

ELECTROMAGNETIC FIELD USED IN PRECISION AGRICULTURE DOES NOT INDUCE LONG-TERM EFFECTS IN WHEAT AND MAIZE

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Abstract

With the increasing application of wireless technologies in crop production, the question whether radiofrequency (RF) electromagnetic fields (EMF) influence crops becomes significant. Effects of RF EMF on plants were studied at genetic, physiological, morphological, anatomical levels, and the obtained results were contradictory. EMF could affect plant growth, biomass accumulation and metabolite synthesis, and thus the quality and quantity of the crop yield. The aim of this study was to examine long-term effects of EMF emitted by wireless sensors used in precision agriculture on economically important wheat and maize varieties, during the sensitive early period of crop development. We investigated morphological (growth rate, biomass) and physiological characteristics (photosynthetic pigments, reducing sugars, anthocyanins and malondialdehyde) 10 days after 2-hour irradiation of sprouts with 900 MHz 370 V/m continuous electromagnetic wave. None of the examined parameters displayed statistically significant difference between control and exposed plants. We concluded that the 900 MHz EMF is safe for crops subjected to precision agriculture technologies even at strong electric field exposures for two hours.

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Abbreviations: A_{530} – Absorbance at 530 nm, ANOVA – Analysis of variance, DW – Dry weight, EMF – Electromagnetic field/s, FW – Fresh weight, H_2O_2 – Hydrogen peroxide, MDA – Malondialdehyde, MRT – Multiple range test, RF – Radiofrequency, ROS – Reactive oxygen species, SEM – Standard error of mean

Introduction. Wireless telecommunication is a fundamental pillar of modern civilization based on data transmission using RF EMF. The widespread use of wireless devices relies on a vast and dense network infrastructure of base stations and communication satellites. All that leads to an increase of electromagnetic background all over the world and concerns about the impact of RF and microwave EMF on living organisms are increasing. Increasing application of new technologies in precision (smart) agriculture as a way to improve crop production raises the question whether RF EMF influence plants. Many authors have investigated effects of exposure to EMF with different frequencies, intensities, time of irradiation etc. on plants at different stages of development (seeds, sprouts, seedlings, developed organisms), tracking changes in various characteristics, obtaining contradictory results. Alterations have been observed at genetic, physiological, morphological and anatomical levels.

TKALEC et al. [1] exposed *Allium cepa* L. seeds to RF EMF with different parameters (frequency: 400 MHz and 900 MHz; electric field: 10, 23, 41 and 120 V/m, continuous waves, period: 2 hours; or 23 V/m, amplitude modulated waves, 4 hours) and did not find significant changes in germination rates and root length. An increase of the mitotic index in root tips, mitotic and chromosomal abnormalities were observed depending on the EMF frequency, intensity and modulation used in the treatment. Nine hundred MHz, stronger and modulated fields were more effective. Induced mitotic abnormalities in root meristematic cells were probably due to mitotic spindle damage.

SENAVIRATHNA and MAIMAITI [2] assessed the 48-hour exposure to 2.45 GHz microwaves (continuous wave, $1.0 \pm 0.1 \text{ W} \cdot \text{m}^{-2}$) on young *Arabidopsis thaliana* (L.) Heynh. plants and found higher levels of photosynthetic pigments, oxidative stress, guaiacol peroxidase and ascorbic peroxidase activities in exposed plants but decreased catalase activity.

EMF could influence the crop production by affecting plant growth, biomass accumulation and metabolite synthesis. Different physical stressors: cold plasma, vacuum and RF EMF, applied on *Crocus sativus* L. stigmas (saffron) did not affect the viability of corms but caused stressor-dependent changes in sprouting, growth parameters, leaf trichome density, and secondary metabolite content in stigmas. EMF was the most effective factor in the increasing of saffron secondary metabolite production [3]. Irradiation of seeds of parsley (*Petroselinum crispum* (Mill.) Fuss cv. Plain leaved 2), dill (*Anethum graveolens* L. subsp. hortorum cv. Common)

and celery (*Apium graveolens* L. cv. Pascal Giant) with WLAN-frequency (2.412–2.48 GHz) resulted in more severe stress than irradiation with GSM-frequency (860–910 MHz) leading to alterations in leaf structure and essential oil content [4]. Exposure of *Zea mays* L. to 1882 MHz EMF (Digital Enhanced Cordless Telecommunications base) for 24 h a day, 7 days a week, pulsed transmission mode, did not affect the sprouting potential, biomass production for the above-ground part and the root, leaf structure and photosynthetic pigment content in the exposed plants but a slight swelling of thylakoids was observed in chloroplasts in the exposed leaves [5].

SHARMA et al. [6, 7] exposed *Vigna radiata* (L.) R. Wilczek (mung bean) seeds to 900 MHz EMF for 0.5, 1, 2, and 4 h and observed time-dependent inhibitory effect on the germination (at ≥ 2 h), and radicle and plumule growth (≥ 1 h). The seedling length and dry weight, as well as the content of proteins and carbohydrates were significantly reduced after exposure for 0.5, 1, 2, and 4 h. The activity of a number of enzymes, including reactive oxygen species (ROS) scavenging enzymes, was increased as a reaction to EMF-induced stress. Malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents were higher in the exposed plants. The increased activities of antioxidant enzymes could not compensate oxidative stress caused by 900 MHz EMF.

Effects of RF EMF reported in available literature are contradictory. Interactions between the EMF and living organisms, in particular – plants, are very complex and depend on many factors – EMF parameters, exposure conditions, plant species, stage of development, examined characteristics, etc. Additional experiments and data accumulation are needed to clarify the mechanisms of RF EMF effects on plants and to ensure safety of ecosystems and crop production.

Evaluation of plant physiological condition includes monitoring molecular markers content. Some of the often analyzed substances are photosynthetic pigments (metabolism markers), anthocyanins (free radical scavengers), total, soluble or reducing sugars (carbohydrate metabolism), malondialdehyde (product of free radical reactions, related to lipid peroxidation). Changes in their concentrations could affect plant cell division, germination, vegetative growth, development, flowering and yield, ageing processes, responses to abiotic and biotic stresses. They give important information about plant reactions to different environmental factors [8]. All monosaccharides (glucose, fructose, galactose, etc.) and many disaccharides (lactose, maltose and cellobiose) can act as a reducing agent, so they are reducing sugars. Sucrose is a nonreducing disaccharide. These soluble sugars are important for plants as nutrients, metabolite signalling molecules; they are involved in stress responses, in ROS production in mitochondria or photosynthesis regulation. They are linked also with anti-oxidative processes, such as the oxidative pentose-phosphate pathway and carotenoid biosynthesis. Stress reactions involving soluble sugars are related to changes in ROS balance [9]. Glucose, as a signalling molecule, regulates all processes of plant growth and development: seed

germination, cell proliferation and death, cell expansion and elongation, primary root length, seedling growth and development, root gravitropism, lateral roots, carbon and nitrogen metabolism and stress responses, root hairs, shoot meristem maintenance, reproduction, senescence, photosynthetic gene expression, crop yield and product quality [10].

The aim of our study was to investigate the effects of 900 MHz electromagnetic field – a frequency used as a carrier wave by wireless sensors used in high-value agriculture – on the growth and synthesis of metabolites during the early development period of wheat and maize.

Materials and methods. *Plant material.* The objects of this study were *Zea mays* L. cv. Knezha-683A (Maize Research Institute, Knezha, Bulgaria) and *Triticum aestivum* L. cv. Enola (Dobrudzha Agricultural Institute, General Toshevo, Bulgaria) – varieties important for Bulgarian agriculture. Seeds of both species were put in 4-liter pots filled with 3 L hand-pressed universal peat-soil mixture, pH 6.5 (Gamma Company Ltd., Sofia, Bulgaria), 2 pots per species, 17 seeds per pot. The plants were grown at uncontrolled average ambient temperature of 20/29 °C (night/day) in a light chamber providing 80 $\mu\text{mol photons}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ photosynthetic photon flux density, at a 12/12 h light/dark photoperiod. The plants were kept watered adequately.

Treatment. The treatment was done 5 days after sowing when seedlings were just few centimetres high. One of the pots was exposed to 900 MHz continuous electromagnetic wave in a semi-anechoic chamber for 2 hours – EMF variant. 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. The antenna was located 2 m above the pot so that:

1. The highly mitotically active apical meristem of the shoots was proximal to the antenna and absorbed the greatest EMF energy amount of all the plant tissues.
2. The polarization and the level of the electric field are well-defined since the pot is in the radiation zone of the antenna (far field).

That way the electric field vector was oriented perpendicularly to the sprouts and its magnitude at the place 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). In the context of smart-sensor, base-station or satellite radiation 370 V/m is a very strong electric field but it was chosen for the study particularly as a conservative safety value since it is 4-times higher than the occupational exposure reference level (90 V/m at 900 MHz for 30 min) [11]. The second pot was transported to the place of irradiation but not exposed – Control variant. Both control and treated plants were returned to the growth chamber right after the irradiation end.

Analyses. Long-term effects of the EMF on the crops were evaluated by monitoring their growth for 10 days after the exposure and determining the content of physiologically important substances in the plants on the last, 10th day of that period. Growth lines were constructed by measuring the height of each individual plant from the soil to the tip of its longest leaf every other day. The growth rate ($\text{mm}\cdot\text{day}^{-1}$) was calculated from the slope of the linear regression of a growth line. On the 10th day the above-ground parts of the plants from each experimental variant were collected by cutting, measured as total fresh weight (FW, g) and put between filter paper sheets to dry at room conditions ($31\text{--}32^\circ\text{C}$, $32\text{--}38\%$ relative humidity). After 4 days the total dry weight (DW, g) was determined and the material was ground in A 11 basic analytical mill (IKA, Germany). The whole above-ground biomass was used for the biochemical analysis of wheat while for the maize the material from the second and from the third leaf (counting from the plant base) were selected and analysed separately.

The photosynthetic pigments – chlorophyll *a* and *b*, and carotenoids ($\text{mg}\cdot\text{g}^{-1}$ DW), and reducing sugars (Glucose Equivalent $\text{mg}\cdot\text{g}^{-1}$ DW) were measured as major components of the crops primary metabolism. Anthocyanins ($A_{530}\cdot\text{g}^{-1}$ DW, A_{530} – absorbance at 530 nm) and malondialdehyde (MDA, $\mu\text{mol}\cdot\text{g}^{-1}$ DW) were used as stress markers. Reducing sugars were assessed by the method of PLUMMER [12]. The other substances were determined following the Rainbow protocol [8]. Three series of samples were prepared: 1) for sugars, 2) for pigments and MDA, and 3) for anthocyanins assays, respectively. Each sample contained about 20 mg dry plant material and the exact DW measured was used for the calculation of the content values.

Statistics. The presented data are average $\pm$ SEM (standard error of mean) values calculated from 3 separate experimental repetitions. Each experiment contained 11–17 replicates (individual plants) per variant for the growth monitoring, 3 – for anthocyanins measurement, and 2 for the other biochemical assays (3 and 2 samples per variant prepared from the dry plant material, respectively). Two-way ANOVA, with factors: treatment (Control and EMF) and plant material (*T. aestivum*, *Z. mays* – Leaf 2 and *Z. mays* – Leaf 3), followed by Duncan's new multiple range test (MRT) were applied to determine statistically significant differences between all the experimental variants ($\alpha = 0.05$). All statistical analyses, as well as the presented graphs, were done in a custom R script.

Results. The growth rates of the *T. aestivum* and *Z. mays* plants during the 10-day period after irradiation are presented in Fig. 1A. The EMF treatment did not affect the growth significantly but interesting tendencies were observed: on average, exposed wheat grew slightly faster than controls, and for maize the trend was reversed – slightly lower rate in EMF-treated plants. Maize grew faster than wheat. The biomass measurements revealed a pattern similar to the growth rate: neither the FW nor the DW of the aerial plant parts differed substantially among treatment variants during the examined period but the trend for higher

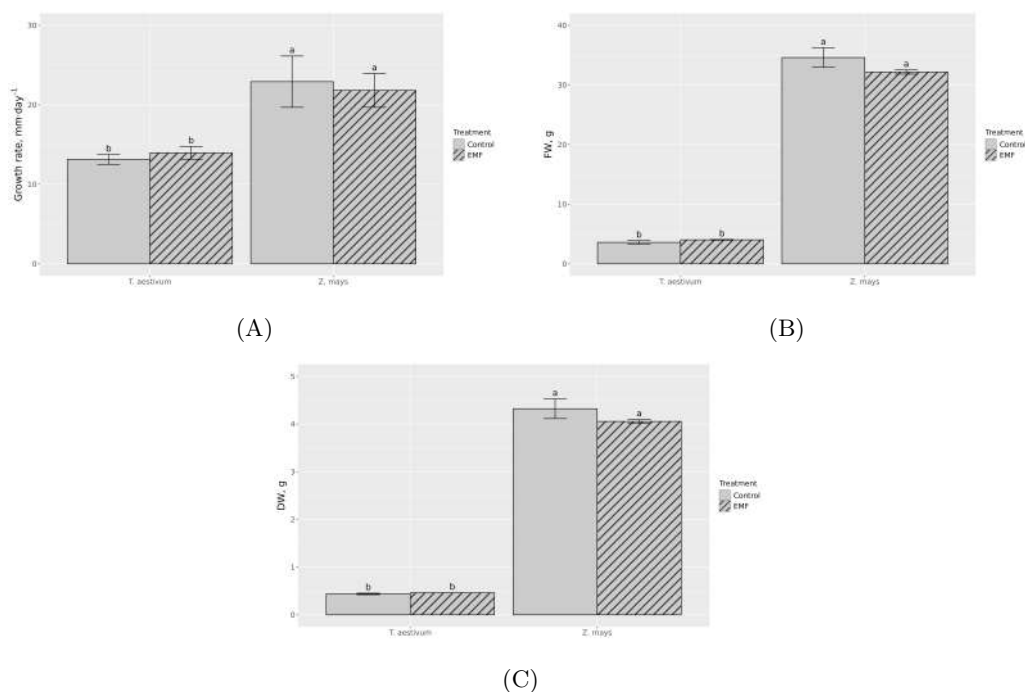


Fig. 1. Growth rate (A), total above-ground fresh weight (FW) (B), and dry weight (DW) (C) of *T. aestivum* and *Z. mays* control plants and plants exposed to 900 MHz 370 V/m continuous electromagnetic wave for 2 hours (EMF). Average $\pm$ SEM values from 3 independent experiments are displayed ($n = 3$). Distinct letter labels denote significantly different experimental variants according to Duncan's MRT ($\alpha = 0.05$)

than control EMF values for wheat and lower – for maize was even more expressed (Fig. 1B, C). Expectedly, maize accumulated more biomass than wheat.

The primary photosynthetic pigments content did not change for 10 days after the EMF exposure in both crops – chlorophyll *a* and *b* concentrations were basically the same among all experimental variants (Fig. 2).

The only difference in carotenoids was that in maize leaves their concentration was twice as much as in wheat seedlings (Fig. 3A). Tendency for lower reducing sugars content in exposed than in control plants was registered but the alterations were not statistically significant (Fig. 3B). Values were close for the investigated species and leaves.

Anthocyanins concentration was significantly different between the two species and among *Z. mays* leaves (Fig. 4A). Its level in wheat was 3–4 times lower than in maize. The older, 2nd maize leaves had more anthocyanins than the younger, 3rd leaves. However, the EMF treatment did not induce alterations in the anthocyanins content. There was a tendency for lower than controls MDA concentration in treated wheat plants and in the 2nd leaf of exposed maize but again the differences were not significant (Fig. 4B).

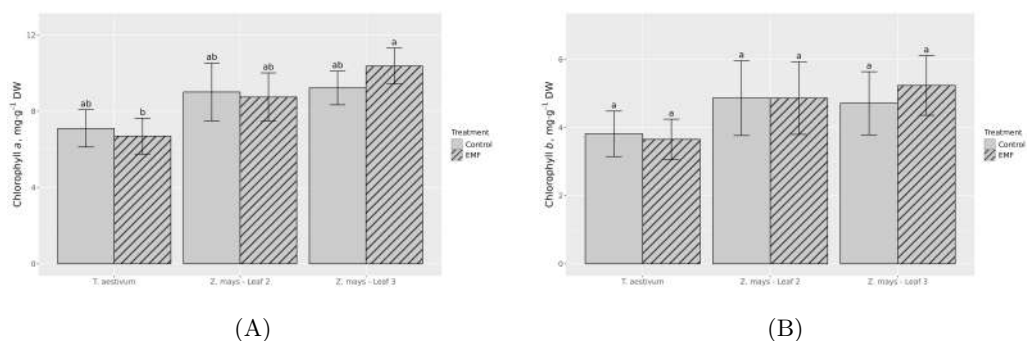


Fig. 2. Chlorophyll *a* (A) and *b* (B) content in *T. aestivum* seedlings and *Z. mays* 2nd and 3rd leaf of control plants and plants exposed to 900 MHz 370 V/m continuous electromagnetic wave for 2 hours (EMF). Average $\pm$ SEM values from 3 independent experiments, 2 replicates per each, are displayed ($n = 6$). Distinct letter labels denote significantly different experimental variants according to Duncan's MRT ($\alpha = 0.05$)

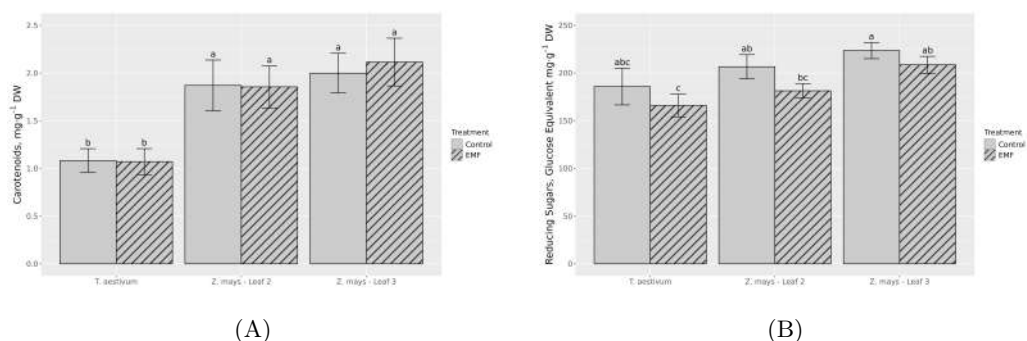


Fig. 3. Carotenoids (A) and reducing sugars (B) content in *T. aestivum* seedlings and *Z. mays* 2nd and 3rd leaf of control plants and plants exposed to 900 MHz 370 V/m continuous electromagnetic wave for 2 hours (EMF). Average $\pm$ SEM values from 3 independent experiments, 2 replicates per each, are displayed ($n = 6$). Distinct letter labels denote significantly different experimental variants according to Duncan's MRT ($\alpha = 0.05$)

Even though morphological characteristics were stable after the applied 900 MHz EMF treatment it is interesting that growth rate and biomass showed similar pattern among experimental groups. For maize the average EMF values were lower than the controls, and for wheat they were higher. That result might be caused by random sampling variability or it could be a clue for a very weak EMF effect, opposite in both species, which cannot express itself clearly because of the particular irradiation conditions, especially the perpendicular to the plants polarization of the electric field, and the relatively short 2-hour exposition – both lowering the absorbed EMF doses. The possibility for an EMF effect should be carefully investigated in future experiments with parallel polarization and longer exposure times. High variability among variants was observed in physiological

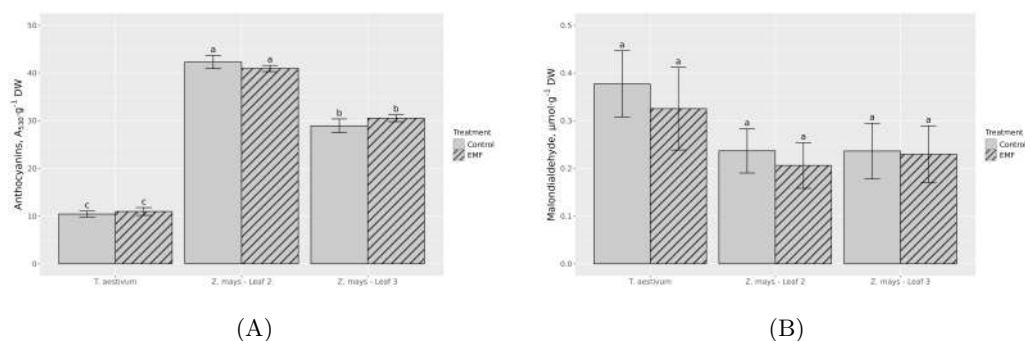


Fig. 4. Anthocyanins (A) and MDA (B) content in *T. aestivum* seedlings and *Z. mays* 2nd and 3rd leaf of control plants and plants exposed to 900 MHz 370 V/m continuous electromagnetic wave for 2 hours (EMF). Average $\pm$ SEM values from 3 independent experiments, 3/2 replicates each, are displayed ($n = 9/6$). Distinct letter labels denote significantly different experimental variants according to Duncan's MRT ($\alpha = 0.05$)

parameters. On the one hand chlorophyll *b*, an accessory photosynthetic pigment, and MDA, a direct measure for lipid peroxidation, were the most conservative variables – their values were the same for all the six experimental variants. A clear lack of oxidative stress due to EMF was registered. On the other hand, the most distinct-among-groups functional parameter was anthocyanin content whose values were determined both by species and leaf age factors, followed by carotenoid content dependent on species only. The other parameters were in-between according to their variability among the groups. Nevertheless, none of the examined characteristics displayed statistically significant difference between control and exposed plants.

Discussion. Contradictory results concerning RF and microwave radiation effects on biological systems, including plants, have been reported in the scientific literature. Some investigations described inhibition of biological processes [6,7,13] while others reported activation [14,15] and a third group failed to find any effects [5,16]. In the context of our experiments few reports are of particular interest. PAL et al. [17] found a suppressive effect of long-term exposure (30 and 60 days) to 2850 MHz EMF of *T. aestivum* on the growth, photosynthetic pigments, and carbohydrate metabolism. Significant decrease in root and shoot length, plant biomass, content of photosynthetic pigments, carbohydrate-metabolizing enzyme activity, water-soluble carbohydrates, and reducing sugars content was observed in the EMF exposed plants. KUMAR et al. [18] presented similar effects in *Z. mays* seedlings 7 days after 4-hour exposure to 1800 MHz EMF – significant reduction in the root and coleoptile length, in contents of photosynthetic pigments and total carbohydrates, but increase in α - and β -amylases activity (starch-hydrolysing enzymes). Shorter exposure times did not induce significant changes in the plants growth. The results of our study did not show significant long-term EMF effects on

the investigated morphological and physiological parameters of wheat and maize under the applied experimental conditions. What is the reason for that discrepancy between our results and the reported ones? The relatively shorter exposure time (2 hours) could be not enough to provoke permanent impairments, and even if there were any, the longer post-irradiation period in our experiments might allow plants to recover. Frequency significance: 900 MHz EMF have lower quantum energy compared to 1800 MHz and 2850 MHz but all these frequencies are in the radiofrequency range of the electromagnetic spectrum. They do not have enough energy to excite electron transition in a molecule but they could induce alterations in the hydrogen bond network of liquid water [19], and in hydration shells of important biomolecules as proteins, and thus to affect enzyme activity and metabolic processes. It is also possible specific organic molecules (enzymes) or their side groups to have vibrations in microwave frequencies and to interact with the 1800 and 2850 MHz radiation, resulting in conformational changes. Water molecules polarizability and heating due to external EMF have a maximum at 1000 MHz – close to 900 MHz, according to dielectric constant models (real and imaginary part) for salty water – environment similar to plant tissues [20]. We did not register temperature increase in plants probably due to lower than expected heating (real dielectric properties of plant tissues may be different from saline solutions), and because of effective thermal dissipation in plants.

Many reports described different observations, depending on exposure conditions (frequency, intensity, duration), as well as the investigated plant species and plant characteristics [4, 5]. Seeking clear biological results, we opted for a treatment with strong electric field (370 V/m), meaning higher power flux density of the EMF, hence more energy interacting with and being absorbed by the plants. Further, in that way we tested for negative impact on crops in the case of a short-lived malfunction of the wireless sensors or the supporting communication network resulting in high levels of electromagnetic pollution with the 900 MHz carrier wave for a limited period of time. We demonstrated that such conditions would not be hazardous for the long-term development of wheat and maize plants targeted by precision agriculture.

Conclusion. Nine hundred MHz EMF, used in telecommunications, even at strong electric field intensity (370 V/m) is safe for crops subjected to precision agriculture technologies given that irradiation lasts up to two hours. More investigations on the effects of different frequencies and signal modulation patterns at longer exposure periods and in situ growing conditions are needed to unravel the full extent of EMF impact on crops.

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