

## Electromagnetic fields in precision agriculture: Do they provoke oxidative stress in maize plants?

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### Abstract

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Precision agriculture is a strategy for managing agricultural activities by using modern technologies, including communication modules, employing radiofrequency (RF) electromagnetic fields (EMF), to increase the farming efficiency. Many studies have shown that plant species respond to RF EMF. This study aims to investigate the effects of 900 MHz EMF, used in precision agriculture on oxidative stress parameters in young maize plants.

*Zea mays* plants, variety Knezha-683A, at the developmental stage of second leaf, were exposed for 2 hours to 900 MHz EMF continuous wave, 370 V/m, in a semi-anechoic chamber. Since EMF—matter interaction and absorbed energy depend on the orientation of the object to the field vectors, two experimental setups were arranged, with the electric field vector perpendicular, or parallel to the plant stems. *Control* plants were transported to the place of irradiation but were not exposed to the EMF. Third group of plants stayed in the growing camera – *referent control*. Total antioxidant activity (TAA), hydrogen peroxide and thiobarbituric acid reactive substances (TBARS) content in the first and second leaf of the plants were determined one and two hours after the end of the exposure. The presented data were averaged from 3 independent experiments.

The obtained results showed differences in the oxidative status between the first and the second leaf. No statistically significant differences between exposed and control plants in the H<sub>2</sub>O<sub>2</sub>, TBARS content or the TAA were found. Under the investigated experimental conditions, 900 MHz, 370 V/m EMF does not induce oxidative stress in young maize plants.

**Keywords:** 900 MHz electromagnetic field; hydrogen peroxide; TBARS; total antioxidant activity

### Introduction

Increasing number of modern technologies, such as radiofrequency (RF) modules, are being implemented in agriculture, including in crop production and forestry. Hence, the interest to the effects of RF EMF on plants increases. Many studies show that a number of plant species, *Phaseolus vulgaris*, *Vig-*

*na radiata*, *Zea mays*, *Pisum sativum*, *Solanum lycopersicon*, *Plectranthus*, *Lemna minor*, etc., perceive and respond to RF EMF (Vian et al., 2016 and References therein).

EMF might induce stress or adaptive responses in plants (Beaubois et al., 2007; Monselise et al., 2011; Tran et al., 2023). Stress responses in plants often include an increase in reactive oxygen species (ROS) (Kaur et al., 2021), which in

turn activate antioxidant system. Therefore, if EMF act as a stressor, they might affect the antioxidant status.

Reported effects of RF EMF on plants involve:

1. Molecular mechanisms: enzyme activity, energy metabolism, oxidative stress markers ( $H_2O_2$ , MDA) alterations in *Lemna minor*, *Triticum aestivum*, *Zea mays*, *Alium cepa*, and *Brassica oleracea* (Tkalec et al., 2007; Dimitrova et al., 2009; Zare and Mohsenzadeh, 2015; Chandel et al., 2017; Handa et al., 2024), cytogenetic changes, effects on gene expression and protein synthesis in tomato and *Lactuca sativa* (Roux et al., 2008; Tran et al., 2023).
2. At the organismal level: stress reactions, morphological changes, alterations in growth rate reported in *Vigna radiata* (Sharma et al., 2009, 2010), in root and stem dimensions, stomatal conductance, photosynthetic activity, heliotropism, yield, content of volatile organic compounds and essential oils (Czerwiński et al. 2020, Kaur et al. 2021).
3. At the ecosystem level: interactions with other plants, insects (pollinators), vertebrates (Czerwiński et al. 2020).

Czerwiński et al. (2023) consider that the exposure to RF EMF at background levels could induce irreversible effects in some plant species growing in the natural environment. Among these are *Trifolium sp.* and other legumes, which are important component in European grasslands. The authors supposed that *Trifolium arvense* could be an indicator of man-made RF EMF in the environment.

Plant responses to EMF could be immediate or delayed (Kaur et al. 2021). Immediate responses include alterations in cytosolic  $Ca^{2+}$  concentration and ROS homeostasis (imbalance between ROS production and scavenging), which lead to changes in metabolic activity and gene expression, and as a result – to rapid cellular effects, e.g. photosynthesis activity, DNA and chloroplast alterations, change in tissue thickness. Delayed plant responses affect growth, which occur days after exposure.

Precision (smart/intelligent) agriculture is an approach for managing agricultural activities by using modern information and communication technologies, and artificial intelligence in order to increase the quantity and quality of products; save time and effort; ensure a more rational use of resources, such as water and fertilizers; reduce costs (Davidova, 2019; IoT in Crop Production, 2019). Many sensors are used to send information about the environmental conditions. The sensors take measurements at predetermined time intervals, and the collected data are periodically sent wirelessly to a control device. This leads to an increase in EMF irradiation of plants, which could affect their development.

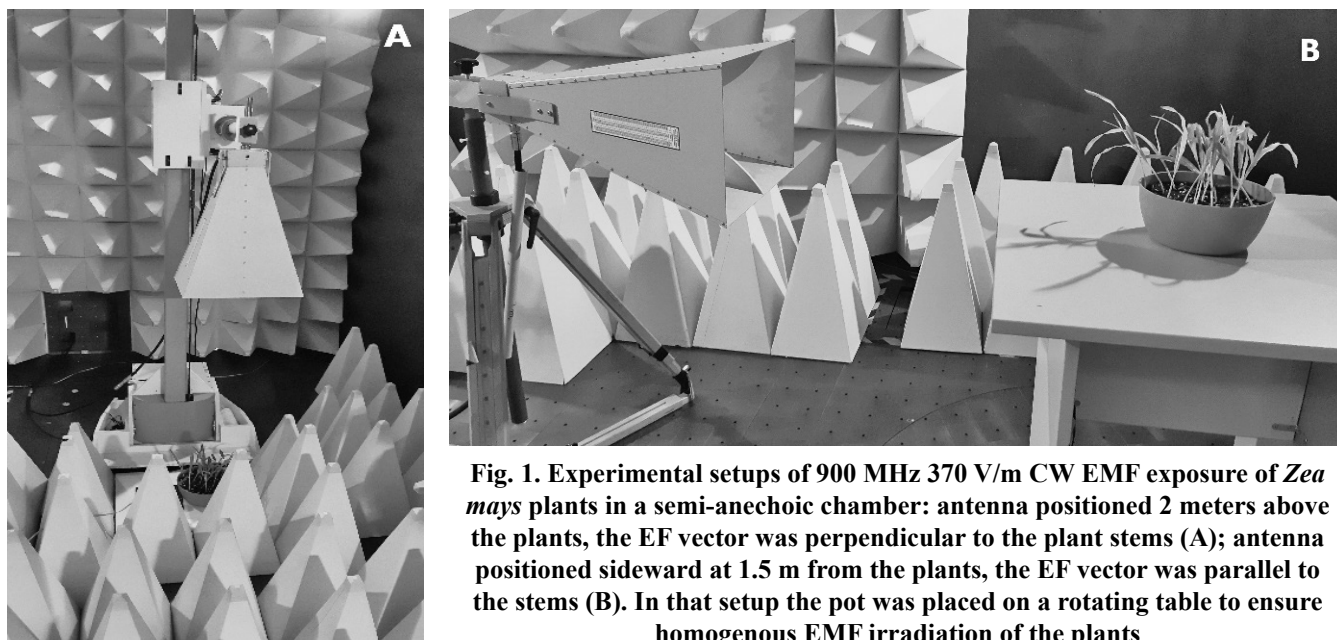
Sensors can be placed in different locations: deep in the soil, on its surface, attached to the plants, on ground vehicles or drones, and even satellites, leading to different directions of the field vectors to the plant axis and higher energy absorption in specific organs (roots, leaves or stem). Since absorbed EMF energy depend on the orientation of the object to the field vectors the polarization of the EM wave should be considered when the biological effects are being analysed.

There are no studies in the available literature comparing the effects of RF EMF with different polarizations on plants. The aim of our research was to investigate the effects of 900 MHz EMF used in precision agriculture on oxidative stress parameters in *Zea mays* plants when the electric field was oriented in different directions to the stem (main axis) of the plants.

## Material and Methods

*Zea mays* variety Knezha-683A (Maize Research Institute, Knezha, Bulgaria) plants were sown in pots with a universal peat soil mixture, pH 6.5 (Gamma Company Ltd., Sofia, Bulgaria) and grown in a chamber under controlled conditions: temperature 23/25°C (night/day), light intensity 80  $\mu\text{mol photons}/(\text{s}\cdot\text{m}^2)$  PPFD (Photosynthetic Photon Flux Density) and photoperiod 12/12 h light/dark.

On the 10<sup>th</sup> day after the sowing, when plants were developing their second leaf, one pot was exposed to 900 MHz EMF continuous wave (CW) for 2 hours in a semi-anechoic chamber. The EMF was emitted by horn antenna connected through power amplifier FLG-50F (Frankonia, Heideck, Germany) with signal generator SMB-100A (Rohde & Schwarz, Munich, Germany) by coaxial cable. Before exposure the electric field at the spot of the pot was set to 370 V/m by tuning the generator output power, while monitoring the electric field with LSProbe 1.2 Field Probe System, (LUMILOOP GmbH, Dresden, Germany). It should be noted that such an electric field would be extreme case in precision agriculture settings since it is 4 times higher than the referent levels allowed for people working in EMF (100 kHz – 300 GHz) environments for 30 minutes – 92 V/m, and 9 times higher than the levels allowed for the general public – 41.2 V/m at 900 MHz (ICNIRP, 2020). However, our study tested for detrimental effects at the upper regulatory limit. Since the interaction between matter and an electromagnetic wave depends on its polarization (Vanegas-Acosta, 2015) and horn antennae emit linearly polarized radio waves, two experimental setups were arranged: 1. antenna positioned above the pot, providing electric field (EF) vector perpendicular to the plant stems (Fig. 1, A); 2. antenna positioned sideward to the pot – electric field vector parallel to the stems (Fig. 1, B).



**Fig. 1. Experimental setups of 900 MHz 370 V/m CW EMF exposure of *Zea mays* plants in a semi-anechoic chamber: antenna positioned 2 meters above the plants, the EF vector was perpendicular to the plant stems (A); antenna positioned sideward at 1.5 m from the plants, the EF vector was parallel to the stems (B). In that setup the pot was placed on a rotating table to ensure homogenous EMF irradiation of the plants**

A second pot – *control* plants, was transported to the place of irradiation but the plants were not exposed to the EMF. A third pot stayed in the growth chamber – *referent control* (RC).

Plant oxidative stress was evaluated by total antioxidant activity (TAA), hydrogen peroxide ( $H_2O_2$ ) and thiobarbituric acid reactive substances (TBARS) content in the first and in the second leaf of the plants at two time intervals: one and two hours after the end of the exposure. At each time point about 300 mg fresh weight (FW) leaf material was collected from six plants (about 50 mg per plant) and homogenized with quartz sand by mortar and pestle in 3 mL 1% w/v trichloroacetic acid (TCA) at 4°C. The suspensions were centrifuged for 20 min at 15000 g. The supernatants were used for preparing the final sample solutions which were measured spectrophotometrically.  $H_2O_2$  concentration (nmol/g FW) was determined from absorbance at 390 nm ( $A_{390}$ ), extinction coefficient  $\epsilon_{390} = 0.28 \mu M^{-1} \cdot cm^{-1}$ , of supernatants diluted with 0.1 M potassium phosphate buffer, pH 7.6, and 1 M KI in ratio 1:1:2 after 1-hour incubation in dark (Alexieva et al., 2001). The samples were shaken vigorously every 15 minutes. TBARS content (nmol/g FW) was calculated from  $A_{532}$ ,  $\epsilon_{532} = 0.154 \mu M^{-1} \cdot cm^{-1}$ , of 1:1:2 supernatant-buffer-reagent solutions after 30-minute boiling water bath followed by cooling at 4°C. The reagent used was 0.5% w/v thiobarbituric acid in TCA (20 % w/v) (modified method of Kramer et al., 1991, described in Velikova et al., 2000). TAA was determined by the ABTS radical cation decolourization assay (Re et al. 1999). Standard curve was constructed with Trolox as an antioxidant and TAA values were expressed as  $\mu mol$  Trolox equivalent (TE)/g FW

from the relative decolourization of samples after 30-minute incubation:  $\Delta A_{734}(\text{Sample}) = \{[A_{734}(\text{Blank}) - A_{734}(\text{Sample})] / A_{734}(\text{Blank})\}$ . For all methods blank samples contained 1% w/v TCA instead of homogenization supernatants.

The presented data are average  $\pm$  SEM (standard error of mean) values calculated from 3 independent experiments for each setup – parallel and perpendicular electric field vector. For every experiment 3 pots were used – 1 *control*, 1 *EMF* and 1 *RC*. Each pot contained 17 plants. 6 of the plants were chosen at random for each treatment variant and time-point for homogenization of the leaves (both 1<sup>st</sup> and 2<sup>nd</sup> leaf from the same plants) and from each extraction 2 samples were prepared for measurement. Two-way ANOVA, with factors: leaf (1<sup>st</sup> and 2<sup>nd</sup>) and combination treatment–time-period-after-treatment (*Control - 1 h*, *Control - 2 h*, *EMF - 1 h*, *EMF - 2 h*, *RC - 1 h* and *RC - 2 h*), followed by Duncan's (new multiple range) test were applied to determine differing values amongst all experimental variants at 0.05 significance level. In figure panels, contrasting variants were denoted by different letters. Pearson correlation coefficients ( $\rho$ ) between values of TAA,  $H_2O_2$  and TBARS from the whole experimental data set ( $n = 36$ ) were calculated. All statistical analyses, as well as the presented graphics, were done in R programming language version 4.4.1 custom script (Paunov, 2024).

## Results and Discussion

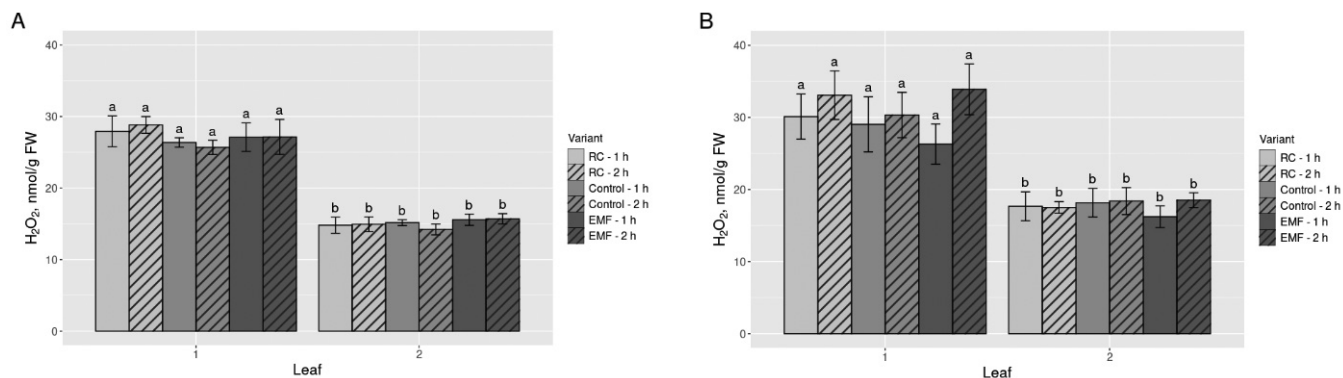
$H_2O_2$  concentrations in the *Z. mays* leaves for all experimental variants are presented in Fig. 2. No significant differ-

ences were observed among the Control, EMF and RC plants independent on the leaf examined or the period after irradiation for both polarization setups. Further,  $H_2O_2$  was not changing significantly in time even though there were some minor fluctuations in the 1<sup>st</sup> leaf for the parallel polarization (Fig. 2B). The only factor determining  $H_2O_2$  levels was the leaf age – the 1<sup>st</sup> contained nearly twice as much  $H_2O_2$  as the 2<sup>nd</sup>.

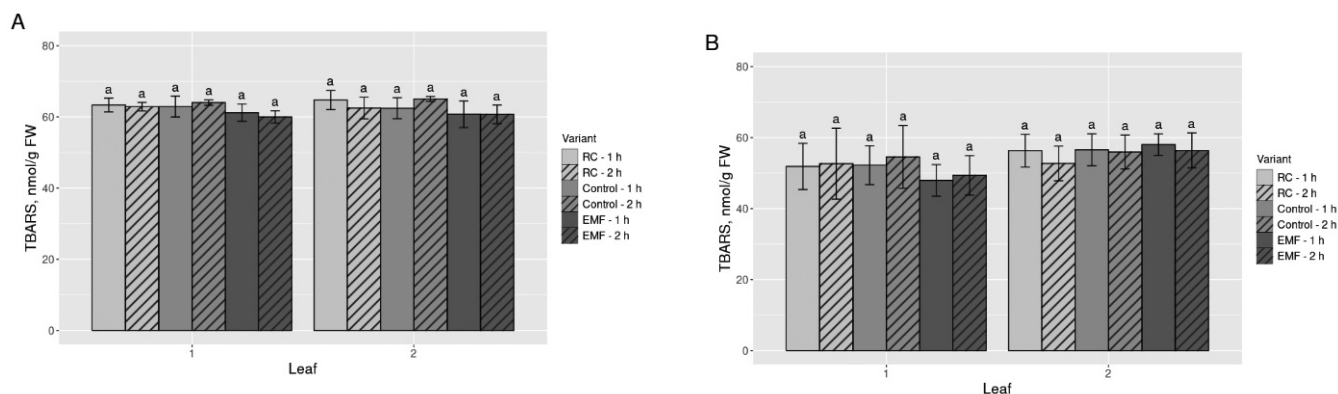
TBARS showed no dependence on any of the factors considered in this study (Fig. 3).

TAA results displayed a pattern similar to that of  $H_2O_2$  (Fig. 4). 1<sup>st</sup> leaf had more antioxidants than the 2<sup>nd</sup> leaf for perpendicular polarization. However, leaf age was associated with significantly higher antioxidants just in two of the six variants for parallel polarization due to the big variance in the experimental data (Fig. 4B). Both the EMF exposure and the time elapsed after it did not affect the TAA.

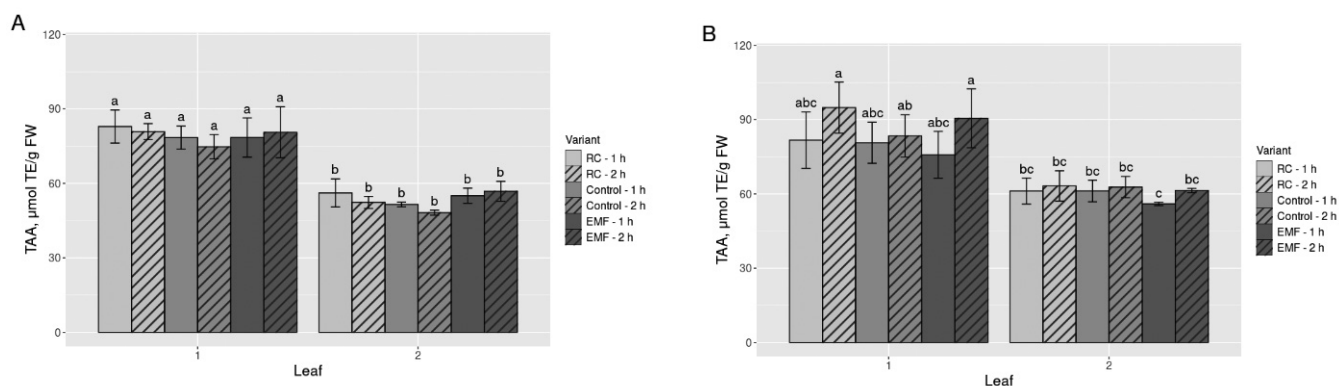
Coefficients of correlation between the examined parameters are presented in Table 1. The relationship between  $H_2O_2$



**Fig. 2.**  $H_2O_2$  concentration in 1<sup>st</sup> and 2<sup>nd</sup> leaf of *Z. mays* plants: *EMF* – irradiated with 900 MHz 370 V/m CW EMF, for 2 hours in a semi-anechoic chamber, with the direction of the electric field vector perpendicular (A) or parallel (B) to the plant stems; *Control* – unexposed plants; and *RC* – referent control plants, which stayed in the growth chamber. The values were determined one (1 h) and two hours (2 h) after the end of the EMF treatment. Average  $\pm$  SEM values from 3 independent experiments are displayed. Different letters denote statistically significant differences between experimental variants ( $p < 0.05$ ) determined by Duncan's test following two-way ANOVA



**Fig. 3.** Thiobarbituric acid reactive substances (TBARS) content in 1<sup>st</sup> and 2<sup>nd</sup> leaf of *Z. mays* plants: *EMF* – exposed to 900 MHz 370 V/m CW EMF, for 2 hours, in a semi-anechoic chamber, with the direction of the electric field vector perpendicular (A) or parallel (B) to the plant stems; *Control* – unexposed plants; and *RC* – referent control plants, which stayed in the growth chamber. The values were determined one (1 h) and two hours (2 h) after the end of the EMF treatment. Average  $\pm$  SEM values from 3 independent experiments are displayed. Different letters denote statistically significant differences between experimental variants ( $p < 0.05$ ) determined by Duncan's test following two-way ANOVA



**Fig. 4. Total antioxidant activity (TAA) in 1<sup>st</sup> and 2<sup>nd</sup> leaf of *Z. mays* plants: *EMF* – irradiated with 900 MHz 370 V/m CW *EMF*, for 2 hours, in a semi-anechoic chamber, with the direction of the electric field vector perpendicular (A) or parallel to the plant stems (B); *Control* – unexposed plants; and *RC* – referent control plants which stayed in the growth chamber. The values were determined one (1 h) and two hours (2 h) after the end of the *EMF* treatment. Average  $\pm$  SEM values from 3 independent experiments are displayed. Different letters denote statistically significant differences between experimental variants ( $p < 0.05$ ) determined by Duncan's test following two-way ANOVA**

**Table 1. Pearson correlation coefficients between pairs among  $H_2O_2$ , TBARS and TAA variables determined their values for all the experimental variants of the two experimental setups.**

Combination of variables	Pearson correlation coefficient, $\rho$	
	Perpendicular polarization	Parallel polarization
$H_2O_2$ – TBARS	-0.04	-0.52
TBARS – TAA	-0.12	-0.39
$H_2O_2$ – TAA	0.94	0.90

and TAA was positive and strong ( $\rho \geq 0.9$ ) for both polarization setups. TBARS correlated weakly in inverse direction with the other two parameters when the electric field was parallel to the stems ( $-0.52 \leq \rho \leq -0.39$ ), but no correlation was observed for perpendicular polarization ( $-0.12 \leq \rho \leq -0.04$ ).

Plant stress at the cellular level is often expressed as oxidative stress – imbalance between oxidant and antioxidant substances in favour of the oxidants (Kaur et al., 2021).  $H_2O_2$  is a molecule associated with different biological roles in the plant cell. On the one hand it is a midpoint in the ROS enzymatic detoxifying pathway, being generated from superoxide anion radical ( $\cdot O_2^-$ ) and decomposed to  $H_2O$  (and  $O_2$ ) (Winterbourn, 2013). However, by the Fenton reaction it can produce the extremely reactive hydroxyl radical ( $\cdot OH$ ), which induce rapid peroxidation of membrane lipids – a main damaging mechanism of oxidative stress (Ayala et al., 2014). On the other hand,  $H_2O_2$  has important roles as a sig-

nalling molecule in the regulation of a variety of biological processes (Kaur et al., 2021). TBARS are major indicator for lipid peroxidation while TAA corresponds to the content of non-enzymatic ROS scavenging substances.

Absence of differences in the TAA, the  $H_2O_2$  and TBARS contents between control and exposed *Z. mays* plants indicates that the *EMF* with the applied parameters had no effect on the oxidative status of the leaves. That finding is in contrast with other studies reporting higher production of  $H_2O_2$  and lipid peroxides in RF *EMF*-irradiated plants outlined in Kaur et al. (2021). Sharma et al. (2009) exposed *Vigna radiata* seeds to modulated 900 MHz *EMF* from cell phones, and reported significant time-dependent inhibition of the germination and radicle growth, and development of oxidative stress, with onset 30 min after the irradiation start, even though the applied field was weak ( $8.55 \mu W cm^{-2}$ , 5.7 V/m). Another investigation followed effects of exposure of *Zea mays* plantlets to DECT base (24 h/day, 7 days, pulsed transmission mode, at 1882 MHz) and found that the sprouting potential, biomass production, leaf structure, photosynthetic pigment content were not affected but the structure of the chloroplasts (Stefi et al., 2017). In our experiment we use *EMF* with the same frequency as Sharma et al. (2009) did – 900 MHz, but we apply CW and with higher electric field (370 V/m); and the same object as Stefi et al. (2017) – *Zea mays*. CW *EMF* is expected to cause lower biological response than modulated EM waves since living organisms are characterized by temporal dynamics maintaining homeostasis, and adaptation is easier when an environmental factor does not change in time. The fact that our and Stefi et al.

results did not show impact of the RF EMF applied could be explained by the possibility *Zea mays* to be more resistant and/or adaptable to external factors, including EMF, than *Vigna radiata* is, and roots could be more susceptible organ in generally.

Another reason we did not observe EMF induced oxidative stress could be the periods after treatment at which we performed measurements – they might be too long to register the cellular stress reactions. Supporting evidence is the lacking dynamics between the first and the second time point. Furthermore, we tested two directions of the electric field vector – perpendicular and parallel to the stems, since polarization is unique parameter for man-made EMF (Panagopoulos et al., 2015). Parallel polarization is expected to result in more energy absorbed by the plants and possibly higher oxidative stress, but all the above-mentioned experimental conditions seem to be more important determinants of biological effects apprehending the manifestation of the role of polarization.

Higher oxidants (H<sub>2</sub>O<sub>2</sub>) and antioxidants (TAA) levels in the first leaf compared to the second one might reveal the higher physiological activity of the fully developed older first leaf. There was a good general correlation between these two parameters, which means the cell redox system was well balanced. As a result, no enhanced lipid peroxidation in both the 1<sup>st</sup> and 2<sup>nd</sup> leaf was observed at both directions of the electric field. Furthermore, EMF did not provoke oxidative stress, regardless of the physiological activity of the leaves.

## Conclusions

Our data showed that under the investigated experimental conditions (two-hour exposure to 900 MHz CW EMF, 370 V/m electric field vector parallel or perpendicular to the plant stems) oxidative stress in young *Zea mays* plants had not been induced. The technologies, which are used in smart agriculture and work on 900 MHz EMF would not have a negative side effects on the maize crops. If there was any activation of ROS generation, it probably was diminished by the antioxidant system, and cell membrane was not interrupted as estimated by lipid peroxidation levels (TBARS content). These results are in agreement with our previous studies of 900 MHz EMF effects on wheat *Triticum aestivum* (Dragolova et al., 2009) and pea *Pisum sativum* (Kouzmanova et al., 2010), but not in conformity with the results of other authors, indicating ROS generation in response to RF EMF (Radić et al., 2007; Tkalec et al., 2007; Chandel et al. 2017; Handa et al., 2024). A compelling reason for this discrepancy could be differences in objects susceptibility (different species),

different sensibility of the plant organs investigated (roots, shoots, leaves) and different EMF parameters (exposure time, intensities, time elapsed after exposure, etc.). Further research is needed to estimate the possible overall effects of RF EMF on crop plants.

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## Conflict of interest

Authors declare no conflict of interest.

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