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ORIGINAL ARTICLE

The effects of electromagnetic field radiation of extremely low frequency on growth parameters and nucleotide substitutions in *L. minor* clones

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Abstract
Current technologies have become a source of electromagnetic pollution resulting from artificially generated electromagnetic radiation (EMR). To understand the influence of the EMR on living organisms, we investigated the long-term effects of EMR of 50 Hz frequency on duckweed (*Lemna minor*) clones. Experimental groups of duckweed were treated directly and indirectly by changing EMR generating magnetic flux (MF) starting from 2 µT (0–11 weeks from the beginning of the experiment) and switching to 300 µT (12–48 weeks) MF density during the second part of the experiment. The growth parameters (plant growth, frond area, and frond number) and the point mutations appearing at the antioxidant genes DNA sequences [ascorbate peroxidase (APx), glutathione peroxidase (GPx), and catalase (Cat)] were analyzed. The significantly enhanced number of nucleotide substitutions in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to indirectly affected clones was revealed at the introns of APx, GPx, and Cat genes starting from the 10th week of the experiment. The results indicate that even low-dose chronic electromagnetic radiation may contribute to the changes in growth parameters and generation of point mutations in antioxidant gene sequences, especially in the intron regions.

Keywords *Lemna minor* · Electromagnetic radiation · Ascorbate peroxidase · Glutathione peroxidase · Catalase

Introduction

Magnetic fields and electric fields *arise naturally* in the environment, but as well it can be *caused or produced by humans, so* this phenomenon is of anthropogenic origin (Minorsky 2007). The rapid development of technology introduced many new devices and technologies in daily life that emit electromagnetic radiation. The most common power frequencies of the electromagnetic field (EMF) range from 50 to 60 Hz, which are classified as extremely low frequencies of the EMF (Behrens et al. 2004). In many cases, this EMF emission of anthropogenic origin should be considered as pollution that may have a much stronger effect on living organisms than any natural sources of electromagnetic fields or radiation. Increased concern about the environment and health has led to a study of the impact of EMF on biological systems. The influence of EMF can be thermal or non-thermal on living organisms. Thermal effects are associated with the heat created by EMF that could damage tissues in a certain area or could affect the whole organism. It happens when there is an interaction between radiofrequency (RF) fields and living tissues causing an energy transfer on various living tissues (Megha et al. 2012). The thermal effect of RF emitted by mobile phones was shown as insignificant (Koyama et al. 2003), but other studies have shown that low-frequency magnetic fields (1–300 Hz) exposed to more than 0.4 µT might be harmful (Touitou and Selmaoui 2012) and can cause some biological changes in targeted cells or tissues (Repacholi 1998). Other effects on living tissues that are not related to variation in temperature are called non-thermal causing various chemical reactions, such as lipid or protein expression changes (Gherardini et al. 2014). It was shown that EMFs

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in their entire frequency spectrum (low to high) induce an increase in oxidative stress in many experimental systems (including plants) (Georgiou 2010). Under these conditions, reactive oxygen species (ROS) such as hydroxyl radical $^{-}$OH and hydrogen peroxide H_2O_2 are generated (Tkalec et al. 2007) in cells. The overproduction of ROS causes peroxidation of lipids, oxidation of proteins, inhibition of enzymes, and damage to DNA/RNA (Ozmen et al. 2006). To study the effect of low-frequency electromagnetic field (LF EMF) radiation on test organisms like *L. minor* defense mechanisms of antioxidative defense preventing damage of plants including glutathione peroxidase (GPx), catalase (CAT) (Atamanalp et al. 2019), and ascorbate peroxidase (APx) should be considered (De Micco et al. 2011). These enzymes catalyze the dismutation of H_2O_2 into water (H_2O) and O_2 (Palma et al. 2020).

Lemna minor was selected as a biological model to study the effects of electromagnetic radiation due to its high phenotypic plasticity in response to environmental conditions (Kendeler 1975). Duckweed (*L. minor*) is a widespread and floating plant in water that grows fast and has a relatively small genome size (Movafegh et al. 2013). These features make them suitable for several applications, such as wastewater treatment (Körner et al. 2003), bioenergy production (Cui and Cheng 2015), and pharmaceutical applications (Zhao et al. 2015).

Changes in different antioxidative enzyme activities in response to duckweed exposure to various electromagnetic radiation have been documented. Peroxidase activity significantly increased (41%) when *L. minor* was exposed for 2 h to 41 V/m at 900 MHz (Tkalec et al. 2005). Another study showed that chronically exposed duckweeds with low-dose rates of ionizing radiation can tolerate it by keeping high levels of photosynthetic activity. In contrast, the high dose rates led to the induction of biological responses like oxidative stress (Van Hoeck et al. 2015).

However, the long-term effects of electromagnetic radiation are still poorly understood. To better understand the effects of EMF on living organisms, the response to stress could be effectively studied in plants modeling more naturally relevant chronic conditions. In this study, we demonstrated the long-term impact of the LF EMF on *L. minor* at the molecular level. The results of the study could be used to evaluate the potential ecological risk of electromagnetic pollution to the biological environment.

Materials and methods

Plant material

Lemna minor indicated as an S2 clone was collected from the Neris River above the city of Vilnius (54°45′48.25″, 25°21′14.53″) and was chosen for testing of LE EMF in laboratory conditions. The sterilized plants were transferred to a Petri dish with Steinberg medium (ISO 20079). The Steinberg medium contains 350 mg KNO_3, 295 mg $Ca(NO_3)_2{\cdot}4H_2O$, 90 mg KH_2PO_4, 12.6 mg K_2HPO_4, 100 mg $MgSO_4{\cdot}7H_2O$, 120 µg H_3BO_3, 180 µg $ZnSO_4{\cdot}7H_2O$, 44 µg $Na_2MoO_4{\cdot}2H_2O$, 180 µg $MnCl_2{\cdot}4H_2O$, 760 µg $FeCl_3{\cdot}6H_2O$, 1500 µg EDTA Disodium-dihydrate per liter of distilled water. The culture of *L. minor* was maintained under continuous light (OSRAM L 36/77), photoperiod of 16 h/8 h day/night of fluorescent light of 90–100 µE m^{-2} s^{-1} intensity at 25 ± 1 °C.

EMF treatment

EMF was generated using an EMF-generating coil. (Fig. 1A). An alternating low-frequency magnetic field (50 Hz) was generated by two identical cylindrical coils. Each coil with 170 turns of isolated copper wire (diameter 0.6 mm) had a diameter of $d = 12$ cm and a height of $h = 9$ cm (Fig. 2). For the generation of alternating electric current in the coil a signal generator-oscilloscope PCSGU250 and power amplifier were used. A sinusoidal 50 Hz electric current was used in this study. Operation of the system was performed by a personal computer with installed software PcLab 2000 LT. Magnetic flux density was measured using meters "DualField 1" (ROM-Elektronik GmbH) and "455 DSP Gaussmeter" (LakeShore Cryotronics).

Three Petri dishes with one duckweed plant in each were exposed to a direct impact (plants were grown on the EMF-generating coil) and the other three Petri dishes with duckweed plants of the same clone were exposed to indirect (plants were grown at a 1.5 m distance from the an EMF-generating coil) EM radiation for 48 weeks (Fig. 1B). During the experiment, plants were transplanted every two weeks. Only one plant frond from each Petri dish was transplanted into the new medium and the remainder fronds were used to extract DNA.

Lemna minor clones were exposed to direct and indirect electromagnetic radiation while growing in Petri dishes. The duration of the experiment was 48 weeks; during the experiment *L. minor* clones were grown in Petri dishes placed on EMF-generating coil (directly affected group) and placed distantly 1.5 m from the coil (indirectly affected group). Preliminary results of the experiments that were carried out by a research group led by prof. Grauda et al. (2015). It was found that when the magnetic flux density was changed from 50 to 400 µT inside the coil it caused changing the fluorescence intensity of gametic cells. Fluorescence was found higher (statistically significant) in comparison to control cells when cells were treated with a density of 100 µT ($p < 0.01$) and 400 µT ($p < 0.01$). Starting from the beginning of the experiment, the magnetic flux density

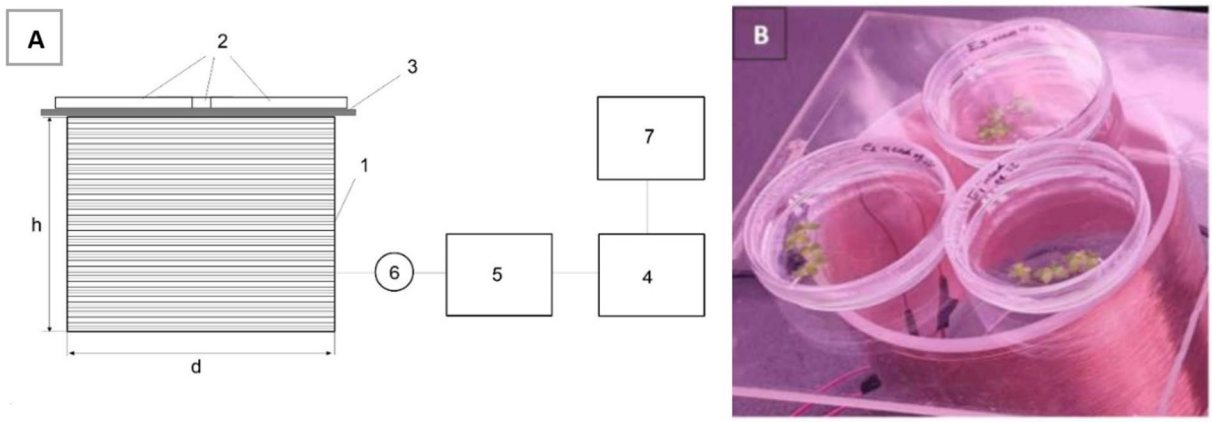

Fig. 1 **A** Scheme of equipment used for exposure to low-frequency EMF: 1—cylindrical coil; 2—Petri dishes with biological materials; 3—plexiglass plate; 4—PC oscilloscope and PC function generator PCSGU250; 5—power amplifier; 6—amperemeter; 7—personal computer. **B** *L. minor* clones growing on EMF-generating coil

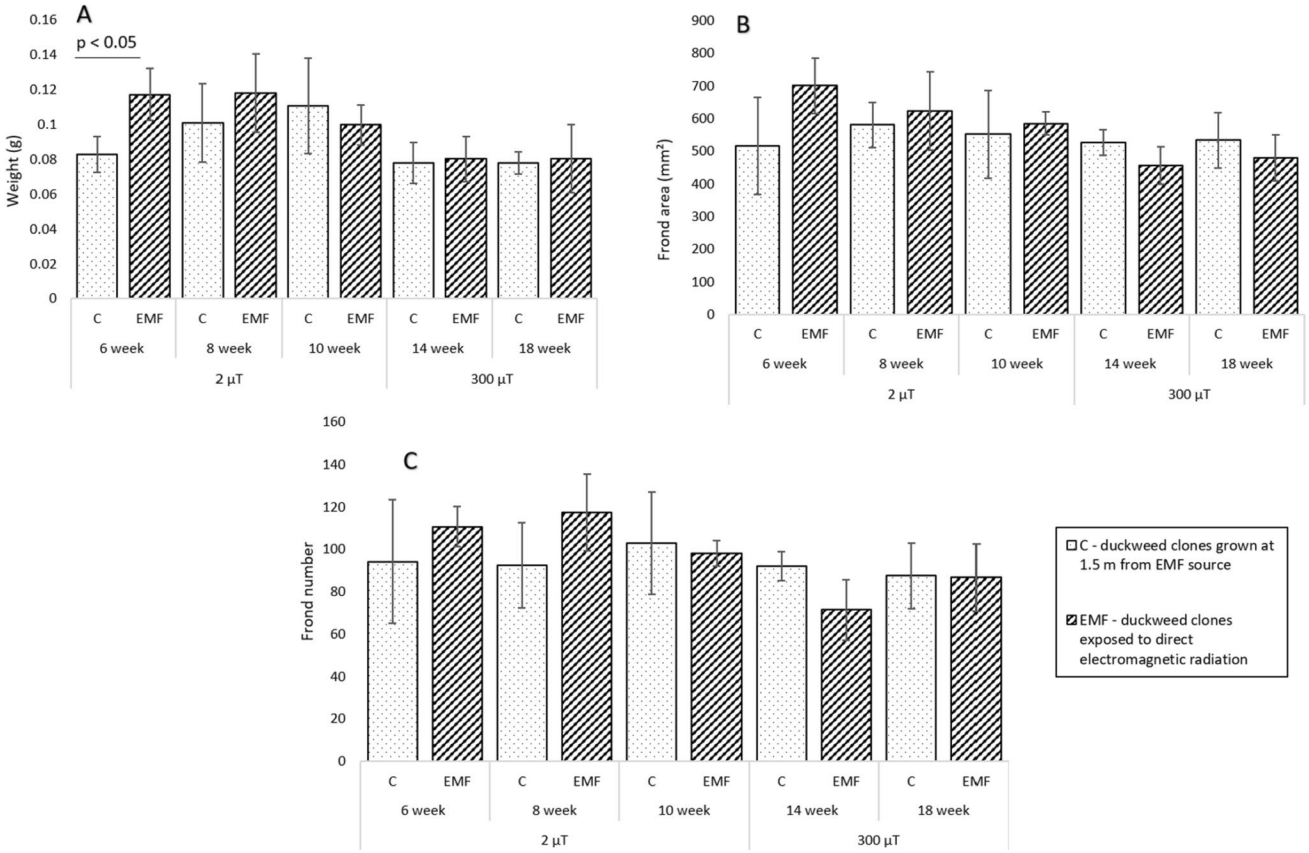

Fig. 2 Comparison of the growth parameters (weeks 6—18) such as weight (**A**), frond area (**B**), and frond number (**C**) of *L. minor* clones grown at 1.5 m from EMF source (C group) and *L. minor* clones exposed to direct electromagnetic radiation (EMF group). The values are presented as the means ± standard errors. Student's *t*-test was performed to compare groups

was set at 2 ÷ 2.3 µT (inside it the center of the coil it was equal to 2.3 µT and in the peripheral area magnetic flux density decreased up to 2.0 µT) until the 11th week of the experiment and it was switched to 300 ÷ 380 µT (inside it the center of the coil it was equal to 380 µT and in the peripheral area magnetic flux density decreased up to 300 µT) for the rest time of the experiment starting from 12th week and continued until the end of 48th week when the experiment

was terminated (Table 1). The current values of magnetic flux density (MFD) were chosen following suggestions that low MFD (up to 1 ÷ 10 µT) have no hazardous effect on cell cultures in a relatively short time of exposure (up to one week of growing cells directly affecting them by LF EMF) although the prolonged effects were not measured so far. Long-lasting effect and higher MFD values (starting from 80 µT) expressed significant effects on DNA concentration and impacted other processes in experimentally affected cell cultures (Alexandrov 2006).

In our experiment, we measured the frond area, frond number, and weight of duckweed after 6, 8, 10, 14, and 18 weeks passed from the beginning of the experiment using the ImageJ program.

DNA extraction

The whole plants with fronds and roots were grounded in liquid nitrogen and total DNA was extracted using the Dneasy Plant Mini Kit method (QIAGEN) according to the manufacturer's protocol. Aliquots of the extracted DNA were used for measuring DNA quantity with NanoDrop ND1000. DNA extracts were diluted to a final concentration of 10 ng/µL in distilled water. DNA was stored at − 20 °C until use.

PCR and sequencing conditions

Lemna minor sequences of antioxidant genes used for the designation of primers were obtained from the CoGe database (https://genomevolution.org/coge/) (Van Heck et al. 2015). Specific primers for the amplification of different parts of the gene including promoter, introns, and exons were designed using the Primer3Plus program (Table 2).

PCR was performed in 10 µL of final solution volume containing 2 µL of DNA (10 ng/µL), 1 µL of each primer (10 µM), 5 µL DreamTaq PCR Master Mix, 1 µL nuclease-free water. The PCR was performed in the Eppendorf Mastercycler thermal cycler. The thermocycling program started from 5 min at 94 °C, followed by 35 cycles of 94 °C for 45 s, annealing temperature (indicated in Table 1) for 45 s and 72 °C for 1 min, and a final extension of 72 °C for 10 min. Amplified products were analyzed by electrophoresis in 1.5% agarose gel in 1X Tris–acetate-EDTA (TAE) buffer using Thermo Scientific Gene-Ruler DNA ladder and visualized by ethidium bromide staining. The PCR products were purified with exonuclease I and FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) and then sequenced by a 3500 Genetic Analyser.

Molecular data analysis

The sequenced DNA fragments were aligned using the MUSCLE (Edgar 2004) option in MEGA-X (Kumar et al. 2018). The aligned sequences were further analyzed indicating rising of point mutations at sequences of affected by EMF *L. minor* colonies of S2 line in comparison to control non-affected colony of the same S2 line kept frozen from the beginning of the experiment and used as a reference sequence. For comparison, the growth parameters of two treatment groups (EMF—directly exposed plant clones and C—indirectly exposed plant clones) were expressed as Log_2FC (EMF/C).

The presented data means ± standard errors of at least three independent measurements for each term. The Student's *t*-test was used to estimate the statistical differences between the two groups directly and indirectly affected by EMF, respectively. The difference was considered significant at p levels lower than 0.05 ($p < 0.05$).

Table 1 Electromagnetic radiation parameters and experimental duration

Test duration, week	Magnetic flux density measured inside coil	Strength of current
0–11	2 ÷ 2.3 µT	2.4 mA
12–48	300 ÷ 380 µT	0.4 A

Table 2 Primer sequences of antioxidant gene markers were used to study the impact of LF EMF on *L. minor*

Gene	Primer name	Primer sequences (5′–3′)	Annealing temperature (°C)	Product length (bp)	Type
Glutathione peroxidase	GPx6	F: TGTGCAAACACATAATCC CAAT R: TGATCATGACCAATAGAT CGTT	49	902	Promoter + exon 1 + intron 1
Catalase	Cat7	F: CGCGGTTTGGTTCAATTCGT R: TGGACTTGATCAGCGGTGAC	55	785	Promoter + exon 1 + intron 1 + exon 2 + intron 2 + exon 3 + intron 3 + exon 4
Ascorbate peroxidase	APx1	F: AAATTCGAGCCGTCAGATTG R: CCGAGATCCGACCTGATAGA	56	772	Promoter 1 + intron 1 + promoter 2 + exon 1 + intron 2 + exon 2

Results

Growth responses

Comparison of the effect of low-frequency electromagnetic radiation on *L. minor* clones expressed as differences in weight, frond number, and frond area between two study groups presented in Fig. 2. The results showed that the general weight of each colony directly affected by electromagnetic radiation was higher after 6 and 8 weeks (0.12 g), in comparison to lower weight (0.08 g after 6 weeks and 0.1 g after 8 weeks) in control group was observed (+0.2 $\log_2$FC(EMF/C)) (Fig. 2A). Starting from the 10th week, the general weight of each affected colony steadily decreased (0.1 g in both treatment groups), and no significant differences were found between duckweeds affected by direct and indirect electromagnetic radiation after 14 and 18 weeks (+0.05 $\log_2$FC(EMF/C)). The frond number was higher in the group affected by direct electromagnetic radiation after 6 and 8 weeks (+0.17 $\log_2$FC(EMF/C)), and it appeared lower after 10 and 14 weeks (−0.18 $\log_2$FC(EMF/C)) compared with clones affected by indirect electromagnetic radiation (Fig. 2B). The frond area of *L. minor* affected by direct electromagnetic radiation was higher after 6, 8, and 10 weeks (+0.21 $\log_2$FC(EMF/C)) and lower after 14 and 18 weeks (−0.18 $\log_2$FC(EMF/C)) compared with clones affected by indirect electromagnetic radiation (Fig. 2C). The largest difference of the frond area between the two study groups was detected after 6 weeks, and this tendency was observed until 14 weeks from the beginning of the experiment.

Antioxidant gene sequences analysis

The fragments of Apx (902 bp), GPx (785 bp), and Cat (772 bp) genes selected for the study contain the promoter, intron, and exon regions. Sequence analysis of three fragments Apx1, GPx6, and Cat7 revealed the appearance of nucleotide substitutions directly and indirectly affected by electromagnetic radiation in *L. minor* clones compared with reference sequence S2 (Fig. 3). The highest number of nucleotide substitutions in the EMF group was detected after the 10th (20 ± 1) and 48th (18 ± 3) weeks from the beginning of the experiment in the Apx1 fragment. The sequence analysis of the GPx6 fragment showed a significant increase in nucleotide substitutions (7 ± 1) after 14th week from the beginning of the experiment in *L. minor* clones directly affected by electromagnetic radiation ($p < 0.05$). However, no nucleotide substitutions were detected after the 18th week in two studied groups. The highest number of nucleotide substitutions (8 ± 6) was detected after the 14th week at the Cat7 fragment directly affected by the EMF sample.

In this section, we analyzed the point mutations detected at APx1, GPx6, and Cat7 genes after growing of *L. minor*

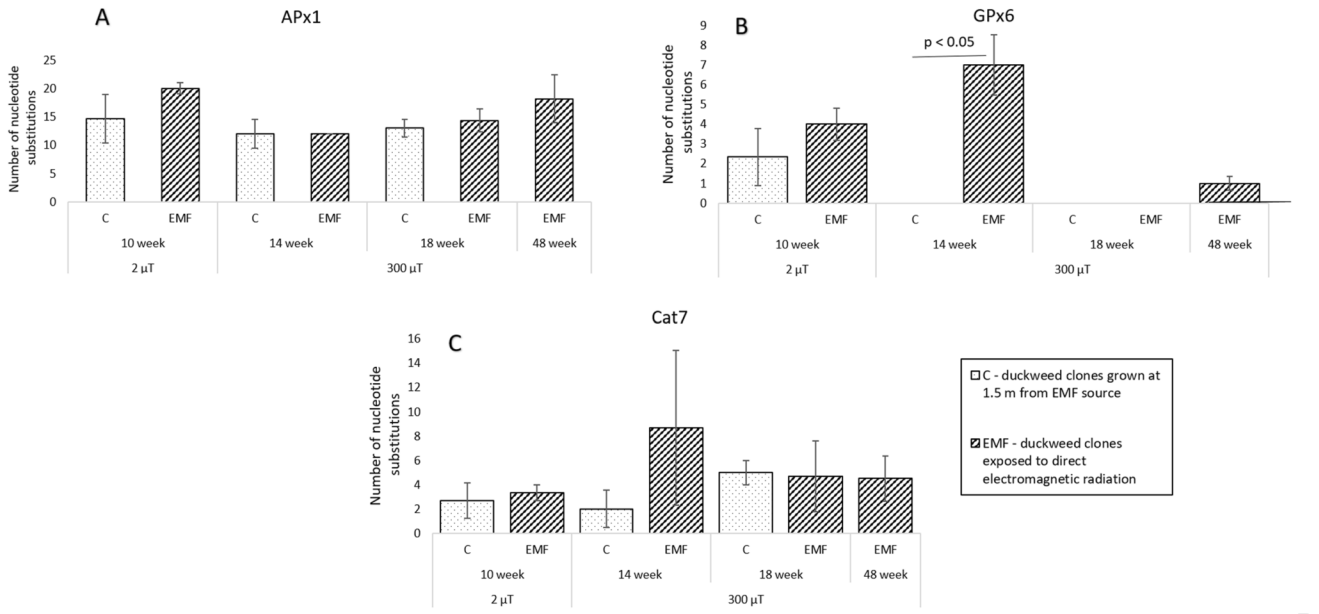

Fig. 3 Number of nucleotide substitutions of ascorbate peroxidase (APx1), glutathione peroxidase (GPx6), and catalase (Cat7) gene fragments after 10, 14, 18, and 48 weeks of treatment. C group represents *L. minor* clones grown at 1.5 m from the EMF source, and EMF group indicates *L. minor* clones exposed to direct electromagnetic radiation. The values are presented as the means ± standard errors. Student's *t*-test was performed to compare groups

clones affected by 2 µT magnetic flux till 11th week and finally grown affected by 300 µT magnetic flux density of EMR until the end of the experiment terminated at the end of 48th week. Figure 4 shows the number of nucleotide substitutions in three different gene fragments including promoter, intron, and exon of APx1, GPx6, and Cat7 genes. We expected that the observed number of nucleotide substitutions in clones of *L. minor* exposed to direct electromagnetic radiation in comparison to substitutions detected in clones grown distantly from the source of electromagnetic radiation will reveal the effect of experimentally generated magnetic flux on a molecular level.

The sequence analysis of APx1 gene fragment showed that the number of nucleotide substitutions in *L. minor* clones grown placed on the coils considered as directly affected by electromagnetic radiation (EMF—*L. minor* clones) decreases in promoter (3.8 ± 0.5 exposed to 2 µT and 0.7 ± 0.5 exposed to 300 µT) but increases in intron (0.3 ± 0.5 exposed to 2 µT and 0.5 ± 0.2 exposed to 300 µT) and exon (1.7 ± 0.5 exposed to 2 µT and 3.2 ± 0.6 exposed to 300 µT) when the magnetic flux density increased from 2 µT to 300 µT (Fig. 4). The appearance of nucleotide substitutions was not observed at C samples in intron. The significant difference between the two study groups was detected in the promoter (1.3 ± 0.5 in C group and 3.8 ± 0.5 in EMF group, $p < 0.05$) and intron (1.3 ± 0.5 in C group and 3.8 ± 0.5 in EMF group, $p = 0.055$) of APx gene. The most common recurrent point mutation is 28911T > A in the exon of APx1, but this position does not change the amino acid sequence. Other point mutations occurring in an exon are detected in only one clone and do not recur in the following weeks. A similar tendency of generation of new point mutations was detected by analyzing a fragment of the GPx gene. The number of nucleotide substitutions decreases in promoter (3 ± 0.5 exposed to 2 µT and 1.4 ± 0.5 exposed to 300 µT) but increases in intron (1 exposed to 2 µT and 1.3 ± 0.5 exposed to 300 µT) and exon (0 exposed to 2 µT and 0.4 ± 0.2 exposed to 300 µT) when the magnetic flux density increased from 2 µT to 300 µT. The appearance of nucleotide substitutions was not detected in the C group of *L. minor* clones in intron and exon. The significant difference in the number of new point mutations between the two study groups was detected in introns ($p < 0.05$) of the GPx gene when the magnetic flux density was 2 µT and 300 µT. Point

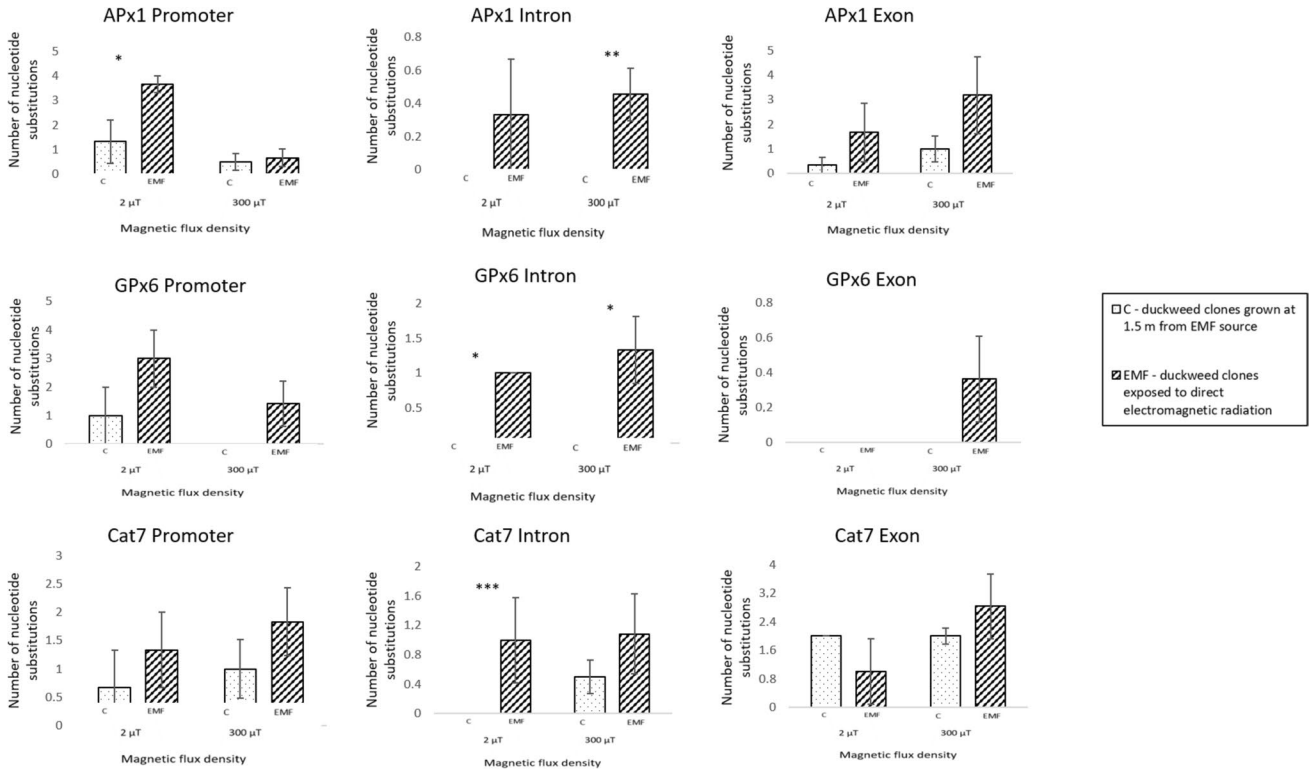

Fig. 4 The overall number of nucleotide substitutions in promoter, introns, and exons of APx1, GPx6, and Cat genes observed in *L. minor* clones exposed to electromagnetic radiation after growing directly and distantly affected by 2 µT magnetic flux and growing affected by 300 µT magnetic flux. Data shown are mean values ± standard error of two independent experiments (C—*L. minor* clones were grown distantly (1.5 m from EMF source), EMF—*L. minor* clones exposed directly as grown placed on the coils representing the source of EMF) performed in 3 replicates. Student's *t*-test was performed to compare groups; *$p < 0.05$; **$p = 0.079$; ***$p = 0.055$

mutations occurring in an exon were detected in only one clone and do not recur in the following weeks. The sequence analysis of the Cat7 gene showed that point mutations were detected in each fragment encompassing promoter, two introns and two exons. The significant difference between the two study groups EMF and C was detected in introns (0 in C group and 1 ± 0.6 in EMF group, $p = 0.079$) of the Cat7 gene exposed to 2 µT. The most common recurrent point mutation is 10119A>C, but this position does not change the amino acid sequence.

Discussion

The impact of nonionizing EMF on various organisms attracted a lot of interest in recent years, because of the possible risks related to chronic exposure to biological systems (Ishikawa 2016). Generally, in studies where plants were exposed to EMF, the attention was focused on physical parameters such as weight, frond number, or frond area in combination with the measurement of alterations of protein activity, related to cell response to oxidative stress (Kouzmanova et al. 2009). However, there is not enough information if the long-term exposure to low-frequency EMF could affect DNA sequences of proteins coding genes especially those that are involved in the processes of cell response to oxidative stress such as ascorbate peroxidase (Maruta et al. 2016), glutathione peroxidase (Waszczak et al. 2018), and catalase (Mhamdi et al. 2012).

The results of the current study indicate that the exposure to the low-frequency electromagnetic field of *L. minor* clones revealed the response of the plants at the molecular and the growing parameters level. In this study, two groups of *L. minor* plants preliminary sterilized and indicated as representatives of clone S2 were directly (grown on a cylindrical coil) and indirectly (grown 1.5 m from a cylindrical coil) exposed to low-frequency 50 Hz electromagnetic radiation for 48 weeks changing the magnetic flux density from 2.3 µT (0–11 weeks) to 380 µT (12–48 weeks), focusing on growth parameters and antioxidant gene sequence analysis.

The greater differences of physical parameters measured between the two treatment groups were observed up to the 8-10th weeks from the beginning of the experiment, but over time the differences between directly and indirectly exposed *L. minor* clones decreased (Fig. 2). For the first 10 weeks from the beginning of the experiment the growth of duckweed increased affected by directly EMR compared to the indirectly affected clones. Similar effects were observed in other studies showing that plant growth could be stimulated by exposure to low-frequency electromagnetic fields (Kato 1988; Racuciu et al. 2008; Abdul et al. 2012; Radhakrishnan and Ranjitha Kumari 2011).

After 14th week of the experiment when the magnetic flux density was increased to 300 µT, we detected a negative effect observed as suppression of growth parameters (Fig. 2) and enhancement of the number of point mutations (Fig. 3) generated by electromagnetic radiation.

Finally, the unification of growth parameters between the two study groups was reached supposing that the long-lasting exposure to LF EMF promoted adaptation of the affected plants: starting from the 18th week from the beginning of the experiment plants may have become adapted to EMF as they were continuously exposed to electromagnetic radiation. A similar phenomenon of the plasticity of various plant species determining adaptations to changing environmental conditions was described earlier (Turner and Begg 1981; Asaeda et al. 2009). Plants exposed to long-term stress pass through different physiological stages: resistance, exhaustion, and regeneration phase (Lichtenthaler 1996). Our study demonstrates three phases of plant responses to increased EMR: positive stimulation (up to first 10 weeks), negative stimulation (up to 14th week from the beginning of the experiment) and supposed adaptation to long-lasting LF EMF exposure of 300 µT Magnetic flux (within 18–48 week) at the physiological (Fig. 2) and the molecular levels (Fig. 3).

It is known that prolonged exposure to electromagnetic radiation can potentially lead to rapid changes in the genetic structures of plant and animal populations (Theodorakis 2001), as well it may affect DNA sequences of genes encoding such enzymes such as catalase, glutathione peroxidase and ascorbate peroxidase playing important role in interaction and adaptation to changing biotic and abiotic environment (Staerck et al. 2017). Consequently, the appearance of new point mutations in these gene structures can be non-accidental, but in some way reduces the response of the cell to oxidative damage. Our results of molecular investigation demonstrate that increasing the number of nucleotide substitutions was higher in directly exposed LF EMF duckweed samples at the period from 10 to 14th weeks (Fig. 3). After 18th week from the beginning of the experiment, we observed a decrease of nucleotide substitutions in the GPx and Cat genes and after 48 weeks decrease of point mutations was observed in the APx gene and this phenomenon corresponded to the supposed adaptation indicated by other authors as the regeneration phase. Previously, nucleotide substitutions of antioxidant gene in its promoters have been detected under oxidative stress experiments (Lubos et al. 2011; Chistiakov et al. 2006) generating point mutations during DNA replication or due to DNA damage (Friedberg 2003).

Our results showed that the number of nucleotide substitutions detected in the promoter, intron, and exon regions of studied antioxidant genes was higher compared to the indirectly exposed to low-frequency electromagnetic

radiation *L. minor* clones (Fig. 4). The intron regions of APx1, GPx6, and Cat genes appeared most sensitive to LF EMF as generated numbers of nucleotide substitutions in directly exposed clones were significantly higher in all three studied genes compared to the promoter or exon regions of the same genes. A significantly higher number of nucleotide substitutions was also detected at the APx1 promoter and exon of catalase gene indicated as Cat7 confirming non-accidental increase of point mutations among directly affected *L. minor* clones and this phenomenon could be supposed as the signals of plant adaptation to changed growing conditions.

Taking into consideration that DNA damage caused by stress is repaired by DNA repair mechanisms in higher plants (Hu et al. 2016) it is likely that such phenomenon as reduction of nucleotide substitutions was also observed in our study. After prolonged growth (up to 48 weeks from the beginning of the experiment), we do not observe accumulation of new point mutations dependent on time in experimentally exposed to LF EMF duckweeds and it could be related to the mechanism of DNA reparation.

Conclusion

The growth parameters of duckweed clones subjected to LF EMF illustrate the positive stimulation of being directly exposed to a 2 µT magnetic flux density group until the 8th week from the beginning of the experiment and the slowing down of growth when magnetic flux was switched to 300 µT starting from 12th till 18th week in comparison to indirectly affected *L. minor* clones. We also detected that the exposure to low-frequency (50 Hz) electromagnetic radiation generated significantly higher number of point mutations in introns of ascorbate peroxidase (APx, $p < 0.079$; at 300 µT), glutathione peroxidase (GPx, $p < 0.05$; at 2–300 µT), and catalase (Cat, $p = 0.055$ at 2 µT) genes in *L. minor* clones directly affected by LF EMF in comparison to indirectly affected treatment group. Some point mutations also were detected in promoters and exons of studied antioxidant genes. The results indicate that even low-dose chronic radiation may contribute to the changes in growth parameters and generation of point mutations in antioxidant gene sequences, especially in the intron regions.

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Author contributions Conceptualization, II, DC, and DB; methodology, II, RG, and VR; software, II, RP and DB; validation, DC, DB; formal analysis, II, RV, VR, and DB; investigation, II, RV, and VR resources, VR, RV, and RP; data curation, II and DB; writing—original draft preparation, II; writing—review and editing, DB; visualization, II, RP; supervision, DB; project administration, DB and DC. All authors have read and agreed to the published version of the manuscript.

Data availability Not applicable

Declarations

Conflict of interest The authors have no conflicts of interest to declare.

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