, Cedar Park, USA). The antenna was set-up 60 cm above the plants, with an incidence angle of 45° (Fig. 1) and plants were exposed for 30 min in groups of 12, arranged in a rectangular area of about 20x30 cm. The resulting EMF amplitudes were measured at the plant level using a tri-axial probe (EP-601, Narda, Cisano sul Neva, Italy) positioned in the center and at each corner of the plants panel and was found to range from 99 to 110 V m^{-1} at the corners and 125 V m^{-1} in the center, Figure 2A). In these conditions, and given the opening of the horn antenna (24 x 13.5 cm), the far field criterion was 95 cm, thus placing the plants close to this distance (about 85 cm at the center of the plate) in the Fresnel zone (between D^2/(2*wavelength) and

158 2*D^2/wavelength) to reach the maximum power value. The spectral characteristics of the
159 EMF (before amplification) was determined by performing a Fast Fourier Transform of the
160 temporal recording of the EMF signal and showed a typical bandwidth at -20 dBm of about
161 30 MHz, from 2.43 to 2.46 GHz (Fig. 2B). Arabidopsis plants are rosette-shaped (the floral
162 stem being most of the time not yet formed), thus creating a more homogeneous biological
163 system for the interaction with the electromagnetic waves than it would be possible to achieve
164 with other plants displaying axis with several different orientations (Kundu et al, 2022). The
165 impact of EMF on plants in terms of thermal effect was assayed through two separate yet
166 complementary methods. First, we measured the leaf surface temperature using a Luxtron®
167 reflectometric probe (LumaSense Technologies, Santa Clara, USA). The recording of the
168 temperature evolution was started after leaf temperature stabilized and performed every 5 s
169 all along the EMF exposure. Second, we monitor the leaf temperature evolution with a thermal
170 imaging camera (FLIR E40, Teledyne Technologies, Thousand Oaks, USA) with a built-in
171 calibration of temperature scale. The Specific Absorption Rate (SAR, in WKg^{-1}) corresponding
172 to the exposure conditions was determined according to equation (1) :

173 $$SAR = C \times (dT/dt)_{t \to 0} \quad (1)$$

174 Where C is the heat capacity ($J^{-1}Kg^{-1}K^{-1}$), T the sample temperature and t the time elapsed
175 since the beginning of the EMF exposure.

176 Plant leaves were harvested 15, 30 and 60 min after the end of the 30 min exposure,
177 immediately frozen in liquid nitrogen and lately used for biochemical assays and molecular
178 biology experiments. Control (*i.e.* non exposed plants) were harvested at the same points of
179 kinetics (they were manipulated similarly, except that they were not subjected to EMF
180 exposure). Each sample per condition (control and exposed) and per time point was
181 constituted with a pool of fully developed leaves from three plants. The sampling of the material
182 was repeated three times after three independent exposure experiments.

183 *RNA isolation, cDNA synthesis and gene expression quantification*

Frozen materials were ground to a fine powder using Retsch® grinder machine prior RNA isolation using the Nucleospin RNA plant Mini Kit including DNAse treatment (Macherey Nagel, Hoerdt, France), according to the manufacturer instructions. The conversion of mRNAs to cDNA was achieved from 500 ng of total RNA, using the iScript Reverse transcription supermix for RT-qPCR (Bio-Rad, Marnes-la-Coquette, France) following the manufacturer instruction. Relative gene expressions were measured using real time quantitative PCR (RT-qPCR) performed in a final volume of 15 µl of PCR mixture containing 3 µl of 50 times diluted cDNA, 1 µl of primers pairs (10 µM), 4 µl iQ SYBR Green supermix (Bio-Rad, Marnes-la-Coquette, France) and 7 µl of ultrapure water. The amplifications were performed using the CFX96 Real Time System (Bio-Rad) with a standard amplification program previously describe in Porcher et al. (2021). Relative transcript abundances were expressed to the control condition (unexposed samples harvested just after the end of the exposure) after $2^{-\Delta\Delta Ct}$ calculation (Livak and Schmittgen, 2001) along with two house-keeping genes (Aldehyde dehydrogenase, *ALDH* and Glyceraldehyde-3-phosphate dehydrogenase, *GAPDH*), taken as internal references (Jin et al*.,* 2019). The primers sequences used in this study are available in the supplementary table 1.

Hydrogen peroxide quantification

Hydrogen peroxide (H_2O_2) levels were assayed using the Amplex™ Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen). Frozen tissue (20 mg) was homogenized in 100 µl of 1X kit reaction buffer and kept 5 min on ice. After centrifugation (5 min at 14,000 x g at 4°C), the supernatant was diluted (10-fold) in 1X reaction buffer. An equal volume of reaction mix freshly prepared according to the manufacturer protocol and mixed to 50 µl of diluted supernatant in a flat bottom black plate (Thermo Scientific™ Nunc™ F96 black MicroWell plate). H_2O_2 levels were determined after a 30 min incubation step (in darkness at room temperature) by fluorescence measurement (570 nm excitation, 590 nm emission) using a microplate reader (BMG Labtech FLUOstar Omega) and a standard curve obtained from known concentrations of commercial H_2O_2 (Sigma).

Quantification of reduced and oxidized forms of glutathione (GSH/GSSG) and ascorbic acid quantifications (AsA/DHA)

Amounts of the reduced (GSH) and oxidized (GSSG) forms of glutathione were determined from 50 mg of frozen leaf tissues using the GSH-GSSG-Glo™ Assay kit (Promega, Madison, WI, USA) adapted to plant tissues (Porcher et al., 2020). Ascorbic acid (AsA) and dehydroascorbic acid (DHA) quantities were determined from 40 mg of frozen tissues using a colorimetric assay described in Gillespie and Ainsworth (2007).

Lipid peroxidation assay

Malondialdehyde (MDA) content was assayed as described by Jin et al (2020). Briefly, 50 mg of finely grinded plant tissue was homogenized in 10% trichloracetic acid and centrifuged for 15 min at 4000xg. The supernatant (300 µl) was added to an equal volume of 0.67% thiobarbituric acid and incubated 30 min at 95°C under gentle shaking in a dry bath. After a centrifugation (15 min at 10,000xg), the supernatant was used to measure absorbances at 450, 532 and 600 nm in a BMG Labtech SpectroStar nano spectrophotometer to determine MDA content (subsequently expressed as $\mu mol.g^{-1}$ FW) accordingly to equation 2:

$$[MDA] = 6.45 \times (A_{532}-A_{600})-0.56 \times A_{450} \quad (2)$$

Statistical analysis

Statistical analysis was performed with the R statistical software (R core Team, 2020) using the statistical tests described in figure captions. Graphics were produced using Veusz 3.4 (https://veusz.github.io/) software.

Results

Leaves temperature evolution after HF-EMF exposure and SAR analysis

The 30 min exposure to the 100 kHz frequency modulated 2.45 GHz HF-EMF did not cause any significant increase in leaf temperature, as demonstrated by measurements performed with the Luxtron reflectometric probe (Fig. 3A). The leaves temperature (about 20.3°C) displayed variations of about 0.1-0.2°C all along the exposure (30 min) and did not show any significant increase after the application of the EMF exposure (Fig. 3 A, vertical line). Similarly, image acquisition with a thermal imaging camera showed that the surface temperature of the leaves was close to 20°C before and after exposure to 2.45 GHz EMF, confirming the temperature measurement performed with the Luxtron reflectometric probe and the absence of detectable temperature increase after exposure (Figure 3 B). However, it cannot be excluded that a slight temperature rise occurred but remained within the temperature measurement uncertainty (0.1°C). Consequently, we considered a potential maximal leaf temperature increase of 0.1°C after 30 min of exposure to calculate the corresponding maximum potential SAR value of 0.21 W kg^{-1} according to Eq. (1), assuming a leaf thermal capacity equal to 3.75 kJ $K^{-1}kg^{-1}$ (Kitaya *et al.*, 2003).

Gene expression after plant exposure to EMF

TCH1 calmodulin transcript remained fairly stable all along the 60 min kinetic in non-exposed plants (Figure 4A, white bars). In contrast, plant exposure to a non-thermic 100 kHz-modulated 2.45 GHz EMF caused a rapid (15 min) increase in the accumulation of calmodulin transcripts that became significant 60 min after the end of the exposure (Figure 4A, grey bars). At the same time, the *ZAT12* transcription factor transcript also rapidly accumulated 15 min after the end of EMF and became significantly different from that measured in control, unexposed plants 30 and 60 min after the end of the EMF exposure (Figure 4B, grey).

We measured the expression level of the NADPH oxidase/respiratory burst oxidase homolog (RBOH), isoforms D and F, that are key enzymes involved in the production of superoxide rapidly dismutates into H_2O_2 by apoplastic superoxide dismutases (SODs), in response to environment constraints (Castro et al., 2021). The expression level of *RBOHD* gene isoform remained essentially constant and unaffected after EMF exposure (Figure 5A). In contrast, if the accumulation of the *RBOHF* isoform transcript displayed slight changes in the control, unexposed plants (Figure 5B, white bars), the exposed plants showed constantly higher amounts of transcripts that became statistically significant 60 min after the end of the exposure (Figure 5B, gray bars), suggesting a higher potential of H_2O_2 production in exposed plants. The expression of the main catalase gene isoform *CAT2* involved in the bulk scavenging of H_2O_2 (Mhamdi et al., 2010), was constant in control plants and not primed by EMF exposure (Figure 5C): a significant decrease in the accumulation of *CAT2* transcript was even noted 30 min after the end of the exposure. In contrast, the expression level of the ascorbate peroxidase *APX1*, involved in the fine tuning of H_2O_2 homeostasis was significantly higher in exposed plants after 60 min (Figure 5D, grey bars). It is worth noting that it remained essentially constant in control, unexposed plants (Figure 5D, white bars).

H_2O_2 accumulation and scavenging

Hydrogen peroxide significantly accumulated 60 min after the end of the exposure, comparatively to that observed in the unexposed control (Figure 6A). The amount of H_2O_2 was about 213.8 nmol.g^{-1} FW while it increased to 293.9 nmol.g^{-1} FW (+37.4 %) in the exposed plants.

The amount of total glutathione increase (however not significantly) in exposed plants 60 min after the exposure (Figure 6B, left), mainly due to an elevation in the content of GSH (about 40%, from 174.52 to 245.53 nmol g^{-1} FW in control and exposed plants, respectively), while the amount of GSSG remains approximately the same (21.47 vs 26.11 nmol g^{-1} FW in control

and exposed plants, respectively). The amplitude of these changes was not sufficient to significantly shift the GSH/GSSH ratio (8.64 vs 9.31 in control and exposed plants, respectively, Fig 6B right). The quantification ascorbic acid (ASA) slightly increased (about 19.6%, from 11.34 to 13.57 µmol g^{-1} FW) after exposure, while being not significantly different from that found in unexposed plants (Fig 6C, right). In contrast, the quantity of dehydroascorbic acid (DHA) was significantly higher (about 124%, from 1.95 to 4.38 µmol g^{-1} FW) 60 min after exposure (Fig 6C, right).

Malondialdehyde formation

The amount of malondialdehyde (Fig 6D) remained very similar in control and exposed plants (4.88 vs 4.67 µmol. g^{-1} FW, respectively) showing that exposing plants for 30 min to a non-thermic 100 kHz-modulated 2.45 GHz EMF did not affect lipid peroxidation.

Discussion

The existence of cellular/molecular and morphological changes after non-thermal electromagnetic exposure is largely a matter of debate in the present literature. An EMF-exposure is generally considered as non-thermal if the tissue temperature rise resulting from the exposure is lower than 1°C after 30 min of exposure (IEEE, 2005; ICNIRP, 2020). Although human cells did not show any significant metabolic changes after such kind of exposure (Roux et al., 2010; Habauzit et al., 2014), the situation is far from being so straightforward in plants. Indeed, several works highlighted that electromagnetic exposures of various frequencies and consistent SAR levels (about 0.5 Wkg^{-1}) caused different kind of cellular responses, including cellular changes and mitotic alteration (Vian et al., 2016; Kaur et al., 2021). Răcuciu et al. (2015) showed that exposing maize seedlings to 1 GHz for 8 h (DAS of 0.47 Wkg^{-1}) resulted in an important decrease in the total pigment (a + b chlorophylls and carotenoids) and a reduction of plantlets growth. Akbal et al (2012) demonstrated that 48 h exposure of dormant *Lens culinaris* seeds to HF-EMF (1800 MHz, SAR 0.76 W kg^{-1}) did not affect germination but

caused a delayed root growth inhibition and an increase in c-mitosis number. Other DNA alterations (increase in micronuclei, ranging from 2.3 to 7-fold compared to non-exposed samples) were found after exposing *Vicia faba* root tips to 915 MHz (SAR: 0.4-1.6 W kg^{-1}) during 72 h (Gustavino et al., 2016). Pesnya and Romanovsky (2013) also observed an increase in mitotic abnormalities after exposing onion root tips to 900 MHz for 9 h (SAR: 1.4 W kg^{-1}). Although it is likely that such alterations are accompanied by changes in gene expression, relatively few studies have investigated this aspect. Engelmann et al. (2008) analyze the global gene expression pattern based on Affymetrix ATH1 microarrays after exposing cultured *Arabidopsis thaliana* cells for 24h to HF-EMF (1.9 GHz, average SAR: 0.75 Wkg^{-1}) and found that 10 genes displayed a significant yet limited variation in their expression (2.5 fold).

Here we used a horn antenna (Fig. 1) to generate an electromagnetic field whose characteristics were deterministic with a fixed incidence, contrary to a finite number of plane waves with random polarization and incidence angle, as in the MSRC that we used in our former studies (Beaubois et al., 2007; Grémiaux et al., 2016; Roux et al., 2008; Vian et al., 2006). This point was essential to ensure that all the waves contributed to the coupling with the plants (here represented by the rosettes of *Arabidopsis* on which the waves reached with an angle of incidence of 45°, Fig. 1). We used in the present work an exposure condition that keep constant the amplitude of the electromagnetic wave (2.45 GHz, about 100 Vm^{-1} at the plant level, Fig. 2A) while the 100 kHz frequency modulation broadens the spectrum of the radiated signal (about 30 MHz bandwidth, Fig. 2B). This exposure condition did not cause any measurable shift in leaf surface temperature over the 30 min of the exposure duration (the temperature oscillation was less than 0.2 °C), as reported by two different measurement methods: the Luxtron reflectometric probe and the thermal imaging (Fig. 3A-B). This absence of tissue heating, that allowed us to selectively study the non-thermal responses of plants to HF-EMF exposure, may be related to the high surface to volume ratio of the plants (Vian et al., 2007) that strongly favor heat dissipation. One could note that the resolution of the thermal

338 camera sensor does not exclude the existence of microscopic temperature rise that are
339 however unlikely to affect the whole leaf temperature.

340 Taking the uncertainty of the temperature measurement as the maximum potential heating
341 value (here 0.1°C) resulted in a maximal potential SAR value of 0.21 W kg^{-1}, assuming a leaf
342 thermal capacity equal to 3.75 kJ $K^{-1}kg^{-1}$ (Kitaya *et al.*, 2003). It is worth noting that this
343 potential maximal value was also comparable to those measured in other works reporting
344 biological effects after exposing plants to HF-EMF: 0.169 Wkg^{-1} (Kumar et al., 2016), 0.282 W
345 kg^{-1} (Chandel et al., 2017), for which no temperature increase was neither observed.

346 We reported here, in this non-thermal exposure condition, that the expression of stress-related
347 genes such as calmodulin *TCH1* (Fig. 4A) or the transcription factor *ZAT12* (Fig. 4B)
348 significantly increased 60 min after the end of the exposure, while remained at a low level in
349 the control, unexposed plants. *TCH1* gene expression was previously reported to increase in
350 tomato after exposure to non-thermal HF-EMF (Roux et al., 2006; Roux et al., 2008) (,a result
351 later confirmed by Rammal et al. (2014) also in tomato and more recently by Kundu et al.
352 (2021a,b) in rice. The expression level of stress-related transcription factor gene *bZIP* was also
353 reported to increase in such condition (Vian et al., 2006; Beaubois et al., 2007), a result also
354 recently confirmed after exposing rice to low power HF-EMF (Kundu et al., 2021a,b). It is worth
355 noting that the accumulations of *TCH1*, *bZIP* and *PIN2* occured more rapidly (0-15 min after
356 the end of the 10 min exposure) when the exposure was performed in a MSRC (Roux et al.,
357 2006, 2008; Beaubois et al., 2007). This difference may result from the multiple reflections of
358 the electromagnetic waves on the metallic walls of the CRBM, which considerably increase the
359 possibilities of coupling of the electromagnetic field with the plant. Changes in the expression
360 levels of calmodulin (*TCH1*) and *ZAT12* genes suggested an activation of calcium and ROS
361 signal transduction pathways that are closely related (Davletova et al., 2005; Gilroy et al.,
362 2016). We therefore analyzed the expression level of several genes corresponding to the main
363 actors of production and scavenging of H_2O_2. We showed that the expression level of the
364 *RBOHD* gene, involved in hypoxia signaling (Yang and Hong, 2015) was not affected after

exposing plants to HF-EMF (Fig. 5A). In contrast, *RBOHF*, which appears as a regulation hub targeted by calcium-inducible protein kinases (Han et al, 2018; Castro et al., 2021), was induced after exposure and became significantly more expressed after 60 min (Fig. 5B), suggesting an increase in H_2O_2 production and confirmed by its direct quantification (Fig. 6A). Interestingly, this was concomitant with the increased expression of *TCH1* (Fig. 4A) that is a marker of an increase in cytosolic calcium concentration.

The expression level of catalase (*CAT2*), an essential pathway of bulk, low affinity H_2O_2 detoxification (Gill and Tuteja, 2010), was not affected by HF-EMF exposure (Fig. 5C), whereas that of *APX1*, which finely regulates the H_2O_2 level due to its high affinity, was significantly increased after 60 min (Fig. 5D), indicating a possible involvement of the AsA/glutathione detoxification pathway. The total quantity of glutathione did not change after exposure (Fig. 6B), as well as the GSH/GSSH ratio. Ascorbic acid (Fig. 6C, left) level did not change either, while the dehydroascorbic acid was the only actor of the AsA/glutathione pathway displaying a significant variation 60 min after exposure (Fig. 6C, right). Taken together, these results showed that non-thermic HF-EMF trigger a low but significant increase in H_2O_2 production which reflects more of a signal transduction mechanism, acting in synergy with possible calcium movements as reported by calmodulin increase, than a stress response. This observation is consistent with the general scheme proposed by Kaur et al. (2021) in which the initial interaction of electromagnetic field with plants triggers both calcium movements and ROS metabolism as initial events of signal transduction cascade that later cause changes in plant development. Modifications in ROS production and/or scavenging were in fact reported among others in wheat (Chen et al., 2009), mung bean (Singh et al., 2012), onions (Chandel et al., 2017), duckweed (Tkalec et al., 2007) and Arabidopsis (Senevirathna et al., 2020). It is also striking that quite similar changes in ROS metabolism have been reported after exposure of plants to a static magnetic field (SMF) or to an extremely low frequency electromagnetic field (ELF-EMF), despite the fundamental differences in the nature of these fields. Indeed, exposing plants to static magnetic field higher than the geomagnetic field generally caused an

increase in H_2O_2 production and ROS-scavenging enzymes activities and H_2O_2 synthesis-related gene expression (see the reviews from Maffei et al., 2014, Saletnik et al., 2022 and Hafeez et al. (2023) for details). In contrast, exposing plants to near null magnetic field (much lower to that of the geomagnetic field) resulted in a drastic reduction of H_2O_2 in roots and shoots (5-folds after 24h), likely related to the up-regulation of gene expression of ROS scavenging enzymes (Parmagnani et al., 2022). ROS metabolism is therefore an essential component in plants' perception of the presence/absence of static magnetic fields, as well as of their exposure to very high frequency electromagnetic fields.

Conclusion

We demonstrated here that a very high frequency electromagnetic field, which we have verified as having no heating effects in plants, was able to induce rapid changes in the expression of several genes involved in signal transduction or ROS metabolism, as well as the production of hydrogen peroxide. This ability appears to be unique to plants, as it is clear that non-thermal exposure does not elicit a molecular response in humans (Roux et al., 2010; Habauzit et al, 2014). These results, in addition to many previous observations, raise the question of the relevance of SAR in plants, insofar as this quantity (which otherwise adequately accounts for biological effects in animals) is based on the thermal effects induced by exposure to electromagnetic fields. This major difference could be the consequence of the fundamental contrast in the logic of development (Vian et al., 2007), as plants create large surfaces, which forces them to immobility but favors interaction with the environment (in this case interaction with electromagnetic waves), whereas animals create volume and comparatively little surface with the environment (which favors their mobility). The interaction mechanism of the electromagnetic field, which clearly does not depend on thermal effects, remains as yet misunderstood although several hypotheses have been proposed (Cifra et al, 2011; Romanenko et al, 2017). Studies are still needed, and we believe that a more comprehensive knowledge of the molecular and biochemical changes (such as those provided by an RNA sequencing or metabolic analyses) taking place after non-thermal exposure could allow us to

419 make effective progress in understanding the mechanism of interaction between plants and
420 electromagnetic field.

421

Figure legends

Fig. 1: experimental set-up. The electric polarization is indicated by a red arrow.

A. Schematic representation. The electromagnetic field was generated by a signal synthesizer, amplified, and then emitted through a horn antenna placed at a distance h=60 cm above the plants with an angle of 45°.

B. Picture of the set-up. Note that the experiments are performed in a shielded room, totally isolated from the electromagnetic background noise.

Fig. 2: electromagnetic field characteristics

A. Field amplitude in the plant panel. The field amplitude was measured using a Narda EP-601 triaxial probe in the center and at each corner of the plant panel.

B. Frequency Bandwidth of the exposure. A Fast Fourier Transform of the temporal signal revealed that the bandwidth of about 30 MHz of the signal is centered on the fundamental frequency (2.45 GHz) .

Fig. 3: Evolution of plant leaf temperature after EMF exposure.

A. The leaf surface temperature was continuously recorded from 2 min before the application of the 100 kHz-modulated 2.45 GHz EMF field (a), during the 30 min exposure between the two red vertical lines (b) and 3.5 min after the exposure stop (c). The measurement was performed using a Lumasense Luxtron modèle STS reflectometric probe. Representative recording out of 3 independent experiments.

B. Thermic imaging of the plant's panel before (left) of after (right) the application of a 30 min 100 kHz-modulated 2.45 GHz EMF. The red cross materializes the possibility

to measure selectively the temperature on a very reduced surface of the leaf. Positioned on hot spots present on both unexposed and exposed samples, the measures revealed similar surface temperatures (24.3°C and 23°C, on unexposed and exposed samples, respectively).

Fig. 4: Effect of HF-EMF exposure on signal transduction-related gene expression

Relative transcript accumulations of *TCH1* calmodulin (**A**) and *ZAT12* transcription factor (**B**) genes, both implicated in rapid responses of plants to various kind of environmental stimuli, in unexposed (control) and exposed plants (15, 30 and 60 min after 30 min exposure to HF-EMF at 2.45 GHz, SAR<0.21 WKg^{-1}). Data are means of n = 3 biological independent replicates ± s.e. Asterisks indicate significant differences after the non-parametric Wilcoxon-Mann-Whitney test at the 95% confidence interval (*: $P < 0.05$; **: $P < 0.01$ and ***: $P < 0.001$) between the exposed and control condition for each different time point.

Fig. 5: effect of HF-EMF exposure on ROS-related gene expression

Relative transcript accumulation of (**A**) NADPH oxidases *RBOHD* and (**B**) *RBOHF*, **(C)** catalase *CAT2* and **(D)** ascorbate peroxidase *APX1* genes, all implicated in H_2O_2 production and scavenging, in unexposed (control) and exposed plants (15, 30 and 60 min after 30 min exposure to HF-EMF at 2.45 GHz, SAR<0.21 WKg^{-1}). Data are means of n = 3 biological independent replicates ± s.e. Asterisks indicate significant differences after the non-parametric Wilcoxon-Mann-Whitney test at the 95% confidence interval (*: $P < 0.05$; **: $P < 0.01$ and ***: $P < 0.001$) between the exposed and control condition for each different time point.

Fig. 6: effect of HF-EMF exposure on ROS metabolism and MDA production

Amounts of (**A**) H_2O_2, (**B**) total glutathione (GSH, GSSG) and GSH/GSSG ratio, (**C**) amounts of ascorbic (ASA) and dehydroascorbic (DHA) acids and (**D**) amounts of malondialdehyde (MDA) in *Arabidopsis thaliana* leaves in unexposed (control) and exposed plants (60 min after 30 min exposure to HF-EMF at 2.45 GHz, SAR<0.21 WKg^{-1}). Data are means of n = 3 biological independent replicates ± s.e. Asterisks indicate significant differences after the non-parametric Wilcoxon-Mann-Whitney test at the 95% confidence interval (*: $P < 0.05$; **: $P < 0.01$ and ***: $P < 0.001$) between the exposed and control condition.

Acknowledgements

The authors are grateful to the French company in charge of the management of the railway infrastructure (SNCF Réseau) for supporting this research through the DéfiOndes project.

484 **Table S1: List of primer sequences used in this study.**

485 Genes in bold were used as housekeeping genes

Gene ontology	Gene name	Locus id	Primer sequences	Amplicon size	References
Glyceraldehyde-3-phosphate dehydrogenase	*GAPDH*	At1G13440	Forward: TCAGGAACCCTGAGGACATC Reverse: CGTTGACACCAACAACGAAC	174 bp	Jin *et al.* (2019)
Aldehyde dehydrogenase	*ALDH*	At3G66658	Forward: TGGGATATTTCCCTGCTCTG Reverse: GAAGAATCCGCAAGAACTGC	123 bp	Jin *et al.* (2019)
Calmodulin 1	*TCH1*	At5G37780	Forward: ATGGAAACGGCACTATCGAC Reverse: ATTGGTCATCACATGGCGTA	161 bp	newly designed
Zinc finger protein/stress responses	*ZAT12*	At5G59820	Forward: TGCGAGTCACAAGAAGCCTA Reverse: GTGTCCTCCCAAAGCTTGTC	127 bp	newly designed
Respiratory burst oxidase homolog D	*RBOHD*	At5G47910	Forward: CGTGGAGATCACGCTAGACA Reverse: AGGAGGTGGTGTTGTTGAGG	149 bp	newly designed
Respiratory burst oxidase homolog F	*RBOHF*	At1G64060	Forward: GGTGCACCAGCACAAGATTA Reverse: TGAAATCCGAGATCGAATCC	148 bp	newly designed
Catalase 2	*CAT2*	At4G35090	Forward: CGAGGTATGACCAGGTTCGT Reverse: CTCCAGGCTCCTTGAAGTTG	114 bp	newly designed
Ascorbate peroxidase 1	*APX1*	At1G07890	Forward: GCACTATTGGACGACCCTGT Reverse: AGCAAACCCAAGCTCAGAAA	117 bp	newly designed

486

487

488

489

490

References

Akbal, A., Kiran, Y., Sahin, A., Turgut-Balik, D., 2012. Effects of Electromagnetic Waves Emitted by Mobile Phones on Germination, Root Growth, and Root Tip Cell Mitotic Division of Lens culinaris Medik. Polish J. Env. Stu. 21(1), 23-29.

Beaubois, É., Girard, S., Lalléchère, S., Davies, E., Paladian, F., Bonnet, P., Ledoigt, G., Vian, A., 2007. Intercellular communication in plants: evidence for two rapidly transmitted systemic signals generated in response to electromagnetic field stimulation in tomato. Plant Cell Environ. 30(7), 834-844. https://DOI:10.1111/j.1365-3040.2007.01669.x.

Castro, B., Citterico, M., Kimura, S., Stevens, D.M., Wrzaczek, M., Coaker, G., 2021. Stress-induced reactive oxygen species compartimentalization, perception and signalling. Nature Plants, 7, 403-412. https://doi.org/10.1038/s41477-021-00887-0.

Chandel, S., Kaur, S., Singh, H.P., Batish, D.R., Kohli, R.K., 2017. Exposure to 2100 MHz electromagnetic field radiations induces reactive oxygen species generation in *Allium cepa* roots. J. Microsc. Ultrastruct. 5, 225-229. https://doi.org/10.1016/j.jmau.2017.09.001.

Chen, Y.P., Jia, J.F., Wang, Y.J., 2009. Weak microwave can enhance tolerance of wheat seedlings to salt stress. J. Plant Growth Reg. 28(4), 381-385.

Cifra, M., Fields, J.Z., Farhadi, A., 2011. Electromagnetic cellular interactions. Prog. Biophys. Mol. Biol. 105, 223-246. https://doi.org/10.1007/s00344-009-9106-7.

Davletova, S., Schlauch, K., Coutu, J., Mittler, R., 2005. The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. Plant Physiol. 39(2), 847-856. https://doi.org/10.1104/pp.105.068254.

Engelmann, J.C., Deeken, R., Müller, T., Nimtz, G., Roelfsema, M.R., Hedrich, R., 2008. Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. Adv. Appl. Bioinform. Chem. 1, 71-83. https://doi.org.10.2147/aabc.s3570.

Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48, 909-930. https://doi.org/ 10.1016/j.plaphy.2010.08.016.

Gillespie, K.M., Ainsworth, E., 2007. Measurement of reduced, oxidized and total ascorbate content in plants. Nat. Protoc. 2(4), 871-874. https://doi.org/10.1038/nprot.2007.101.

Gilroy, S., Białasek, M., Suzuki, N., Górecka, M, Devireddy, A.R., Karpiński, S., Mittler, R., 2016. ROS, calcium, and electric signals: Key mediators of rapid systemic signaling in plants. Plant Physiol. 171, 1606-1615. https://doi.org/ doi: 10.1104/pp.16.00434.

Grémiaux, A., Girard, S., Guérin, V., Lothier, J., Baluška, F., Davies, E., Bonnet, P., Vian, A., 2016. Low-amplitude, high-frequency electromagnetic field exposure causes delayed and reduced growth in *Rosa hybrida*. J. Plant Physiol. 190, 44-53. https://doi:10.1016/j.jplph.2015.11.004.

Gustavino, B., Carboni, G., Petrillo, R., Paoluzzi, G., Santovetti, E., Rizzoni, M., 2016. Exposure to 915 MHz radiation induces micronuclei in *Vicia faba* root tips. Mutagenesis 31(2), 187-192. https://doi.org/10.1093/mutage/gev071.

Habauzit, D., Le Quément, C., Zhadobov, M., Martin, C., Aubry, M., Sauleau, R., Le Dréan, Y., 2014. Transcriptome Analysis Reveals the Contribution of Thermal and the Specific Effects in Cellular Response to Millimeter Wave Exposure. PLoS ONE 9(10), e109435. doi.org/10.1371/journal.pone.0109435

Hafeez, M.B., Zahra, N., Ahmad, N., Shi, Z., Raza, A., Wang, X., Li, J., 2023. Growth, physiological, biochemical and molecular changes in plants induced by magnetic fields: a review. Plant Biol. 25, 8-23. doi.org/10.1111/plb.13459

Halgamuge, M.N., Yak, S.K., Eberhardt, J.L., 2015. Reduced growth of soybean seedlings after exposure to weak microwave radiation from GSM 900 mobile phone and base station. Bioelectromagnetics 36, 87-95. https://doi: 10.1002/BEM.21890.

Han, J.P., Köster, P., Drerup, M.M., Scholz, M., Li, S., Edel, K.H., Hashimoto, K., Kuchitsu, K., Hippler, M., Kudla, J., 2019. Fine-tuning of RBOHF activity is achieved by differential phosphorylation and Ca^{2+} binding. New Phytol. 221(4), 1935-1949. https:\\doi.org\10.1111/nph.15543.

IEEE, 2006. Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz," in IEEE Std C95.1-2005 (Revision of IEEE Std C95.1-1991), pp.1-238. https://doi.org/10.1109/IEEESTD.2006.99501.

Israel, M., Zaryabova, V., Ivanova, M., 2013. Electromagnetic field occupational exposure: Non-thermal vs. thermal effects. Electromagn. Biol. Med. 32(2): 145-154. https:// DOI: 10.3109/15368378.2013.776349.

Jin, Y., Liu, F., Huang, W., Sun, Q., Huang, X., 2019. Identification of reliable reference genes for qRT-PCR in the ephemeral plant *Arabidopsis pumila* based on full-length transcriptome data. Scientific Reports 9, 8408. https://doi.org/10.1038/s41598-019-44849-1.

Jin, M., Wang, H., Liu, H., Xia, Y., Ruan, S., Huang, Y., Qiu, J., Du, S., Xu, L., 2020. Oxidative stress response and proteomic analysis reveal the mechanisms of toxicity of imidazolium-based ionic liquids against Arabidopsis thaliana. Environ Pollut. 260, 114013. https://doi.org/ 10.1016/j.envpol.2020.114013.

Kaur, S., Vian, A., Chandel, S., Singh, H., Batish, D., Kohli, R., 2021. Sensitivity of plants to high frequency electromagnetic radiation: cellular mechanisms and morphological changes. Rev. Environ. Sci. Biotechnol. 20, 55-74. https://doi.org/10.1007/s11157-020-09563-9.

Keppler, D., Rudigier, J., Decker, K., 1970. Enzymic determination of uracil nucleotides in tissues. Anal Biochem 38, 105-114.

Kitaya, Y., Kawai, M., Tsuruyama, J., Takahashi, H., Tani, A., Goto, E., Saito, T., Kiyota, M., 2003. The effect of gravity on surface temperatures of plant leaves. Plant, Cell and Environ. 26**,** 497-503. https://doi.org/10.1016/S0273-1177(01)00375-1.

Kaur, S., Vian, A., Chandel, S., Singh, H.P., Batish, D.R., Kohli, R.K., 2021. Sensitivity of plants to high frequency electromagnetic radiation: cellular mechanisms and morphological changes. Rev Environ Sci Biotechnol 20, 55-74. https://doi.org/10.1007/s11157-020-09563-9.

Kumar, A., Pal Singh, H., Batish D.R., Kaur, S., Kohli, R.K., 2016. EMF radiations (1800 MHz)-inhibited early seedling growth of maize (*Zea mays*) involves alterations in starch and sucrose metabolism. Protoplasma 253(4), 1043-1049. https://doi.org/10.1007/s00709-015-0863-9.

Kundu, A., Vangaru, S., Bhattacharyya, S., Mallick, AI., Gupta, B., 2021a. Electromagnetic Irradiation Evokes Physiological and Molecular Alterations in Rice. Bioelectromagnetics 42(2), 173-185. https://doi.org/10.1002/bem.22351.

Kundu, A., Vangaru, S., Bhowmick, S., Bhattacharyya, S., Mallick, AI., Gupta, B., 2021b. One-time Electromagnetic Irradiation Modifies Stress-sensitive Gene Expressions in Rice Plant. Bioelectromagnetics 42(8), 649-658. https://doi.org/10.1002/bem.22374.

Kundu, A., Gupta, B., & Mallick, A. I., 2022. Dependence of specific absorption rate and its distribution inside a homogeneous fruit model on frequency, angle of incidence, and wave polarization. Frequenz 76(1-2), 109-119. https://doi.org/10.1515/freq-2021-0049

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25(4), 402-408. https://doi.org/10.1006/meth.2001.1262.

Mhamdi, A., Queval, G., Chaouch, S., Vanderauwera, S., Van Breusegem, F., Noctor, G., 2010. Catalase function in plants: a focus on Arabidopsis mutants as stress-mimic models. J. Exp. Bot. 61(15), 4197-4220. https://doi.org/10.1093/jxb/erq282.

Mittler, R., Zandalinas, S.I., Fichman, Y., Van Breusegem F., 2022. Reactive oxygen species signalling in plant stress responses. Nat. Rev. Mol. Cell Biol. 23, 663–679. https://doi.org/10.1038/s41580-022-00499-2.

590 Parmagnani, A.S., Mannino, G., Maffei, M. E., 2022. Transcriptomics and Metabolomics of
591 Reactive Oxygen Species Modulation in Near-Null Magnetic Field-Induced Arabidopsis
592 thaliana. Biomolecules, 12(12), 1824. https://doi.org/10.3390/biom12121824

593 Pesnya, D.S., Romanovsky, A.V., 2013. Comparison of cytotoxic and genotoxic effects of
594 plutonium-239 alpha particles and mobile phone GSM 900 radiation in the Allium cepa test.
595 Mutation Res. 750, 27-33. http://dx.doi.org/10.1016/j.mrgentox.2012.08.010.

596 Porcher, A., Guérin, V., Montrichard, F., Lebrec, A., Lothier, J., Vian, A., 2020. Ascorbate
597 glutathione-dependent H_2O_2 scavenging is an important process in axillary bud outgrowth in
598 rosebush. Annals of Botany 126(6): 1049-1062. https://doi.org/10.1093/aob/mcaa130.

599 Porcher, A., Guérin, V., Lebrec, A., Leduc, N., Lothier, J., Vian, A., 2021. Glutathione synthesis
600 and AsA-GSH pathway, mediated by cytokinins, manage H_2O_2 level in bud burst photocontrol.
601 Plant Physiol. 186, 910-928. https://doi.org/ https://doi.org/10.1093/plphys/kiab123.

602 Răcuciu, M., Iftode, C., Miclaus, S., 2015. Inhibitory effects of low thermal radiofrequency
603 radiation on physiological parameters of *Zea mays* seedlings growth. Rom. J. Phys. 60(3-4),
604 603-612.

605 Rammal, M., Jebai, F., Rammal, H., Joumaa, W.H., 2014. Effects of long term exposure to
606 RF/MW radiations on the expression of mRNA of stress proteins in *Lycopersicon esculentum*.
607 *WSEAS Transactions on Biology and Biomedicine* 11, 10-14.

608 R Core Team, 2020. R: A language and environment for statistical computing. R Foundation
609 for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

610 Romanenko, S., Begley, R., Harvey, A.R., Hool, L., Wallace, V.P., 2017. The interaction
611 between electromagnetic fields at megahertz, gigahertz and terahertz frequencies with cells,
612 tissues and organisms: risks and potential. J. R. Soc. Interface 14: 20170585.
613 https://doi.org/10.1098/rsif.2017.0585.

614 Roux, D., Vian, A., Girard, S., Bonnet, P., Paladian, F., Davies, E., Ledoigt, G., 2006.
615 Electromagnetic fields (900 MHz) evoke consistent molecular responses in tomato plants.
616 Physiol. Plant 128, 283-288. https://doi.org/10.1111/j.1399-3054.2006.00740.x.

617 Roux, D., Vian, A., Girard, S., Bonnet, P., Paladian, F., Davies, E., Ledoigt, G., 2008. High
618 frequency (900 MHz) low amplitude (5 V m^{-1}) electromagnetic field: a genuine environmental
619 stimulus that affects transcription, translation, calcium and energy charge in tomato. Planta
620 227(4), 883-891. https://DOI:10.1007/s00425-007-0664-2.

621 Roux, D., Girard, S., Paladian, F., Bonnet, P., Lalléchère, S., Gendraud, M., Davies, E., Vian,
622 A., 2010. Human keratinocytes in culture exhibit no response when exposed to short duration,
623 low amplitude, high frequency (900 MHz) electromagnetic fields in a reverberation chamber.
624 Bioelectromagnetics 32, 302-311. https://DOI:10.1002/bem.20641.

625 Saletnik, B., Saletnik, A., Słysz, E., Zaguła, G., Bajcar, M., Puchalska-Sarna, A., Puchalski, C,
626 2022. The Static Magnetic Field Regulates the Structure, Biochemical Activity, and Gene
627 Expression of Plants. Molecules 27, 5823. https://doi.org/10.3390/molecules27185823

628 Senevirathna, M.D.H.J., Nagahage, I.S.P. , Muhetaer, G., 2020. Stimulatory effect of exposure
629 to low-power-density 2.45 GHz microwaves on *Arabidopsis thaliana* seedlings *in vitro*. Braz. J.
630 Bot 43, 459-467. https://doi.org/10.1007/s40415-020-00618-3.

631 Singh, H.P., Sharma, V.P., Batish, D.R., Kohli, R.K., 2012. Cell phone electromagnetic field
632 radiations affect rhizogenesis through impairment of biochemical processes. Environ. Monit.
633 Assess, 184, 1813-1821. https://doi.org/10.1007/s10661-011-2080-0.

634 Tkalec, M., Malarić, K., Pevalek-Kozlina, B. Exposure to radiofrequency radiation induces
635 oxidative stress in duckweed *Lemna minor* L. Sci Total Environ. 388(1-3), 78-89.
636 https://doi.org/10.1016/j.scitotenv.2007.07.052.

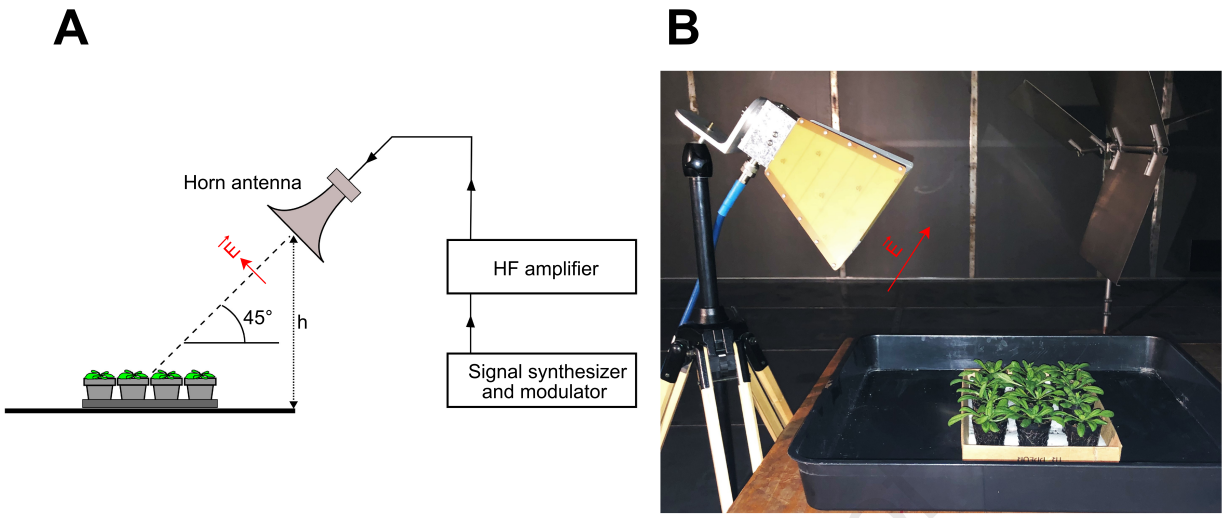
637 Upadhyaya, C., Upadhyaya, T., Patel, I., 2022. Attributes of non-ionizing radiation of 1800
638 MHz frequency on plant health and antioxidant content of Tomato (*Solanum Lycopersicum*)
639 plants. J. Rad. Res. App. Sci. 15(1), 54-68. https://doi.org/10.1016/j.jrras.2022.02.001.

640 Vian, A., Roux, D., Girard, S., Bonnet, P., Paladian, F., Davies, E., Ledoigt, G., 2006.
641 Microwave irradiation affects gene expression in plants. Plant Signal. Behav. 1(2), 67-70.
642 https://doi: 10.4161/psb.1.2.2434.

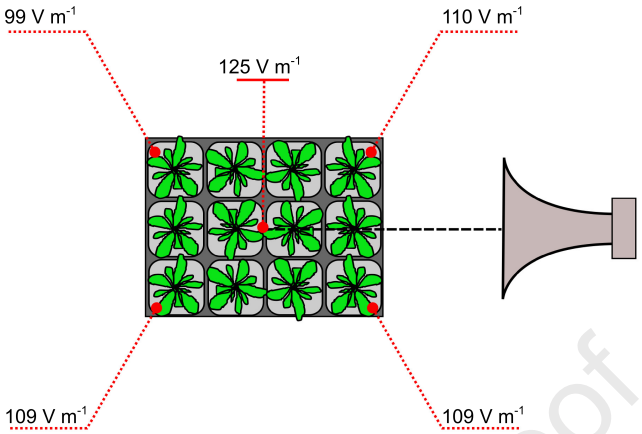
643 Vian, A., Faure, C., Girard, S., Davies, E., Hallé, F., Bonnet, P., Ledoigt, G., Paladian, F., 2007.
644 Plants respond to GSM-like radiation. Plant signal. Behav. 2(6), 522-524.
645 https://doi.org/10.4161/psb.2.6.4657.

646 Vian, A., Davies, E., Gendraud, M., Bonnet, P., 2016. Plant Responses to High Frequency
647 Electromagnetic Fields. Biomed Res Int., 2016, 1830262. https://doi:10.1155/2016/1830262.

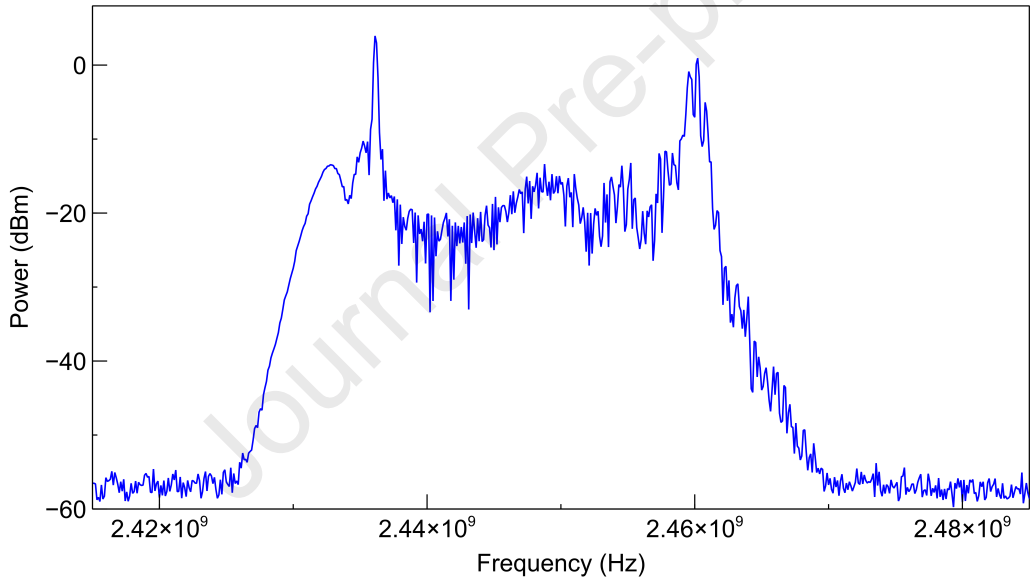
648 Yang, C.Y., Hong, C.P., 2015. The NADPH oxidase Rboh D is involved in primary hypoxia
649 signalling and modulates expression of hypoxia-inducible genes under hypoxic stress. Env.
650 Exp. Bot. 115, 63-72. https://doi.org/10.1016/j.envexpbot.2015.02.008.



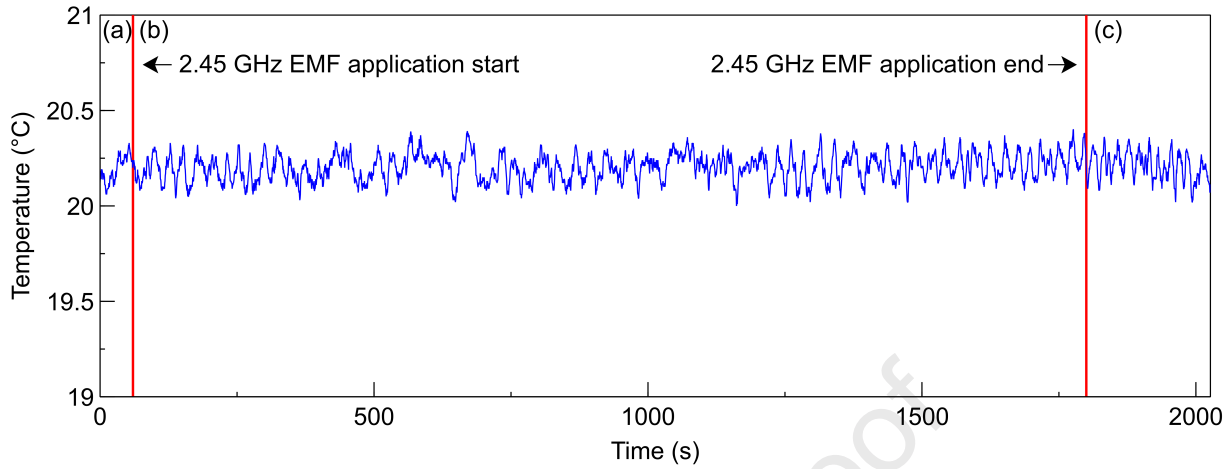
A



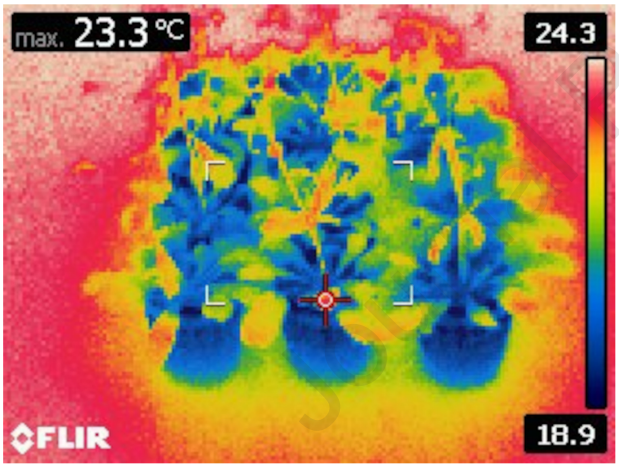
B



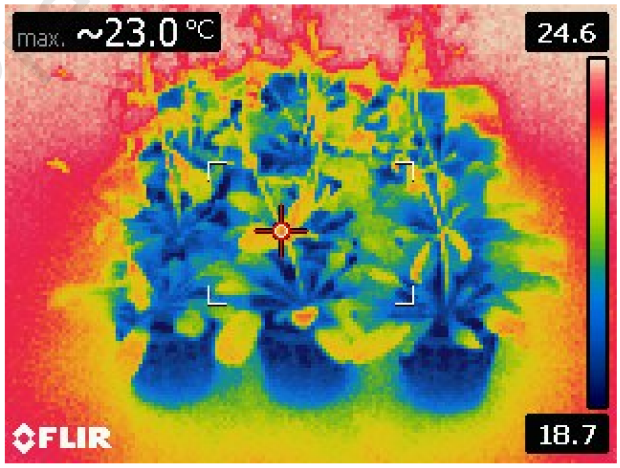
A



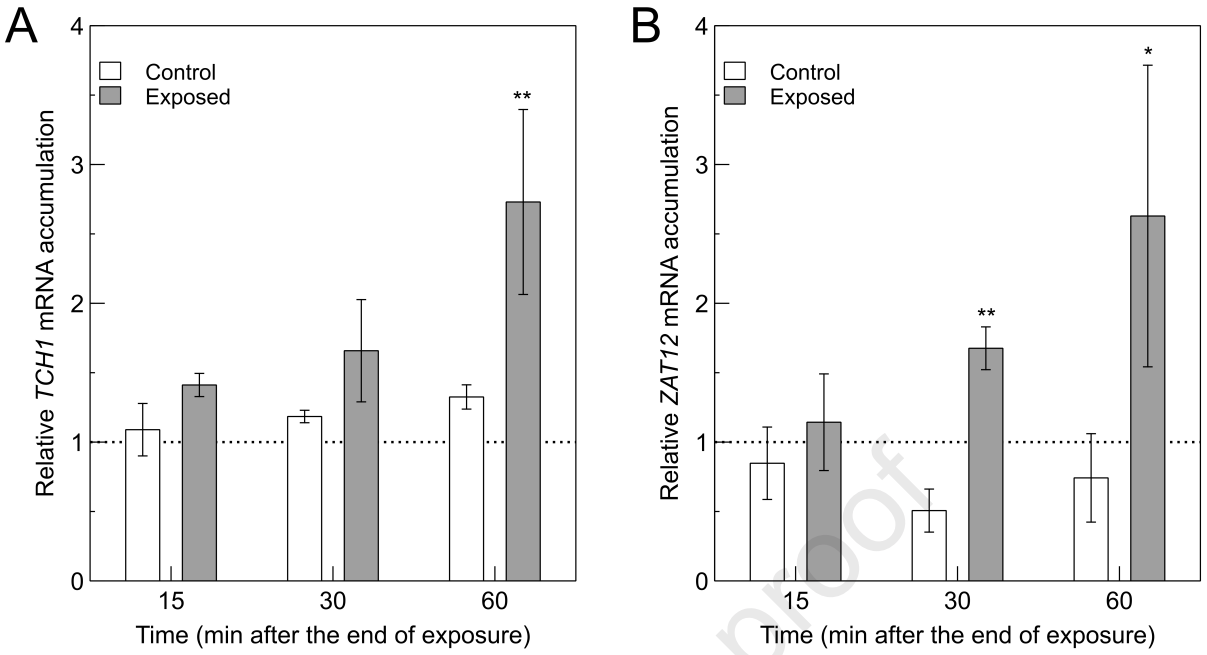
B

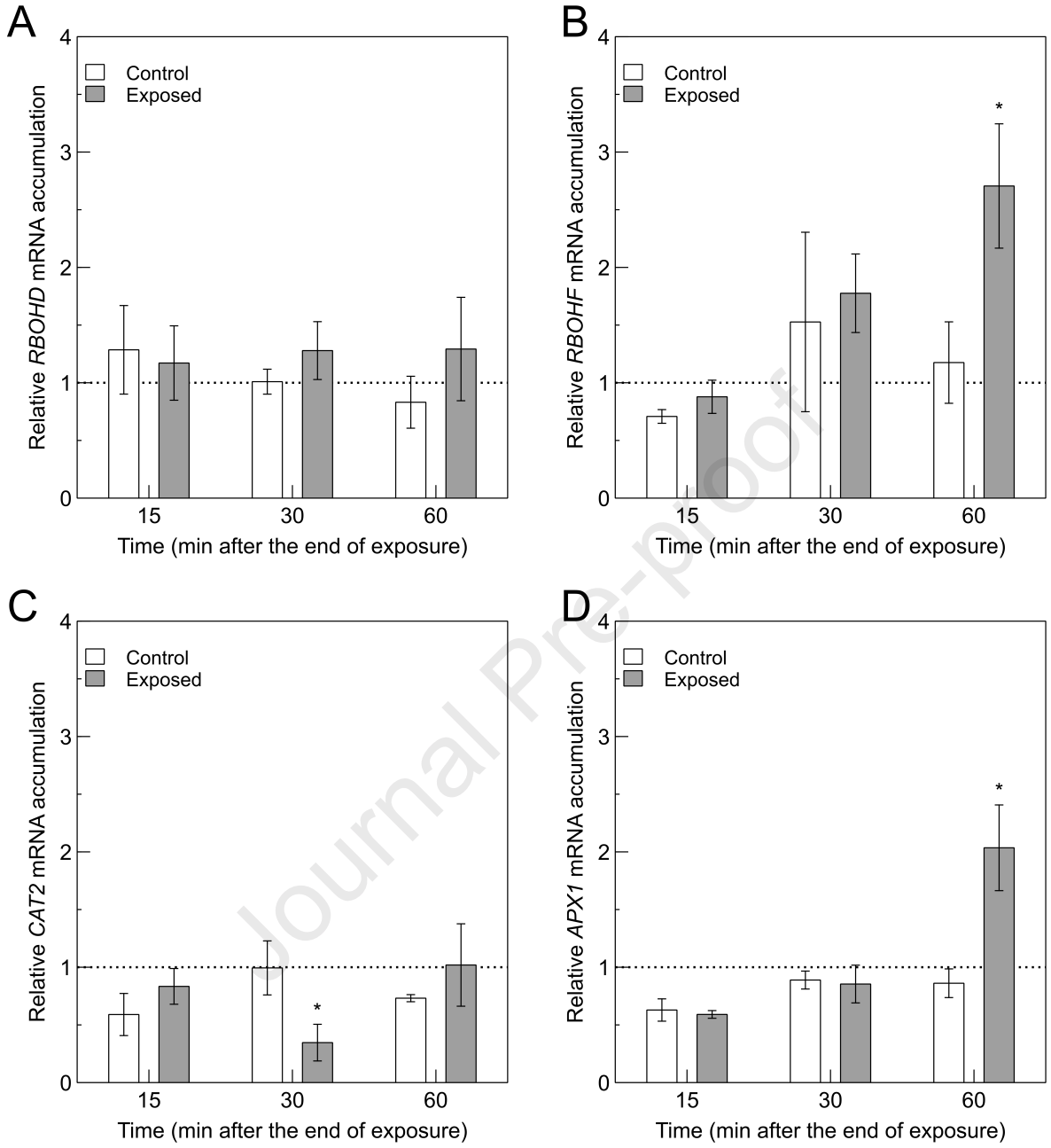


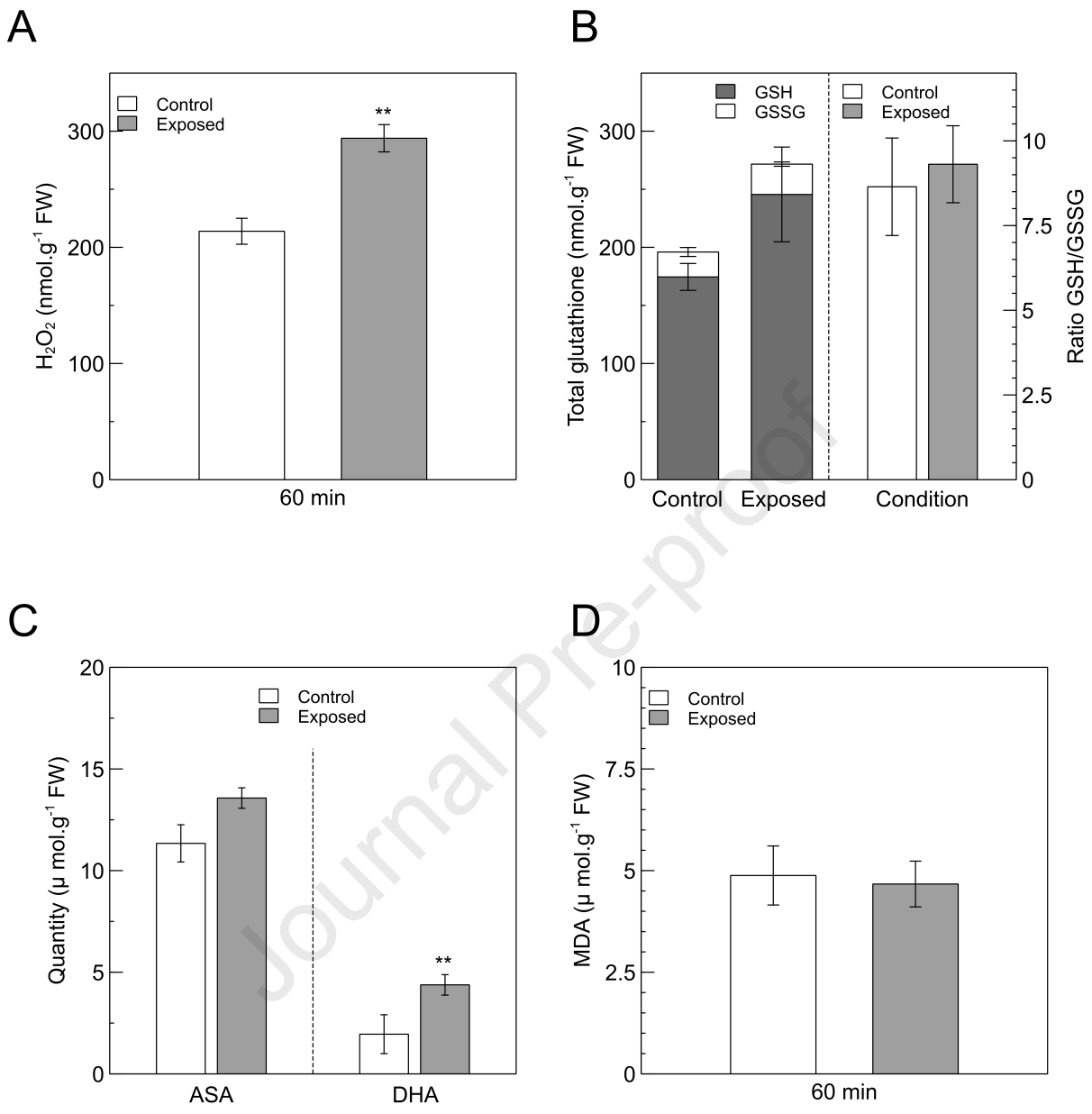
Before exposure



After 30 min EMF exposure







Highlights

- In our condition, 2.45 GHz HF-EMF exposure did not induce thermal effect in plants
- We observed rapid and transcient changes of gene expression and H_2O_2 metabolism
- Non-thermal biological responses to EMF exposure do exist in plants

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Francoise Paladian reports was provided by SNCF Réseau.