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Effects of Low Frequency Electromagnetic Radiation on *Lemna minor* growth parameters and generation of point mutations at GPx, CAT and APx genes

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Abstract: Development of new technologies distributing electric power from power stations to our homes through a *network* of cables and wires, including numerous electric devices at working places and home environment become a source of electromagnetic radiation (EMR) much stronger than EMR of natural origin. To provide a better understanding of the impact of the EMR of anthropogenic origin on living organisms, we investigated the long-term effects of EMR on *Lemna minor*. In this study, plants of the *L. minor* laboratory clone were exposed to low frequency (50 Hz) electromagnetic radiation (LF EMR) growing clones in Petri dishes placed on the coils initially generating magnetic flux (MF) of 1 μT for first three weeks, 2 μT until 12th week and after 12th week of the experiment MF was enhanced up to 300 μT. We examined the response of the plants by sequencing DNA fragments that included promoter, intron, and exon regions of ascorbate peroxidase (APx), glutathione peroxidase (GPx), and catalase (Cat) genes) as well measured growth parameters growth intensity, frond area, and number of fronds.

Comparison of growth parameters of *L. minor* clones exposed to 2 μT and 300 μT magnetic flux revealed positive effect stimulating growth of experimentally affected plants at 2 μT After the first 14 weeks of treatment, the growth parameters were lower in the directly exposed by LF EMR group than in the group grown distantly from the source of EMR. However, after 18 weeks from the beginning of the experiment no significant difference was observed between two groups of *L. minor* including directly and indirectly affected by LF EMR plants. Moreover, the signals of the impact of LF EMR on the plants as rising of new point mutations were detected. The significantly enhanced number of variations in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to indirectly affected clones were revealed at the introns of APx ($P = 0.011$), GPx ($P = 0.009$), and Cat ($P = 0.044$) genes starting from the 10th week of the experiment

In conclusion, the comparison of DNA sequencing data together with measurements of growth parameters revealed differences in response at molecular and physiological levels of directly and

indirectly affected *L. minor* clones providing evidence that response to the impact of LF EMR depended on the prolongation of the impact and the magnetic flux density.

Key words: Low frequency electromagnetic radiation, duckweed, point mutations, catalase, glutathione peroxidase, ascorbate peroxidase

Introduction

Lemna minor is an aquatic plant with all parts of the plant floating in water. This plant belongs to the Lemnaceae family, which consists of five genera: *Landoltia, Lemna, Spirodela, Wolffia and Wolffiella*. Currently, 37 species belonging to this family have been identified (Les et al., 2002). The small float has one to four leaves that attach to a root that floats in the water. Their roots are 1–2 cm long and their floating leaves are oval shaped, 1–8 mm long and 0.6–5 mm wide (Wolverton and McDonald, 1980). *L. minor* is often used as a model organism in various studies (Hoeck et al., 2015). For example, due to its ability to absorb heavy metals from water and adapt to different environmental conditions, *L. minor* is an excellent organism for studying water pollution levels (An et al., 2018).

Although the variability of glutathione peroxidase (GPx) genes of *L. minor* has not been investigated so far, several studies related to stress and changes in GPx enzyme activity have been conducted. For example, Tjidjen and colleagues studied the effect of oxidative stress on *L. minor* and used the GPx enzyme as a biomolecular marker. During the study, *L. minor* was exposed to the herbicide diclofop-methyl at different concentrations of 17.5 µg/L, 35 µg/L, 70 µg/L. After 21 days, the obtained results showed that GPx activity is directly dependent on the duration of cultivation with herbicide and its concentration (Tlidjen et al., 2012). Another group of researchers cultivated *L. minor* for 21 days exposed to biopesticides at different concentrations (4 µL/L, 80 µL/L, 120 µL/L). Compared to the control, GPx activity also increased significantly with biopesticide concentration and cultivation time (Atamanalp et al., 2019). A considerable amount of research has been done with enzymatic activity of catalase (Cat) using *L. minor* as a model organism. Unfortunately, investigations of *L. minor* DNA sequences coding Cat gene starting from construction of primers for amplification of DNA fragments that could be suitable genetic marker to investigate impact of various factors on stability of DNA strand including generation of new point mutations have not been developed yet. Available publications mostly represent results related to measurements of changes of Cat enzyme activity under stress conditions. For example, in one study, smallmouth bass fishes were exposed to mercury, cadmium, and chromium at concentrations ranging from 0.02 to 20 mg/L. The obtained results showed that Cat activity began to increase significantly in the case of mercury, cadmium, and chromium when their concentration reached 0.2 mg/L (Varga et al., 2013). Ascorbate peroxidase (APx, EC 1.11.1.11) is one of

the key enzymes that detoxifies H_2O_2 in plants. This APx gene belongs to a family of multigene coding peroxidases that have a heme group and can catalyse the H_2O_2-dependent oxidation of various organic molecules (Lazzarotto et al., 2011).

Oxidative stress to the body can also be caused by an electromagnetic field (EMF), the background level of which is low in the natural environment, and the effect is almost imperceptible. EMFs of various frequencies are caused by human activities and are increasing every year due to the use of wireless technologies, including mobile phones, Wi-Fi, and other related devices. Since the numbers and variety of devices emitting non-ionizing radiation in the living environment is increasing significantly more and more attention is paid to their effects depending on the EMF amplitude, frequency, wavelength, tissue distance from the source of EMF or other parameters (Vian et al., 2016). To provide a better understanding of the impact of the EMR of anthropogenic origin on living organisms, we investigated the long-term effects of EMR on *Lemna minor* clones based on changing growth parameters as well screening of appearance of new point mutations in DNA sequences of candidate genes involved into regulation of oxidative stress in cells.

Materials and methods

Plant material

L. minor indicated as S2 clone was collected from the Neris River above city of Vilnius (54° 45‘48.25“, 25° 21‘14.53“) and was chosen for testing of LF EMF in the laboratory conditions. The sterilized plants were transferred to Petri dishes 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 litter 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

For the test, representatives of one colony (3 clones) of *L. minor* S2 were grown in 15 ml of Steinberg’s medium in a Petri dishes. Every seven days, the plants were transplanted into a new medium. After 2 weeks, the material was fixed, leaving one clump from each variant plate for continuation of the test by transferring to a new medium. Lighting conditions ~70–80 µmol. Temperature 25 °C ± 2 °C. For each test option, 3 Petri dishes were placed on the coils representing plants indicated as directly exposed to EMF, these samples were coded as group EMF, and the clones experiencing remote EMF exposure (plants grown in Petri dishes at 1.5 m distance

from the EMF generating coils) were coded as group C. To create EMF conditions for the cultivation of *L. minor*, a generator with a frequency of 50 Hz was used (Fig. 1).

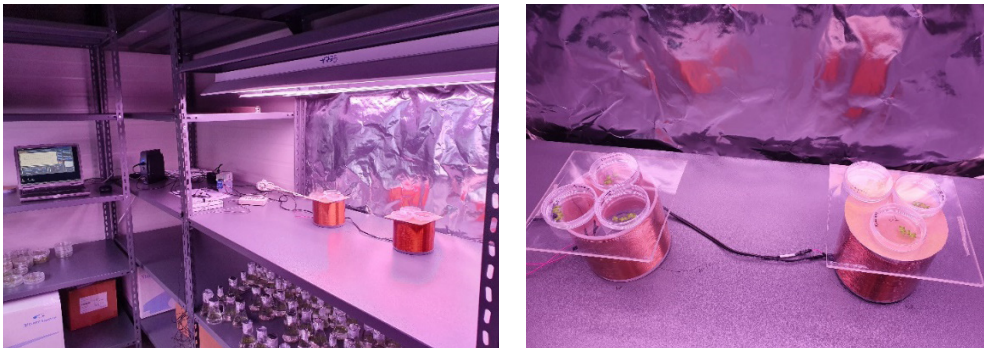


Figure 1. Cultivation of *L. minor* on EMF generating equipment. *L. minor* samples are grown on electromagnetic radiation generating coil (10 cm in diameter and 10 cm in height)

The plants were transplanted every week and the experimental conditions changing the effect of EMF on *L. minor* clones are described in Table 1. Growing parameters of experimentally affected plants were analysed after 3, 6, 8, 10, 14 and 18 weeks from the beginning of the experiment. After each week, the green mass of the plant was weighed, and the number and area of nodules were measured by ImageJ software. For DNA analysis, the material was fixed at –20 °C.

Table 1. Electromagnetic radiation parameters and experimental duration

Test duration, week	EMF induction	Strength of current
1–3	1 μT	1.2 mA
4–11	2 μT	2.4 mA
12–18	300 μT	0.4 A

DNA extraction

The whole plants with fronds and roots were grounded in liquid nitrogen and total DNA was extracted using Dneasy Plant Mini Kit method according to manufacturer's protocol (QIAGEN). 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

L. minor sequences of antioxidant genes were used for designation of primers were obtained from CoGe database (https://genomevolution.org/coge/) (Van Heck et al., 2015). Specific primers for amplification of different parts of gene including promoter, introns and exons were designed using 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 Eppendorf Mastercycler thermal cyclers. 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 3500 Genetic Analyser.

Table 2. Primer sequences of antioxidant gene markers used to study 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: TGTGCAAACACATAATCCCAAT R: TGATCATGACCAATAGATCGTT	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

Molecular data analysis

The sequenced DNA fragments were aligned using MUSCLE approach (Edgar, 2004) option in MEGA-X (Kumar et al., 2018). The aligned sequences were further analysed indicating rising of point mutations at sequences of affected by EMF colonies of S2 line in comparison to control grown distantly (1.5 m) from the source of EMF colony of the same S2 line. Before the start of the experiment plants representing

non-affected by EMF colony were collected and kept frozen and used as a source of reference DNA sequences. The presented data are means ± standard errors of at least three independent measurements for each term. The Student's t-test was used to estimate the statistically differences between the two groups representing directly and indirectly affected by EMF groups. The difference was considered significant at p levels lower than 0.05 ($p < 0.05$).

Results and Discussion

The entire length of the GPx gene is 3167 bp, therefore ~~bso~~ to make easier amplification of shorter sequences of the GPx gene, it must be broken down into smaller fragments that overlap. In this case, we constructed primers for amplification of 7 overlapping fragments: GPx1, GPx2, GPx3, GPx4, GPx5, GPx6, GPx7 (Fig. 2). All generated fragments contain at least one exon and one intron. Of the seven GPx primer pairs generated, one fraction is obtained only with the GPx6 and GPx7 primer pairs. Sequencing of these gene fragments resulted in clean sequences that were used for further analysis.

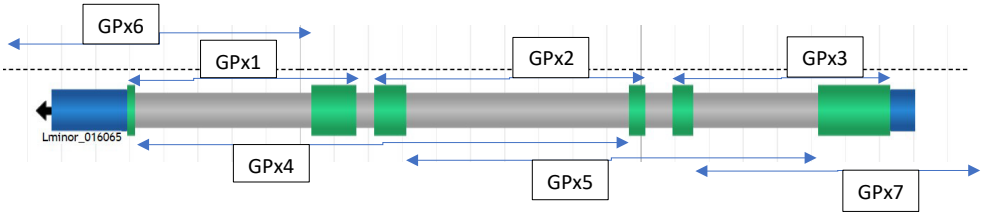


Figure 2. Positions of 7 DNA fragments encompassing whole sequence of glutathione peroxidase gene. Exon region is shown in green, intron in grey, promoter, and terminator regions in blue. Arrows indicate the relative length of the corresponding fragments for which primer pairs were created for amplification

The length of the Cat gene is 3788 bp, therefore, to sequence the entire gene, it must be broken down into smaller fragments that overlap. In this case, 9 fragments were created: Cat1, Cat2, Cat3, Cat4, Cat4a, Cat4b, Cat5, Cat6, Cat7 (Fig. 3). Fragments with Cat4, Cat4b, Cat7 primer pairs were selected for further analysis. Sequencing of fragments of this gene resulted in clean sequences that were used for further analysis.

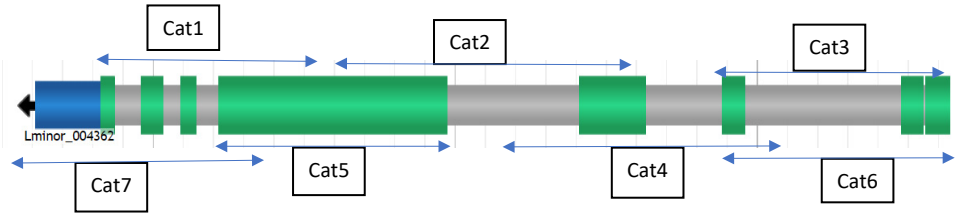


Figure 3. Positions of 9 DNA fragments encompassing whole sequence of catalase gene. The exon region is shown in green, the intron region in grey, and the promoter region in blue. Arrows indicate the relative length of the corresponding fragments for which primer pairs were created for amplification

Since the length of ascorbate peroxidase gene is 2085 bp, to perform sequencing of the entire gene, it must be split into smaller fragments that overlap. In this case, 4 fragments were obtained: APx1, APx2, APx3, APx4 (Fig. 4). Each fragment with designed primer pairs includes at least two exon and two intron regions. Sequencing results in each case showed impure sequences, making such sequences ineligible for further analysis. Primer pairs Apx1 and Apx2 that yield clean sequences are selected for further analysis

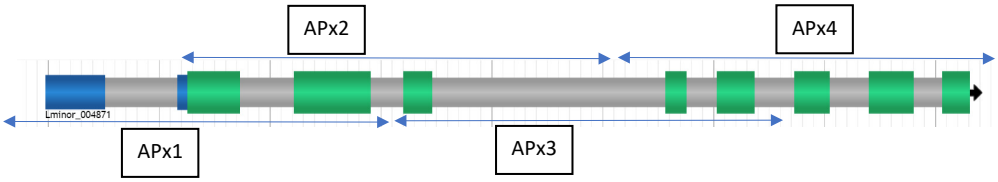
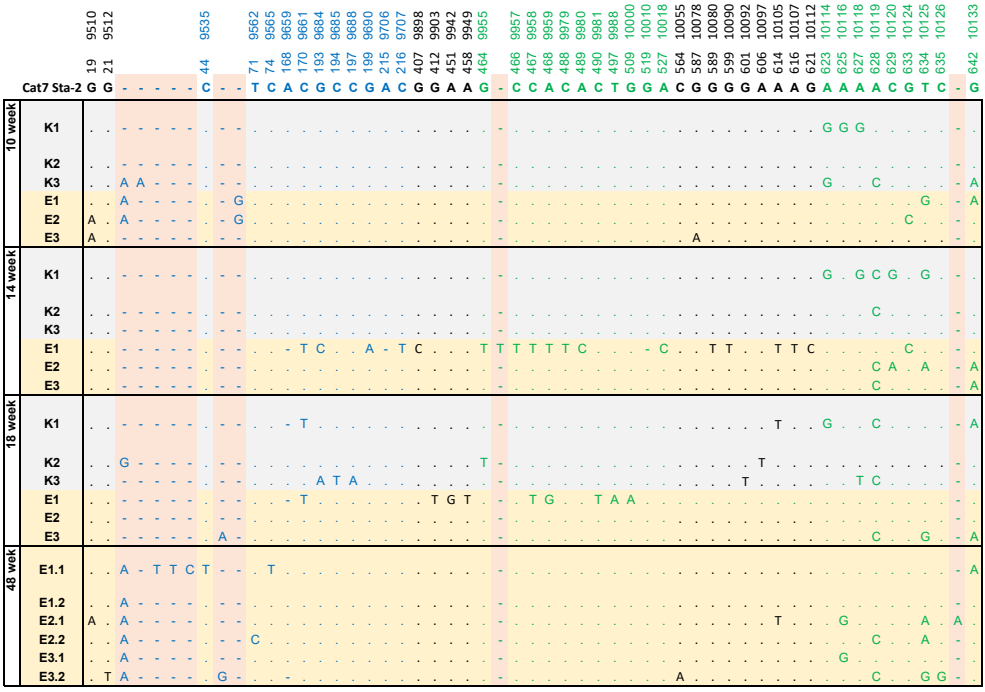


Figure 4. Positions of 4 DNA fragments encompassing whole sequence of ascorbate peroxidase gene. The exon region is shown in green, the intron region in grey, and the promoter region in blue. Gene fragments marked with arrows were amplified with the help of appropriate primers

When analysing gene markers encoding antioxidant enzymes, it was observed that the cleanest sequences were obtained with the Cat7 primer pair for all tested samples of the wild population *L. minor* (Table 3). This marker was also chosen for the study of experimental clones related to electromagnetic radiation. In the fragments amplified with the Cat7 primer pair, most nucleotide substitutions were observed in exon (623, 625, 627, 628, 629, 633, 634) in both treatment groups.

Table 3. Nucleotide substitutions and deletions detected at Cat7 fragments when comparing plants exposed to EMF with the reference sequence S2. E1, E2, E3 – plants exposed to direct electromagnetic radiation, K1, K2, K3 – plants exposed to indirect electromagnetic radiation. The exon region is marked in green; blue – promoter; grey – intron



In the fragments amplified with the GPx6 primer pair, nucleotide substitutions were observed at several positions (95, 97, 99, 101, 102, 199, 202 – promoter, 226, 228, 242 – exon) after 14 weeks and no point mutations were detected after 18 weeks in EMF S2 clones exposed independently to electromagnetic radiation (Table 4).

Table 4. Nucleotide substitutions and deletions of GPx6 fragments when comparing plants exposed to EMF with the reference sequence S2. E1, E2, E3 – plants exposed to direct EMR, K1, K2, K3 – plants exposed to indirect EMR. The exon region is marked in green; blue – promoter; grey – intron

		9244	9246	9248		9250	9251					9348	9351	9375	9377	9391					9444	9494	9710	9858	9908			9988	9989	9997		10009	10010
		95	97	99		101	102					199	202	226	228	242					295	345	561	709	759			839	840	848		860	861
	GPx6 Sta-2	A	C	A	-	G	T	-	-	-	-	G	A	G	A	G	-	-	-	-	T	A	A	A	G	-	-	C	A	A	-	A	C
10 week	K1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	K2	.	.	.	C	.	.	A	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E1	.	.	.	-	.	.	-	T	T	-	.	.	.	.	.	-	-	-	-	.	.	.	T	-	-	-	.	-	.	-	.	.
	E2	.	.	.	G	T	G	A	-	-	-	.	.	.	.	.	-	-	-	-	.	C	.	.	-	-	-	.	-	.	-	.	.
14 week	K1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	K2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	K3	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E1	G	T	C	-	-	-	-	-	-	-	-	-	.	C	-	C	-	C	-	C	.	C	.	-	-	-	.	-	.	-	T	.
	E2	.	.	.	G	T	C	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	A
	E3	-	-	-	-	-	-	-	-	-	-	T	G	A	.	A	-	T	T	T	.	.	.	.	-	-	-	.	-	.	A	.	.
18 week	K1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	K2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	K3	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E3	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
48 week	E1.1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	G	-	.	.
	E1.2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E2.1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	.	-	.	-	.	.
	E2.2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	A	-	.	-	.	-	.	.
	E3.1	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	A	-	A	.	-	.	-	.	.
	E3.2	.	.	.	-	.	.	-	-	-	-	.	.	.	.	.	-	-	-	-	.	.	.	.	-	-	-	A	C	.	-	.	.

In the fragments amplified with the APx1 primer pair, nucleotide substitutions were observed at several positions in promoter, exon, and intron after 10, 14 and 18 weeks in EMF S2 clones exposed independently of electromagnetic radiation exposure distance (Table 5). The new point mutations that do not persisted till the end of the experiment should be considered as repaired by the DNA repair mechanism.

Table 5. Variable positions and nucleotide substitutions of APx1 fragments when comparing plants exposed to EMF with the reference sequence S2. E1, E2, E3 – plants exposed to direct EMR, K1, K2, K3 – plants exposed to indirect EMR. The promoter region is marked in blue, the rest is the intron

		28286	28318	28319	28320	28438	28446	28629	28712	28729	28828	28833	28854	28876	28911	28915	28916	28924	28925	28941	28964	28977
		39	71	72	73	191	199	382	465	482	581	586	607	629	664	668	669	677	678	694	717	730
	Apx1 Sta-2	G	-	T	C	T	-	G	A	G	-	-	C	T	-	-	G	-	-	C	-	C	-	-	G	-	-	G	-	-	C	-	-	-	G
10 week	K1	-	-	.	.	C	T	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	A	.	-	-	-	.	-	-	-	.
	K2	.	-	.	.	.	C	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	K3	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	E1	-	-	-	-	-	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	E2	.	-	-	-	-	-	.	.	C	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	T	-	T	.
	E3	-	-	-	-	-	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	-	-	-	.
14 week	K1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	K2	.	-	.	.	C	C	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	-	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	K3	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	T	C	.	-	-	.	.	-	-	-	.	-	-	-	.
	E2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	C	.	.	-	-	.	.	-	-	-	.	-	-	-	.
	E3	.	-	.	.	.	-	.	G	.	-	-	.	.	-	-	.	-	-	.	-	-	.	G	.	-	-	.	.	-	-	-	.	-	-	-	.
18 week	K1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	C	.	.	-	-	.	.	-	-	-	.	-	-	-	A
	K2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	K3	.	A	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	E1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	E2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	A	.	-	-	-	.	-	-	-	.
	E3	.	-	.	.	C	-	T	.	T	.	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	.	.	-	-	T	.	-	-	-	.
48 week	E1.1	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	E1.2	.	-	.	.	.	-	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	A	.	-	-	-	.	-	A	-	.
	E2.1	A	G	.	.	.	-	.	.	C	.	.	T	-	.	G	C	A	T	.	-	A	A	A	A	A	T	.	.	.	G	G	.	G	T	-	G	C	G	T	A
	E2.2	.	-	.	.	.	-	.	.	.	C	.	-	-	.	-	.	-	.	.	-	-	.	-	.	-	-	.	.	-	-	-	.	-	-	-	.
	E3.1	.	-	.	.	.	-	C	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	A	T	C	T	C	T	-	A	-	-	T	.	-	-	-	A
	E3.2	.	-	C	T	C	T	.	.	.	-	-	.	.	-	-	.	-	-	.	-	-	.	-	.	-	-	.	.	-	-	-	.	-	-	-	.

Comparison of growth parameters of *L. minor* clones exposed to 1 μT, 2 μT, and 300 μT magnetic flux revealed positive effect stimulating growth of experimentally affected plants at 2 μT. After the first 14 weeks of treatment, the growth parameters were lower in the directly exposed to LF EMR group than in the group grown distantly from the source of EMR. However, after 18 weeks from the beginning of the experiment no significant difference was observed between two groups of *L. minor* including directly and indirectly affected by LF EMR plants. Moreover, the signals of the impact of LF EMR on the plants rising point mutations were detected. The significantly enhanced number of variations in DNA sequences of *L. minor* clones directly affected by LF EMR in comparison to indirectly affected clones were revealed at the introns of APx ($P = 0.011$), GPx ($P = 0.009$), and Cat ($P = 0.044$) genes starting from the 10th week of the experiment.

In conclusion, the comparison of DNA sequencing data together with measurements of growth parameters revealed differences in response at molecular and physiological levels of directly and indirectly affected *L. minor* clones providing evidence that response to the impact of LF EMR depended on the prolongation of the impact and the magnetic flux density.

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