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RESEARCH ARTICLE

The Impact of Low and Medium Intensity Electromagnetic Field Exposure on Pepper Seed Germination

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ABSTRACT The interaction processes of electromagnetic fields with biological tissues have been extensively studied over the last decades. However, while the exposure of humans and animals to electromagnetic radiation has been the subject of numerous research papers, it is not so common to find studies in the scientific literature that address the effects of plant exposure to radioelectric emissions. In this paper, 24 different seed sets were exposed to an electromagnetic field continuous wave at 2.45 GHz of low and medium power levels in a controlled environment. Radiated power densities at seed location for low levels range from 3.5 to 3820 μW/cm^2 in free space inside an anechoic chamber. For medium power levels a waveguide was used, which produced power density levels at seed location from 502.7 to 2097.9 mW/cm^2. Exposition time was also controlled from periods of just 4.7 seconds to 16 full days. Subsequently, the samples were transferred to a culture chamber for germination keeping growth conditions constant for all samples. Great care was taken in the strict protocols of sample preparation, radiation, and growth tests. Finally, different growth parameters were analyzed. The results show that, under certain exposure conditions, the germination process is affected. Generally, most of the seeds exposed to medium power, completed their germination earlier than the untreated control. In the other hand, seeds treated at low power and high exposure times generally slowed down their germination.

INDEX TERMS Radioelectric emissions, electromagnetic field exposure, biomass accumulation, Capsicum annuum L., germination.

I. INTRODUCTION

The incessant demand for mobile communications services, that is spreading at dizzying speed the different standards of wireless systems (4G/5G, Wi-Fi, Bluetooth and others), means a progressive increase in the presence of electromagnetic fields (EMF) in our environment. For decades, this has led to a continuous interest in the scientific community to understand the effects that living organisms can manifest when exposed to EMF. In this sense, while there are numerous studies in relation to exposure to animals or people, studies on plants exposure and its effects are relatively scarce. Considering the way in which radiation is received by plants, in their natural environment, the interaction presents clear differences with respect to animals or human beings: they are static, they maintain the same orientation with respect to the origin of the radiation and they present a specific topology given that the exposed surface in relation to its volume is larger [1].

A recent study [2] presents the results of a survey that examined the policy option preferences of key stakeholders in Europe, drawing from the European Parliament's report. The findings show a strong agreement on the importance of increasing funding for research into the impact of EMF exposure on plants, animals, and other living organisms.

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Additionally, it underscores the necessity for further discussion about alternative policy choices that could offer solutions for regulatory bodies, government agencies, the private sector, Non-Governmental Organizations, and academic professionals.

The agricultural sector is a strategic sector in many countries, with great economic, social, territorial, and environmental importance. Currently, there are endless products, mostly of chemical origin, that act as stimulators for crops, helping to improve their correct development, to increase the vigor, or the caliber of the fruits, to facilitate flowering, or to overcome adversities that cause stress in the plants. The success of the agricultural industry is linked to the capacity to guarantee the economic viability of their holdings and its competitiveness in the international markets. For this, it will be needed to increase productivity and the efficiency of production but stimulating the practice of sustainable agricultural techniques, both economically and environmentally.

The biological effects of electromagnetic (EM) radiation constitute a fundamental scientific problem, clearly oriented towards practical applications [3]. The seed is an extremely complex system, and its state cannot always be controlled. Biological processes unfold concurrently and may react in different directions to a stimulus from an EMF. Knowledge of seed parameters (moisture content, growth rate, storage period) and EMF parameters (field intensity, frequency, exposure time, polarization), as well as environmental parameters (temperature, light, soil moisture content), is necessary to understand the correlations between the physiology and cytology of growing seedlings and the electromagnetic stimulus [4]. Probably, there is no external factor that has as powerful an effect on living organisms as EM radiation [3].

The study of EMF as a stimulating agent in the treatment of agricultural crops is an emerging issue that has received some attention in the scientific community in recent years. Thus, for example, in [5] an exhaustive review of the scientific literature is carried out regarding the response of plants to exposure to high-frequency EMF at the cellular and molecular levels. The studies we can find in the scientific literature point different approaches, in aspects such as the way in which the exposure takes place (use of reverberation chambers [6], [7], [8], coil cylinders [9], or modified microwave ovens [10]), emission frequencies (5.28 MHz [11], 900 MHz [7], [8], 2100 MHz [6], [12], 2.45 GHz [10], [13]), power levels (low power [6], [7], [8], [9], [11], [12], [13] or high power [10]), and the duration of radiation exposure (<60 minutes [7], [8], [10], [17], 1 to 4 hours [6], [12]).

Likewise, the object of study in literature is also very diverse. In tomato plants (Lycopersicon esculentum Mill) the abundance of three stress-related mRNA changes soon after EMF exposure [8]. In [7] it is shown that EMF exposure causes delay and reduces growth in rose (Rosa hybrida L.). Other authors have focused on the oxidative damage in onion (Allium cepa L.) roots [6], the dielectric effect enforced on charged ions and dipolar molecules by the oscillating electric field of microwaves [13], the potential of radiofrequency (RF) radiations to act as cytotoxic and genotoxic agent [11], the previous treatment of common sunflower (Helianthus annus L.) seeds with RF fields and cold plasma [11], the assay of biochemical components, enzymatic and non-enzymatic antioxidant systems of the EMF treated samples of tea [10], the effect of RF treatment of water on the growth of pepper (Capsicum annuum L.) plants [15], or the effects of distance to base station on flower and cone yield and germination percentage in Pinus individuals [16]. In [17] a review is presented where it is summarized the potential applications of magnetic fields and the key processes involved in agronomic applications. Other applications for microwave and plants have also been investigated like changes in standing wave ratio (SWR) caused by the presence of stem water and magnetic particles in the stem water flow as the basis of plant monitoring systems [18].

Pretreatment with EM radiation is a non-invasive stimulation technique that has also been studied to improve or accelerate germination processes in seeds as well as improve the performance of certain biological components. These treatments cover EMF of microwave frequency band, optical spectrum, or even ultraviolet radiation. Thus, for example, some authors have verified the effect that pretreatments to EM radiation have on the regulation of the germination process [19], [20], [21], [22]. EM radiation, at a certain power, can activate the action of some enzymes involved in the seed germination process [23], [24] or increase the synthesis of biological components [25]. These studies infer that subjecting the seeds to a treatment prior to germination can have a direct effect in accelerating the process.

However, no clear correlation has been established regarding the exposure time, the electric field level, the radiated power, the energy or even the specific absorption rate to which the sample is exposed before proceeding to its culture. In this work, a total of 240 sweet pepper seeds (Capsicum annuum L. cv. Celta F1) grouped in sets of 10 seeds were exposed to treatments with different levels of radiation and exposure times, to be able to draw conclusions about the specific energy values that can be a real stimulus in the plant germination process.

II. METHODS AND PROCEDURES

A. LOW POWER EXPOSITION

For low-power treatments, the seeds were exposed to a radiation process inside a high-frequency anechoic chamber, with dimensions 3.36 m × 2.56 m × 2.64 m. The chamber is made of metal walls covered in its inner side with absorbent cones. This provides a reflectivity less than −32 dB for frequencies above 1 GHz and less than −50 dB above 4 GHz, so that echoes are not generated inside, and EM radiation resembles that which would occur in a free space situation.

A Rohde & Schwarz SMATE200A vector RF signal generator was used at a frequency of 2.45 GHz and with output power levels of up to 0.1 W. Moreover, to increase the level of radiated power, a WSPS-2450-100M 2.45 GHz solid state generator with output power levels from 1 to 100 W was used. These signal generators were connected to a NARDA horn antenna via a four-meter-long low-loss RF cable. Cable losses were measured offering a value of 4.7 dB at 2.45 GHz. The antenna was supported by a wooden positioning mast that minimized interaction with electromagnetic fields inside the chamber. The horn antenna factor at 2.45 GHz was 37 dB/m. Figure 1 shows the assembly diagram of these devices.

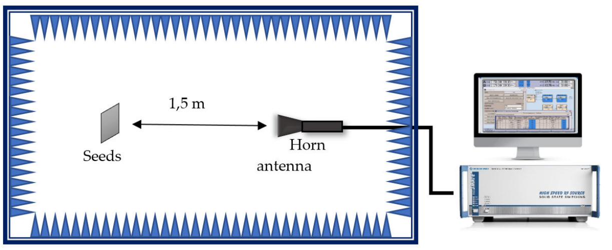


FIGURE 1. Block diagram for seeds irradiation inside the anechoic chamber for low power exposition.

The distance between the antenna and the seeds was 1.5 meters (12.25 λ for 2.45 GHz) which guaranteed exposure in the far field region (> 3λ). However, measurements were made to check the uniformity of the field in the region of the anechoic chamber in which the samples had been placed for their radiation. Specifically, a 3 × 3 matrix was established in a plane perpendicular to the direction of incidence of the electromagnetic wave of 40 × 40 cm and centered on the direction of maximum radiation. The electric field level was measured for those 9 points at 1.5 meters from the transmitting antenna with a level of radiated power of 100 mW (20 dBm). For these measurements, two vertically polarized omnidirectional antennas (QMS-00017 from Steatite) were used as transmitting/receiving antenna. Receiving antenna was connected to a Rohde & Schwarz FSH4 spectrum analyzer to measure the electric field. Resolution bandwidth was set to 100 kHz and sweep time to 200 μs. A correction factor of 0.2 dB was applied according with [26]. Figure 2 shows the results of the field uniformity measurements. The electric field radiated, in the region where the samples were placed, had a variation of less than 0.5 dB.

The samples were located on a dielectric grid using only the central cells. Each set was made up of 10 seeds. For each set, 2 seeds were placed in each of the five central horizontal cells of the grid. Figure 3 shows the uniformity measurements and radiation set-ups, and detail of the grid box for seed samples. In the anechoic chamber, 17 different sets were irradiated according to the time and power distribution shown in Table 1.

An additional control set of 10 seeds was also prepared, which was placed inside the anechoic chamber in a null electric field area in which it was not subjected to radiation but maintained the same conditions of temperature (26.4 °C ± 0.5 °C), relative humidity (45% ± 2%) and artificial light exposure (full darkness). In this way, comparisons could be made between the seeds exposed to the different radiation conditions and those of the control set.

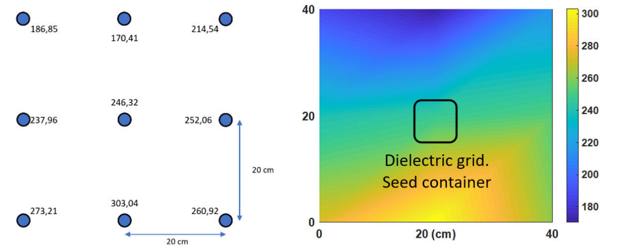


FIGURE 2. Electric field (mV/m) in the plane where seeds were radiated.

TABLE 1. Low power exposures.

Set	Power (W)	P_{dens} (μW/cm^2)	Time (min)	W_{dens} (mJ/cm^2)
1.2	0.1	3.5	1440	302.4
1.3	0.1	3.5	2880	604.8
1.4	0.1	3.5	5760	1209.6
1.5	0.1	3.5	11520	2419.2
1.6	0.1	3.5	23040	4838.4
2.1	0.9	97.78	26	152.54
2.2	0.9	97.78	52	305.09
2.3	0.9	97.78	104	610.18
2.4	0.9	97.78	208	1220.35
3.1	8.8	980.56	2.6	152.97
3.2	8.8	980.56	5.2	305.94
3.3	8.8	980.56	10.4	611.87
3.4	8.8	980.56	20.8	1223.74
4.1	34.1	3819.72	0.66625	152.69
4.2	34.1	3819.72	1.3325	305.39
4.3	34.1	3819.72	2.665	610.77
4.4	34.1	3819.72	5.33	1221.55

Transmitted power, radiated power density (P_{dens}), time, and energy density (W_{dens}) for radiation to different seed sets.

B. MEDIUM POWER EXPOSITION

Although the maximum power delivered by the Wattsine WSPS-2450-100M 2.45 GHz solid state generator is 100 Watts, it was limited to around 41 Watts due to a limited current in the power supply. This power produced a low level on power density in the anechoic chamber set-up. In order to test higher power densities, the radiation on the samples was confined by means of a waveguide set-up, which is shown in Figure 4 (scheme and picture).

The solid-state microwave generator was connected to a coaxial-waveguide adapter followed by a circulator-based isolator (Muegge MW1003A-210EC), which protected the generator from possible reflections. The water load of the isolator was connected to a pump to circulate the water. After the isolator, a directional coupler (Muegge MM1002C) was used for sampling the direct wave with an attenuation of 60.2 dB. These samples were measured by a power detector (Muegge MM1001B-110AB), whose DC output voltage was measured by a voltmeter (Vitecom 75-MY64). The sample holder (a 5 cm long WR340 waveguide section) was connected to

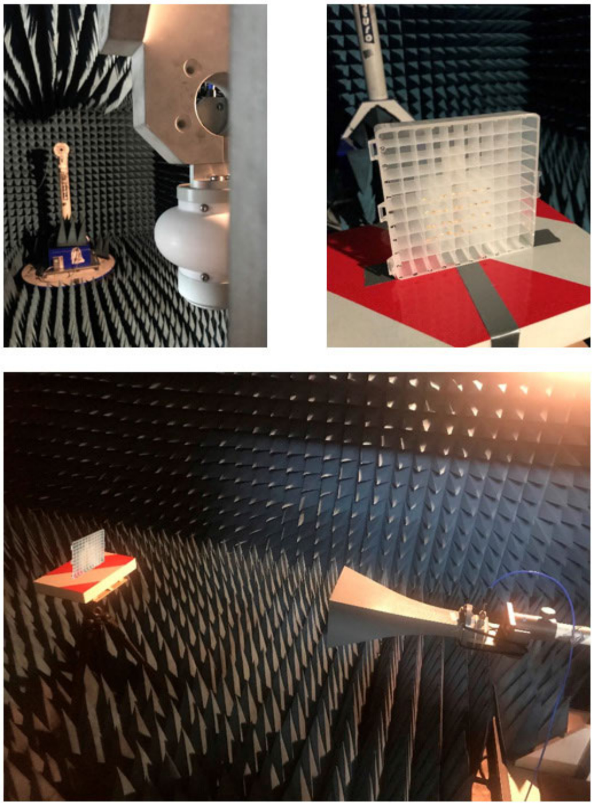
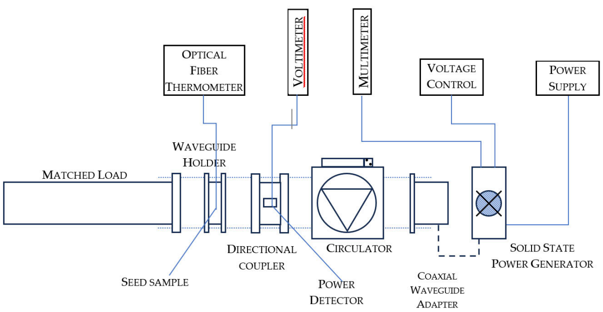
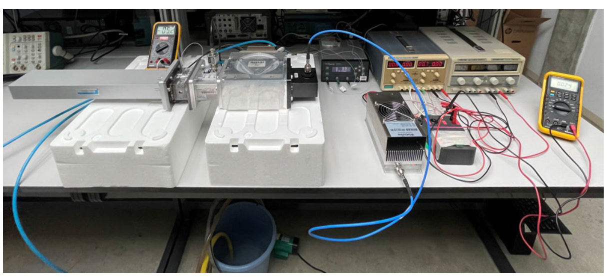


FIGURE 3. Up left: Set-up for uniformity measurements. Up right: Grid box with seeds samples. Down: set up for EMF radiation.



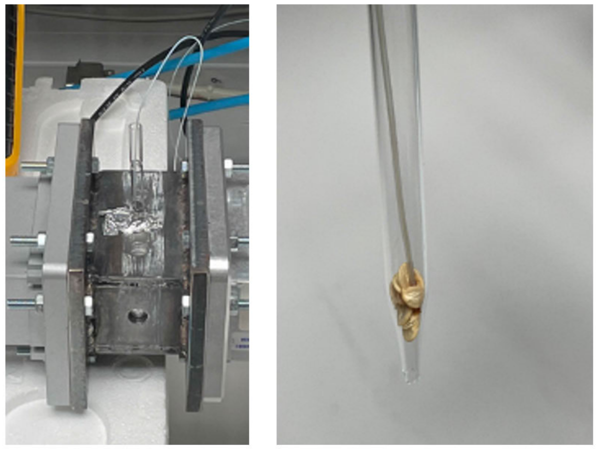
(a)



(b)

FIGURE 4. Waveguide setup for high power expositions: a) scheme, b) actual set-up.

the coupler, and it held a soda lime glass pipette with the corresponding batch of 10 seeds (see Figure 5). To avoid reflections, this waveguide was connected to a matched load (Continental PLPT340).



a) b)

FIGURE 5. Waveguide holder: a) waveguide with the inserted pipette, b) detail of the pipette with 10 seeds and an optical fiber sensor for temperature measurement.

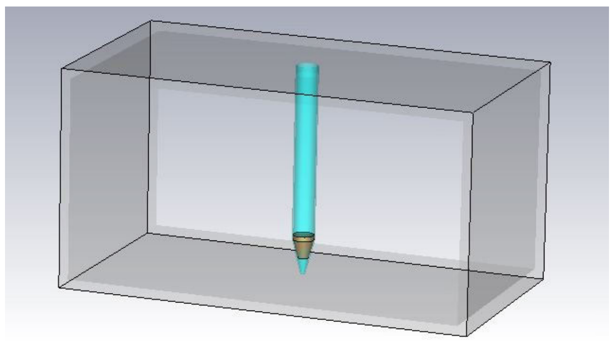


FIGURE 6. Modelling of the seeds and air assembly inside the pipette.

The temperature of the seeds was monitored by an optical fiber sensor, as Figure 5.b shows, by inserting the fiber optic inside the pipette between the seeds and connecting this fiber to an OPsens TMS-6B-10-100-ST-L thermometer.

The power of the microwave generator was controlled by the voltage provided by a Protek 3033B power supply and powered by a Freak M10-380T-303C power supply. This power supply delivers a maximum of 11 Amps, and this limited the maximum power of the source to 40.9 Watts. Compared to magnetrons, the spectrum of solid-state generators around the operating frequency is very narrow and stable.

Moreover, in order to check that the electric field uniformity on the seeds set could be considered acceptable and, so, the power density could be considered constant, EM simulations of the experiment were performed with the commercial electromagnetic simulator CST Studio Suite [27]. Figure 6 depicts the simulated experiment, where the waveguide walls are considered perfect electric conductor. The properties used to characterize the lime glass soda pipette were density of 2.44 g/cm^3, dielectric constant of 7.75 and specific resistivity of 7.9·10^{17}-7.9·10^{18} $\mu\Omega$cm taken from [28]. For the dielectric characterization of the mix of pepper seeds + air, 3 measurements of the effective permittivity of the mixture were realized by means of the ITACA vial dielectometer, based on [29], obtaining the results shown in Table 2.

TABLE 2. Dielectric constant, resonance frequency and quality factor of the seed + air mixture.

Mass (gr)	Volume (cm^3)	Dielectric Constant	Loss factor
2.3	4.3	1.86	0.1052
2.3	4.3	1.86	0.1054
2.3	4.3	1.86	0.1053

The electric field distribution on the seeds sample is shown in Figure 7, and the power density in Figure 8.

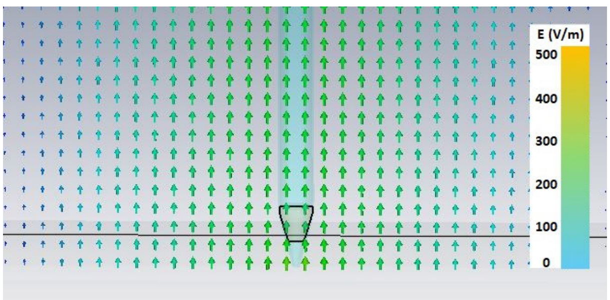


FIGURE 7. Electric field distribution (V/m).

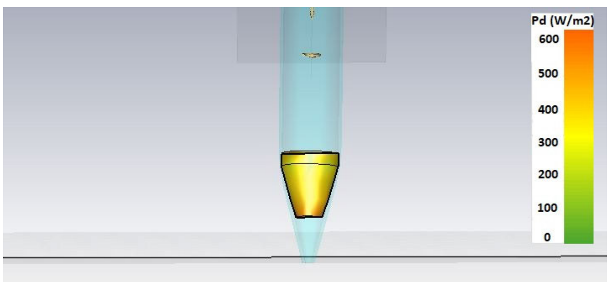


FIGURE 8. Power density distribution (W/m2).

After the simulation in CST, for an input power of 1 Watt, the maximum and minimum values of the electric field in the volume of the mix seeds + air were 333.7 and 306.1 V/m, respectively. The mean was 319.9 V/m having a variation of ±13.8 V/m, that is ±4.3%. In terms of delivered power, for 1 Watt the values obtained were a maximum of 539.1 W/m^2 and a minimum of 471.9 W/m^2, giving an average of 505.5 W/m^2 with a variation of ±33.6 W/m^2, ±6.64%.

Since the obtained variation was small, it was assumed that the electric field and the delivered power were constant for the whole mix of seeds + air. Therefore, the averages of both parameters were used to calculate the power density.

After these previous measurements and simulations, the radiation exposures were performed for two different levels of power density and four exposure times each, for a total of eight sets of ten seeds. Table 3 summarizes the main parameters for each exposition.

TABLE 3. Medium power exposures.

Set	Power (W)	P_{dens} (mW/cm^2)	Time (s)	W_{dens} (J/cm^2)	Δt (ºC)
5.1	9.8	502.7	21.0	10.6	0.2
5.2	9.8	502.7	42.0	21.1	0.2
5.3	9.8	502.7	84.0	42.2	1.7
6.1	40.9	2097.9	4.7	10.6	0.5
6.2	40.9	2097.9	9.5	21.1	0.5
6.3	40.9	2097.9	19.0	39.9	1.8

Transmitted power, radiated power density (P_{dens}), time, energy density (W_{dens}), and temperature variation in the sample for radiation to different seeds sets.

C. GROWING CONDITIONS AND MEASUREMENTS

After all the exposition times were completed, for each set, 10 seeds of sweet pepper (Capsicum annuum L.) cv. Celta F1 [30] were sown individually in 70 cm^3 seedling pots, containing coconut fiber wetted with tap water. Seedling pots were randomly placed inside the growth chamber and relocated every 10 days.

In the first stage, to analyze germination, the growth conditions established were a 12/12h day/night photoperiod, at 23/18 °C and 60% relative humidity.

The sets were analyzed for germination evolution every 10 days, classifying every seed into: "non-germinated", "emerged" and "fully expanded cotyledons" (FEC) following the international rules for seed testing classification [31]. Data were expressed for each treatment as a percentage of FEC according to the Eq. (1):

$$n_{FEC}\,(\%) = \frac{n_{FEC}}{n_{sowed}}100 \tag{1}$$

where n_{FEC} is the number of seeds in the category of fully expanded cotyledons, and n_{sowed} the total number of seeds for each set.

Relative germination rate coefficient (W_c) with respect to the control treatment was calculated for each day as the ratio between the fully number of expanded seeds of each treatment ($n_{gtreatment}$) and the germinated control seeds ($n_{gcontrol}$) of the non-radiated treatment at the same day (Eq. 2) [32]:

$$W_c = \frac{ng_{treatment}}{ng_{control}} \tag{2}$$

Germination rate index (GRI) [33] was calculated as the summatory of daily percentage of germinated seeds (n_{FEC}) divided into its corresponding days after sowing (t):

$$GRI\left(\%day^{-1}\right) = \frac{n_{FEC_i}}{t_i} + \frac{n_{FEC_j}}{t_j} + \ldots + \frac{n_{FEC_x}}{t_x} \tag{3}$$

For the second stage of the trial, to analyze vegetative growth, the growth conditions were changed to 12/12 h day/night photoperiod, 25/21 °C. The substrate was watered to field capacity and plants were fertilized monthly with 0.84 mg N per plant. When plants reached a suitable size for transplanting, 7–8 leaves, vegetative growth was analyzed. The aerial part was cut off and the roots were carefully

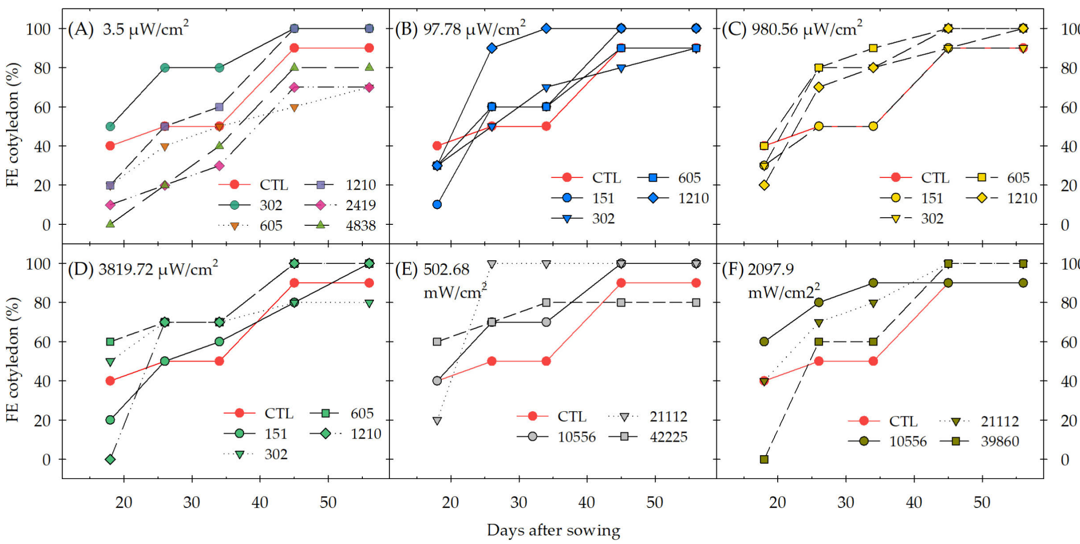


FIGURE 9. **Evolution of the germination percentage of seeds as fully expanded cotyledons (FE cotyledon). Each plot shows the null radiation exposure evolution (CTL) and the seed sets corresponding to different incubation times within the same power exposure: (A) 0.1 W, 0.9 W (B), 8.8 W (C) 34.1 W/ (D), 9.8 W (E) and 40.9 W (F). Each set is label with the power density (P_{dens}) received in μJ/cm^2 or mJ/cm^2.**

cleaned. For dry biomass measurements, tissue was oven dried at 65 °C until a constant weight was reached.

D. STATISTICAL ANALYSIS

First, descriptive procedures were performed on the germination indexes for the raw data: n_{FEC}, W_c and GRI of the seeds. Subsequently, data was grouped based on the energy density (W_{dens}; mJ/cm^2) as treatments: control set (null electric field); [0-500]; [500-1000]; [1000-2000]; [2000-10,000]; [10,000-30,000] and [30,000-100,000]. When statistical analysis of variables obtained from counts, proportions or binary data is performed, one or more assumptions made prior to the analysis of variance are usually not satisfied, which affects the results [34]. In this sense, the class of Generalized Linear Models (GLMs) is a general frame for incorporating statistical models for normal and non-normal data [35], for instance, the proportion of germinated seeds. The individual variability effect of the experimental units, or any possible correlation between them, results in overdispersion of the germination data [36], and not considering this effect in the models will underestimate the standard errors and therefore lead to a wrong data interpretation and conclusions [37]. An extension of GLMs is the Generalized Linear Mixed Models (GLMMs), which include random effects in the linear predictor and can be used to properly adjust for additional variability [37]. In our case, the outcome of interest is the yes or no fully expanded cotyledons proportion and, assuming that each seed has the same probability of germination, we can suppose that the variable has a binomial distribution [37]. As in linear models, with the GLMMs we try to ''explain'' the expected value of an observation by using known predictors given one or more random effects. The linear component is defined by a linear predictor $\eta_i = x_i'\beta$; where x_i' represent the transposed vector of characteristics for the i^{th} observation and β is a vector of unknow parameters. Nevertheless, in GLMMs the relationship between the outcome of a variable Y and the linear predictor requires a link function g, which describes how the expected value of the mean μ_i of Y is related to the linear predictor $g(\mu_i) = x_i'\beta$. Finally, in our model the random component corresponded to a binomial distribution of aggregated data, with a systematic component $\eta_i = x_i'\beta$ and a logit-link function $g(\mu_i) = \log(\mu_i/1 - \mu_i)$. The energy density, days after sowing and their interaction were included as fixed effects, and the seed sets as random effects, to test for differences in the fully expanded cotyledons proportion.

A principal component analysis (PCA) was performed to explore the observations variability and the correlations between the vegetative growth, as aerial and root dry biomass of the seedlings, power density and energy density at 56 days after sowing. Since the variables have different units of measurement, the data were previously standardized, and the correlation matrix r-Pearson was used.

GLMM and PCA were carried out using the InfoStat software with the R-language interface [38].

III. RESULTS AND DISCUSSION

A. GERMINATION

Generally, most of the seeds exposed to low and medium power (Figure 9, B-F), completed their germination earlier than the untreated control. In the other hand, seeds treated at the lowest power (0.1 W) and high exposure times (Figure 9, A), sets 1.5 (2419 mJ/cm^2) and 1.6 (4839 mJ/cm^2) generally slowed down their germination.

The relative germination rate coefficient (W_c) allows us to compare that on the first day of sampling, only a few sets (1.2, 3.3, 4.2, 4.3, 5.1, 6.1, 6.2) have germinated as much

TABLE 4. Relative germination rate coefficient (W_C) and final gemination rate index (GRI).

Set	T exposure (s)	P_{dens} (μW/cm²)	W_{dens} (mJ/cm²)	W_c					GRI
				18	26	34	45	56	56
1.2	86400	3.5	302.4	1.25	1.60	1.60	1.11	1.11	1.22
1.3	172800	3.5	604.8	0.50	0.80	1.00	0.67	0.78	0.67
1.4	345600	3.5	1209.6	0.50	1.00	1.20	1.11	1.11	0.88
1.5	691200	3.5	2419.2	0.25	0.40	0.60	0.78	0.78	0.50
1.6	1382400	3.5	4838.4	0.00	0.40	0.80	0.89	0.89	0.52
2.1	1560	97.78	152.54	0.25	1.20	1.20	1.11	1.11	0.86
2.2	3120	97.78	305.09	0.75	1.00	1.40	0.89	1.00	0.90
2.3	6240	97.78	610.18	0.75	1.20	1.20	1.00	1.00	0.93
2.4	12480	97.78	1220.35	0.75	1.80	2.00	1.11	1.11	1.21
3.1	156	980.56	152.97	0.75	1.00	1.00	1.00	1.11	0.88
3.2	312	980.56	305.94	0.75	1.60	1.60	1.00	1.00	1.07
3.3	624	980.56	611.87	1.00	1.60	1.80	1.11	1.11	1.20
3.4	1248	980.56	1223.74	0.50	1.40	1.60	1.11	1.11	1.02
4.1	40	3819.72	152.69	0.50	1.00	1.20	0.89	1.11	0.84
4.2	80	3819.72	305.39	1.25	1.40	1.40	0.89	0.89	1.07
4.3	160	3819.72	610.77	1.50	1.40	1.40	1.11	1.11	1.21
4.4	320	3819.72	1221.55	0.00	1.40	1.40	1.11	1.11	0.88
5.1	21.00	502,680	10.56	1.00	1.40	1.40	1.11	1.11	1.10
5.2	42.00	502,680	21.11	0.50	2.00	2.00	1.11	1.11	1.19
5.3	84.00	502,680	42.23	1.50	1.40	1.60	0.89	0.89	1.16
6.1	4.73	502,680	84.45	1.50	1.60	1.80	1.00	1.00	1.27
6.2	9.45	2,097,900	10.56	1.00	1.40	1.60	1.11	1.11	1.13
6.3	19.00	2,097,900	21.11	0.00	1.20	1.20	1.11	1.11	0.81

Radiated power density (Pdens), energy density (Wdens). The two indicators, GRI and Wc, were calculated based on fully expanded cotyledons.

TABLE 5. A. Model fit summary and hypothesis testing for the fixed effects. B. Fully expanded cotyledons proportion.

N	AIC	BIC	logLik	Deviance
120	381.7	423.5	-175.8	108.4

Term	F-value	DF	*p*-value
W_{dens}	2.5	6	0.028
DAS	161.2	1	<0.0001
W_{dens} × DAS	2.6	6	0.023

W_{dens}: energy density and DAS: days after sowing. BIC: Bayesian information criterion and, logLik: log-likelihood

Energy density mJ/cm²	Days After Sowing				
	18	26	34	45	56
Null electric field	0.40 ±0.17	0.50 ±0.16 ab	0.50 ±0.16 bc	0.90 ±0.09	0.90 ±0.09
]0-500]	0.31 ±0.06	0.63 ±0.06 a	0.67 ±0.06 ab	0.89 ±0.04	0.94 ±0.03
]500-1,000]	0.37 ±0.08	0.63 ±0.06 a	0.68 ±0.07 ab	0.87 ±0.05	0.90 ±0.05
]1,000-2,000]	0.17 ±0.06	0.70 ±0.07 a	0.77 ±0.07 ab	1.00 ±0.00	1.00 ±0.00
]2,000-10,000]	0.05 ±0.05	0.20 ±0.09 b	0.35 ±0.11 c	0.75 ±0.10	0.75 ±0.10
]10,000-30,000]	0.40 ±0.08	0.80 ±0.06 a	0.85 ±0.06 a	0.98 ±0.02	0.98 ±0.02
]30,000-100,000]	0.30 ±0.11	0.65 ±0.11 a	0.70 ±0.10 ab	0.90 ±0.07	0.90 ±0.07

Values correspond to the original scale mean ± standard error according to a GLMM. Different letters for the same day indicate significant differences according to the LSD Fischer test ($p < 0.05$).

or more than the untreated control. This coefficient changes daily, as the germination stage progresses, being higher than the control for most of the sets in the latest sampling (Table 4).

By 56 DAS, the control set had a GRI value for FEC of 0.92, which indicates that the treatments with higher values of GRI (1.2, 2.4, 3.2, 3.3, 3.4, 4.2, 4.3, 5.1, 5.2, 5.3, 6.1, 6.2) germinated at a faster rate.

In the GLMM developed to determine the proportion of seeds reaching the fully expanded cotyledon (FEC) phase over time based on the energy density, each term of the model was significantly different from zero (Table 5). Likewise, the ratio between the relative deviance (108.4) and the residuals degrees of freedom (112) was 0.96, so it is possible to assume that there was no overdispersion [39] (Table V.A). To develop the most parsimonious model, hypothesis tests were performed for linear combinations of the estimated parameters and confirmed the assumption that the regression models are distinguishable ($p < 0.05$) from each other in all combinations (data not shown) [40].

The progress of the FEC proportion for each energy density in the original scale are shown in Table V.B). No differences were detected between the energy density

TABLE 6. Principal component analysis (PCA).

	PC 1	PC 2
Eigenvalue	2.33	1.11
Variance (%)	58.3	27.9
Cumulative variance (%)		86.1
Power density (μW/cm^2)	0.500	**–0.505**
Energy density (mJ/cm^2)	**0.512**	–0.460
Roots (g dry weight)	**0.535**	0.401
Aerial (g dry weight)	0.449	**0.610**

Eigenvalues, percentage of variation accounted by the first two principal components, and eigenvectors of the aerial and root biomass and main parameters of radiation exposure at 56 days after sowing. Values with higher absolute weights on the determination of the PCA axes are reported in bold. n = 84.

TABLE 7. Correlation matrix.

Variable	Power density	Energy density	Roots
Power density (μW/cm^2)	1.00		
Energy density (mJ cm^2)	0.72***	1.00	
Roots (g dry weight)	0.40***	0.39***	1.00
Aerial (g dry weight)	0.18^{ns}	0.26*	0.70***

Correlation matrix r-Pearson of the seedling's biomass and the main parameters of radiation at 56 days after sowing. *: $p < 0.05$; ***: $p < 0.001$ and ns: not significant. $n = 84$.

intervals established at 18 days after sowing (DAS). However, at 26 DAS, the seeds exposed to an energy density in the range of [2,000-10,000] mJ/cm^2 had a significantly lower proportion of seedlings with the FEC than the rest of the seeds exposed to radiation. Likewise, the seeds of the control set showed only 50% of FEC, in contrast to those exposed to [10,000-30,000] mJ/cm^2, whose seedlings already showed an 80%. This trend was maintained until 35 DAS, when the seeds exposed to [10,000-30,000] mJ/cm^2 showed the maximum proportion of FEC, specifically 85%, being significantly higher than the seeds of the control set and those exposed to [2,000-10,000] mJ/cm^2. Once 45 DAS had been reached, no differences were detected between the energy density intervals or with the control set. Similarly, at 56 DAS, all seedlings exceeded 90% FEC, except those exposed to [2000-10,000] mJ/cm^2 whose maximum value corresponded to 75% (Table V.B).

B. VEGETATIVE GROWTH AND PCA

The principal component analysis (PCA) results explained 86.1% of the total variability of the observations in its first two components (Table 6). If we consider the association coefficients between the original and transformed variables (eigenvectors), PC1 (58.3%) showed differences mainly in the seedlings root dry weight and the energy density. At the PC2 level, which accounted for 27.9% of the variability, the variables with the highest weight were the seedlings aerial dry weight and the power density (Table 6 and Figure 10).

As expected, the radiation parameters were significantly correlated with each other. In terms of vegetative growth, power density was significantly positively correlated with the seedling root dry weight biomass accumulation 56 days after sowing. Similarly, a significant and positive correlation was detected between power density with aerial and root biomass, but more pronounced for roots (Table 7). In the same sense, set 6.3, showed higher biomass, which coincides with a higher power density and energy density compared to the rest of the seeds (Figure 10 and Table 7 seed sets description).

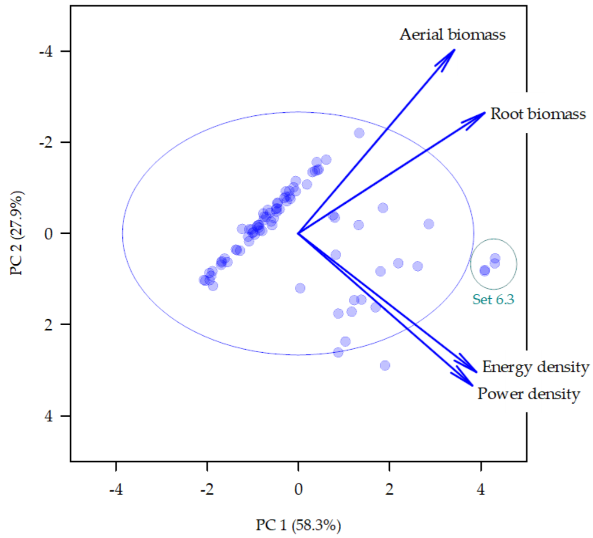


FIGURE 10. Biplot of the principal component (PC) analysis obtained with standardized data at 56 days after sowing. Blue arrows represent the variables evaluated and the blue circles each seedling. n = 84.

IV. CONCLUSION

In summary, the findings of this study underscore the significant impact of different power densities on both seed germination and vegetative growth processes. Notably, seeds subjected to medium power exposure exhibited a notable advancement in germination, outpacing the untreated control group. Conversely, seeds treated with low power and prolonged exposure durations experienced a deceleration in their germination rates, suggesting a potential inhibitory effect under these conditions. Moreover, an intriguing pattern emerged wherein seeds exposed to medium power densities demonstrated consistently higher Germination Rate Index (GRI) values across various treatment combinations, indicating a favorable response to increased energy input. This trend not only highlights the sensitivity of germination processes to variations in power intensity but also suggests the potential for optimizing germination outcomes through controlled energy exposure. Furthermore, the relationship between power density and root dry weight biomass accumulation, particularly evident in seeds from set 6.3, provides valuable insights into the influence of electromagnetic energy on subsequent vegetative growth stages. The observed positive correlation between power intensity and root biomass accumulation underscores the importance of energy dosage in promoting robust vegetative development. This opens up new possibilities for improving plant growth and accelerating germination in crops through controlled exposure to electromagnetic fields.

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