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Pre-Sowing Static Magnetic Field Treatment of Vegetable Seeds and Its Effect on Germination and Young Seedlings Development

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Abstract: The pre-sowing treatment of seeds with an α static magnetic field has been reported in the literature as a means of enhancing plant development. In this work, we have designed, characterized, and constructed a setup for exposing small vegetable seeds to a static magnetic field. In a series of experiments, we have treated the seeds of vegetables that are important for the Mediterranean diet, i.e., tomato, lettuce, and salad rocket. The results show that tomato seedlings significantly benefit from a pre-sowing treatment with a magnetic flux density of 45 mT, for both an exposure time of 60 and 90 min compared to control, while the time of treatment that leads to improved growth is 90 min. In order to improve the growth of salad rocket seedlings the magnetic field had to be 150 mT, whereas the results for lettuce seeds were a bit inconsistent, i.e., it is not clear whether a lower (45 mT) or a higher (300 mT) magnetic flux density should be applied.

Keywords: Faraday cage; early growth; *Solanum lycopersicum* L.; *Lactuca sativa* L.; *Eruca sativa* L.

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1. Introduction

It is well known that most growers focus on the parameter of yield, in order to achieve higher income, as well as to satisfy the demand for food of the continuously increasing world population. Therefore, in recent years most vegetable growers have chosen to use transplants instead of direct seeding for satisfactory plant establishment [1–3]. Other important advantages of transplant use are the uniformity of the plants, better weed control, absence of manual plant removal in high-density seeded rows, better reaction of the plant to environmental stress, and minimization of the required time until harvest [3–5]. It is clear that continuous improvement of the transplant industry is required to satisfy market demands. One of the challenges for the industry is to achieve higher germination and vigour with the application of various seed treatments [3,6]. The use of magnetic fields for seed treatment is a rather new technique in this direction.

Maffei [7] and Pietruszewski and Martinez [8] give an overview of the studies that have addressed how magnetic fields can affect plants. Several other reviews have stressed the beneficial effects on seed invigoration and seedling performance of "magneto-priming", i.e., a pre-sowing treatment of seeds with magnetic fields [9], classifying them under eustressors [10], as well as the better reaction of the plant to environmental stress [11]. This method is critical because it offers important advantages compared to chemical treatments of the seeds [12].

Among the most common vegetables that are grown with the use of transplants are tomatoes and lettuce. The tomato is known as one of the most important vegetable crops that can be cultivated either in an open field or in a greenhouse [12] where transplants

are the only choice for the grower. Additionally, for lettuce, one of the most popular leafy vegetables worldwide, poor plant establishment when directly using seeds in the field has led to the use of transplants by growers [1]. Lately, there is an increasing demand for rocket, since it is a vegetable with increasing popularity all over the world [13]. Moreover, rocket sprouts contain a higher amount of glucosinolates than mature leaves [14], while their positive effect on human health [15] is well documented.

Although the effect of magnetic field on germination parameters has been studied in some plants [16–19], information on tomatoes, rocket, and lettuce are relatively scarce with limited data [20], brief exposure time [21], or regarding the use of pulsed magnetic field [22]. Improvement of germination or growth rate is very important for the sprouts industry and is of high value for a sustainable horticultural economy, especially in the Mediterranean area. Therefore, in this current study, we investigate the effects of a static magnetic field on the seeds of three popular vegetables (tomato, rocket, and lettuce). The seed exposure to a static magnetic field was performed with the use of permanent magnets. This type of exposure setup is not used very often in the literature [16]. In most cases, the seeds are exposed to a magnetic field generated by electromagnets [19]. However, the spectral content of the electric current in the coil is rarely reported [23] in such a setup, and neither is the potential temperature rise in the coil due to ohmic losses [24].

The rest of this work is structured as follows. First we describe the exposure set-up that has been fabricated, as well as the experimental design, the parameters, and the statistical analysis that have been applied to perform the characterization of static magnetic field treatment in vegetable seeds. Next, we present the results of this work for the treated vegetable seeds of tomato, lettuce, and salad rocket, and discuss our findings compared to other published works in the literature. Finally, we conclude this work with some useful remarks.

2. Materials and Methods

2.1. Exposure Set-Up

The exposure setup was enclosed inside a Faraday cage (Figure 1) with dimensions of 910 mm × 250 mm × 240 mm, made of a metallic wireframe (grid dimensions: 12 mm × 12 mm), in order to minimize the electromagnetic radiation of external sources that reaches the seeds. The magnet boxes were mounted on a non-metallic (wooden) base inside the Faraday cage and were equally spaced from each other.

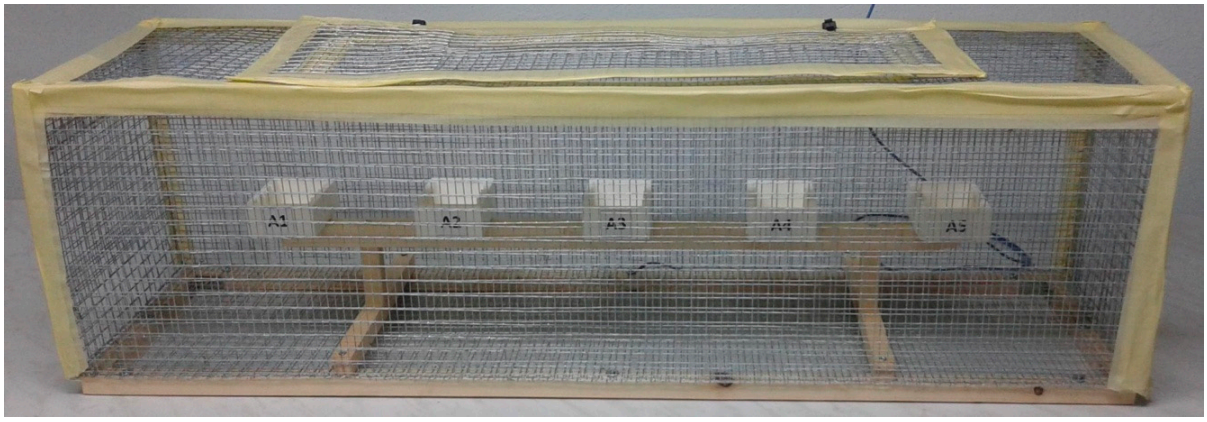


Figure 1. The Faraday cage containing the five exposure boxes in which the seeds were placed. The boxes were properly mounted on a wooden base.

The magnetic field was generated by the use of rectangular sintered neodymium ($Nd_2Fe_{14}B$) magnets with a permanent magnetic field of 1.33 Tesla (NEOTEXX, Andreas Kerskes und Jessica Albrecht GbR, 12487 Berlin, Germany). Each nickel-plated magnet was 50 mm × 30 mm × 3 mm in dimensions. The magnets were placed inside a plastic exposure box in three different configurations (Figure 2), generating the required magnetic

flux density values. The plastic boxes were fabricated using a 3D printer (ZMorph 25 v. 2.0, ZMorph sp. z o.o., 53-601 Wroclaw, Poland) and polylactic acid (PLA) material. The dimensions of the magnet box were 60.2 mm × 82 mm × 30 mm. The seeds of each treatment were placed inside the seed case (50.2 mm × 11.8 mm × 25 mm), which was also made of PLA.

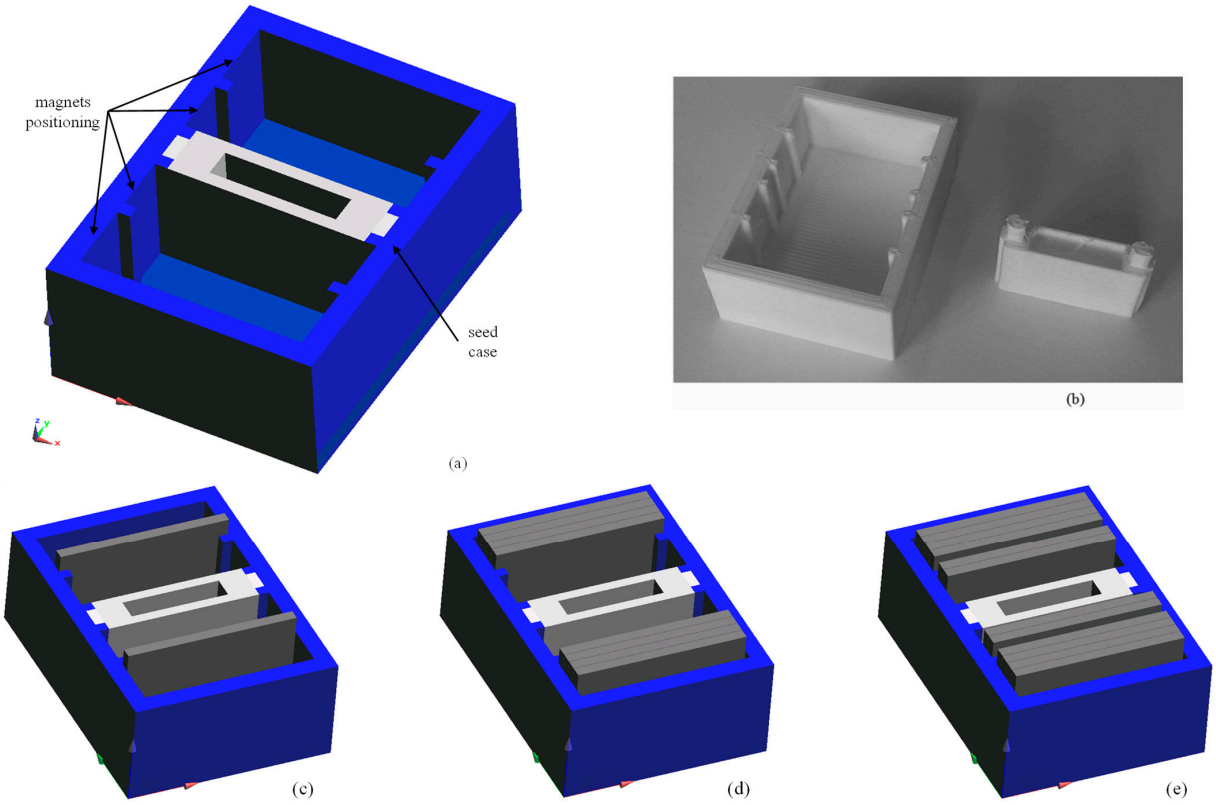


Figure 2. Positioning of permanent magnets inside the exposure box, in order to achieve the required magnetic flux density values; (**a**) exposure box, (**b**) picture of the 3D printed exposure box with the seed case, (**c**) M1 configuration (4 magnets), (**d**) M2 configuration (8 magnets), and (**e**) M3 configuration (12 magnets).

2.2. Magnetic Field Characterization

Since the static magnetic field which was produced by the rectangular magnets was non-uniform, field mapping was required to assess the exact field exposure of the seeds. The magnetic field measurements were performed on the vertical mid-plane of each exposure box (seed case), using a three-axis Hall magnetometer (THM 1176-MF, MetroLab Technology SA, CH-1228 Geneva, Switzerland). The probe was designed for measuring magnetic fields with frequencies from DC to 1 kHz, providing the total magnetic flux density irrespective of its orientation. The dimensions of the probe (including the sensor cap) were 113 mm × 16 mm × 10 mm. The probe movement was controlled by a set of two-step motors with a spatial resolution of 1.6 μm along with a FireBall V90 CNC Router (Probotix, Peoria, IL, USA). A MATLAB (The MathWorks, Natick, MA, USA)-based program was written to control the movement of the probe on the two axes in the desired vertical mid-plane. At predefined positions (measurement grid points), a magnetic field measurement was performed and recorded by the software.

Figure 3 illustrates the magnetic flux density distribution in the vertical mid-plane of the seed case, i.e., the part of the exposure box where the seeds are held, for the three different configurations used in the experiments (Figure 2). The seeds of the three vegetables were so small that they all lay on the bottom of the seed case in the outlined areas. The solid line marks the space occupied by the tomato and rocket seeds, which are much

smaller than the lettuce seeds. The latter took up the area from the bottom of the case up to the dashed line. The average magnetic flux density in these areas was (mean ± std. deviation) 44.59 ± 1.84 mT, 146.22 ± 6.53 mT, and 302.53 ± 7.56 mT for the small seeds and three cases M1, M2, and M3, respectively. The above values changed for the lettuce seeds to 45.53 ± 2.09 mT, 148.54 ± 6.90 mT, and 307.96 ± 9.31 mT. The local geomagnetic field flux density was measured at 51.70 ± 16.7 μT with the same equipment. This value is in agreement with the one estimated online by the NOAA calculator [25] for the geographical coordinates of experimentation, which are 46.76 ± 0.15 μT. Moreover, during the experiment, the maximum value of the magnetic flux density in the seed case where the control group was kept was less than 0.29, 1.18, and 1.85 mT for the treatment groups of 45, 150, and 300 mT, respectively. It is acknowledged that the exposure of the control group to a magnetic field higher than the local geomagnetic field is a limitation of this study. However, in this way it was possible to obtain a control-exposed group of seeds, which was simultaneously present in the exposure setup, an approach missing from all previous studies, where the control group was placed in the exposure setup (if placed at all) at different times than the exposed groups.

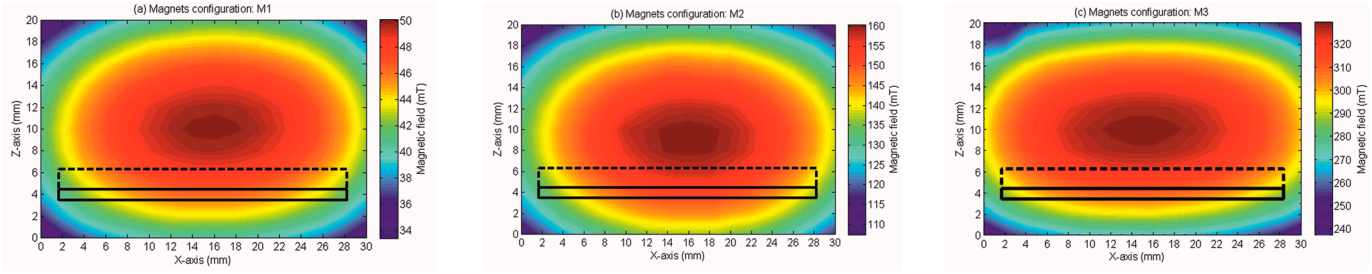


Figure 3. Magnetic flux density distribution for the three different cases of seed exposure, (**a**) M1 distribution, (**b**) M2 distribution, and (**c**) M3 distribution.

2.3. Experimental Design

Certified tomato (cv. 'Ace 55 VF'), lettuce (cv. 'Paris Island'), and rocket (*Eruca sativa* L.) seeds were used for the experiment. The experiments were carried out over the last two months of 2014. Seeds were exposed to three different magnetic field flux densities (M1, M2, M3) for four different periods (ET1: 30 min, ET2: 60 min, ET3: 90 min, ET4: 120 min). For each vegetable species, a repeated (four times) exposure of 25 seeds at each magnetic flux density and the exposure time was used, plus a control. All treatments in the experiments were performed under similar environmental conditions (temperature 22.72 ± 1.12 °C; relative humidity (RH) 48.46 ± 8.96%). Following exposure, the four replicates of 25 seeds were sown on moistened filter paper disks in Petri dishes and kept in an incubator (GRW-500 CMP, Emmanuel E. Chryssagis, 17341 Ayios Dimitrios Athens, Hellas) at 21 °C in an upright position and for seven days according to the guidelines issued by the International Seed Testing Association, with a modification of 7 days of germination instead of 14 days [24].

The experiment was performed in a blinded fashion, i.e., the Petri dishes were labeled with numbers and placed randomly in the exposure boxes with the help of a random generator in a computer. The exposure parameters for each Petri dish were noted. Subsequently, the growth parameters were evaluated for the seedlings of each Petri and only then was the exposure related to them.

2.4. Germination Parameters

The daily number of germinated seeds was recorded with the criterion that germination be a visible radicle protrusion. At the end of each experiment, germination percentage

was calculated based on normal seedlings. The speed of germination was calculated according to Maguire [25] as

$$\text{Speed of germination} = \sum\left(\frac{n}{t}\right) \quad (1)$$

where n is the number of seeds newly germinating at time t and t is days after sowing.

2.5. Growth Parameters

On the last day of the experiment, all seedlings of each replicate root length, shoot length, as well as total seedling length, were determined. Subsequently, the fresh weight of the above seedlings was measured and then the seedlings were left to dry in an oven at 72 °C for 48 h for dry weight determination. Seedling vigour was calculated according to Abdul-Baki and Anderson [26] as

$$\text{Vigour (Index) I} = \text{Germination percentage} \times \text{Seedling length} \quad (2)$$

$$\text{Vigour (Index) II} = \text{Germination percentage} \times \text{Seedling dry weight} \quad (3)$$

2.6. Statistical Analysis

For each vegetable, the control average value of each parameter was considered as 100 and all raw data were accordingly expressed. An analysis of variance was performed assuming the magnetic field and exposure time as changing factors to reveal their contribution to the germination of the different vegetable species. Data were analyzed by using a completely randomized design with four replications of 25 seeds each and the means were compared by Duncan's multiple range test at the 0.05 level with MSTAT version 4.00/EM (Michigan State University).

3. Results

3.1. Effect of Pre-Sowing Treatment on Tomato (Solanum lycopersicum L.)

Statistical analysis for tomato seeds showed that the magnetic field had a significant effect on germination, speed of germination, seedling fresh weight, and Vigour I, while exposure time did not affect any of the determined parameters (Table 1). However, magnetic field and exposure time interaction significantly affected root and seedling length, seedling fresh weight, and Vigour I.

Table 1. Analysis of variance for germination, speed of germination, root length, shoot length, seedling length, seedling fresh weight, seedling dry weight, Vigour index I, and Vigour index II of tomato seeds treated with different doses of magnetic field (0, 45, 150, and 300 mT) and various exposure times (0, 30, 60, 90, and 120 min) before sowing.

Source of Variance	DF [Z]	Germination	Speed of Germination	Root Length	Shoot Length	Seedling Length	Seedling f. w.	Seedling d. w.	Vigour I	Vigour II
Magnetic field (A)	3	***	***	NS	NS	NS	***	NS	***	NS
Exposure time (B)	4	NS	NS	NS	NS	NS	NS	NS	NS	NS
A × B	12	NS	NS	*	NS	*	***	NS	***	NS
Error	60									

[Z] Degree of Freedom; *, *** Significant effect at the 0.005, and 0.001 levels, respectively; NS not significant.

A significantly negative effect on tomato seed germination was observed with the application of 150 and 300 mT magnetic flux density (Figure 4A). Similarly, the above magnetic field levels led to a significantly lower speed of germination compared to seeds exposed to the low magnetic field of 45 mT (Figure 4B). Moreover, the speed of germination for tomato seeds exposed to high magnetic flux density was significantly lower than control (Figure 4B). On the contrary, a significant increase in seedling fresh weight was observed at

150 mT, while a significantly lower fresh weight was taken at 45 mT (Figure 4C). Seedling Vigour I was significantly lower for exposure to 150 and 300 mT magnetic flux density (Figure 4D).

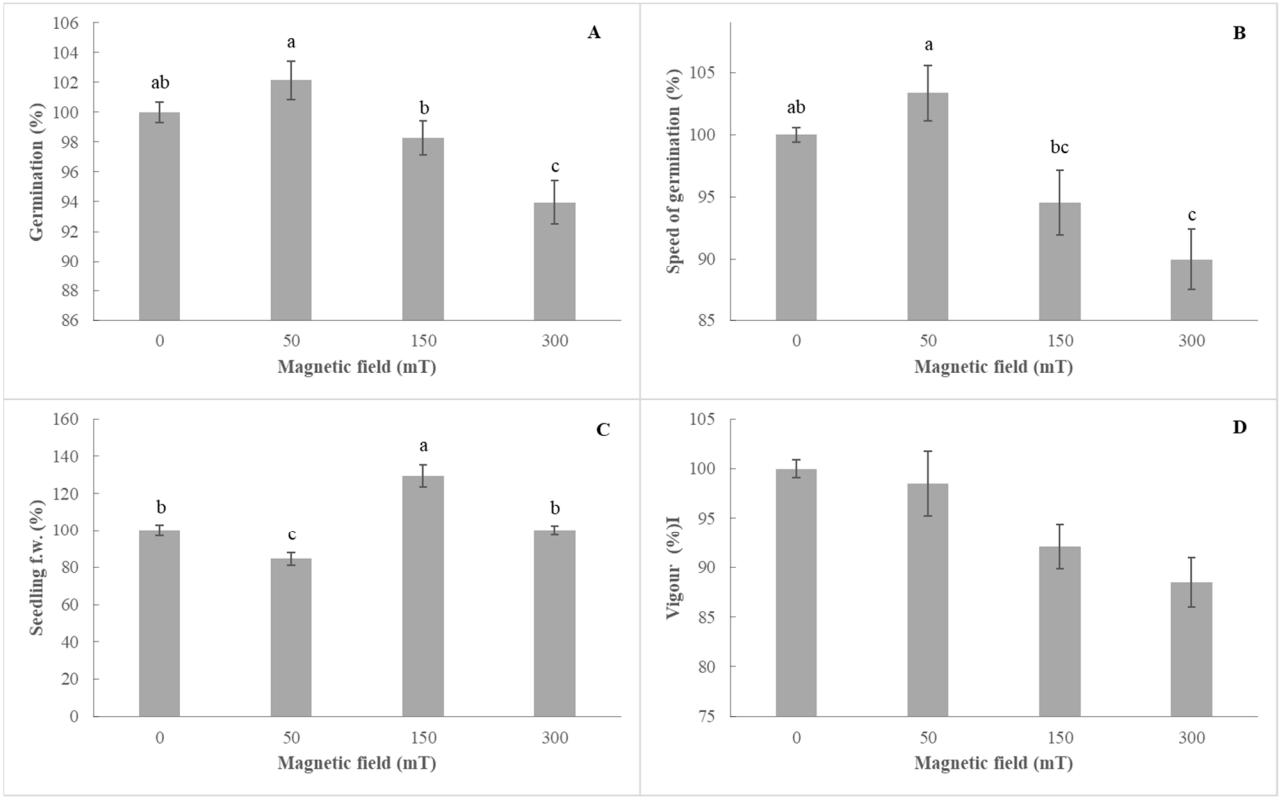


Figure 4. Germination characteristics ((**A**) percentage. (**B**) speed, (**C**) seedling f.w., (**D**) Vigour Index I) of tomato seeds exposed to different levels of the magnetic field. Each value is the mean of four replicates ± S.E. Different letters at each column denote significant differences between means in the same parameter (Duncan's multiple range test, $p < 0.05$). The control average value of each parameter was considered as 100 and all raw data were accordingly expressed.

From the mean comparison of the interaction of magnetic field and exposure time a significantly positive effect only for seedling dry weight was observed at 150 mT and 90 min (Figure 5C). Additionally, root length, seedling length, and Vigour I were higher, from 2.5% to 9.5%, for exposure at 45 mT for 60 and 90 min compared to control (Figure 5A,B,D). However, there was a significant reduction compared to control for some treatments and parameters, such as in root length when seeds were exposed to 300 mT for 60 min (Figure 5A), seedling dry weight for exposure at 45 mT for 30 min (Figure 5C), and Vigour I for exposure at 45 mT for 30 min (Figure 5D).

3.2. Effect of Pre-Sowing Treatment on Rocket (Eruca sativa L.)

The analysis of variance for the rocket seeds showed that only magnetic field strength had a significant effect on growth parameters (root length, shoot length, seedling length, seedling dry weight, and Vigour I) (Table 2). Significant improvement was observed during exposure to 150 mT magnetic flux density for the root and seedling lengths (Figure 6A,C), as well as for Vigour I (Figure 6E). On the contrary, the application of 300 mT magnetic field had a negative effect on seedling dry weight compared to untreated seeds (Figure 6D).

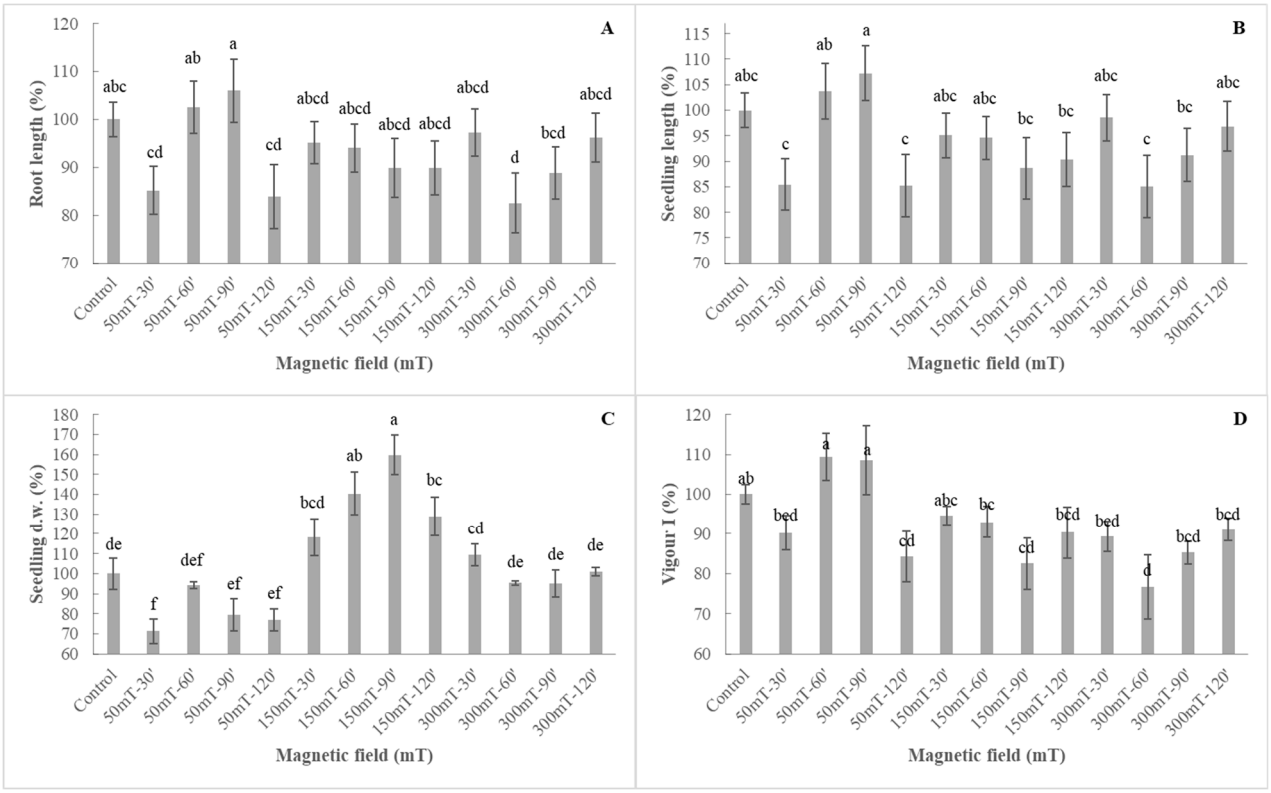


Figure 5. Germination characteristics ((**A**) root length. (**B**) seedling length, (**C**) seedling d.w., (**D**) Vigour Index I) of tomato seeds exposed to different levels of magnetic field for various exposure times before sowing. Each value is the mean of four replicates ± S.E. Different letters at each column denote significant differences between means in the same parameter (Duncan's multiple range test, $p < 0.05$). The control average value of each parameter was considered as 100 and all raw data were accordingly expressed.

Table 2. Analysis of variance for germination, speed of germination, root length, shoot length, seedling length, seedling fresh weight, seedling dry weight, Vigour index I, and Vigour index II of rocket seeds treated with different doses of magnetic field (0, 45, 150, and 300 mT) and various exposure times (0, 30, 60, 90, and 120 min) before sowing.

Source of Variance	DF [Z]	Germination	Speed of Germination	Root Length	Shoot Length	Seedling Length	Seedling f. w.	Seedling d. w.	Vigour I	Vigour II
Magnetic field (A)	3	NS	NS	**	*	**	NS	***	**	NS
Exposure time (B)	4	NS	NS	NS	NS	NS	NS	NS	NS	NS
A × B	12	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error	60									

[Z] Degree of Freedom; *, **, *** Significant effect at the 0.005, 0.01, and 0.001 levels, respectively; NS not significant.

3.3. Effect of Pre-Sowing Treatment on Lettuce (Lactuca sativa L.)

Magnetic field strength significantly affected four out of the nine growth parameters (germination, speed of germination, root length, and seedling length) of the lettuce seeds, whereas exposure time did not have any effect (Table 3).

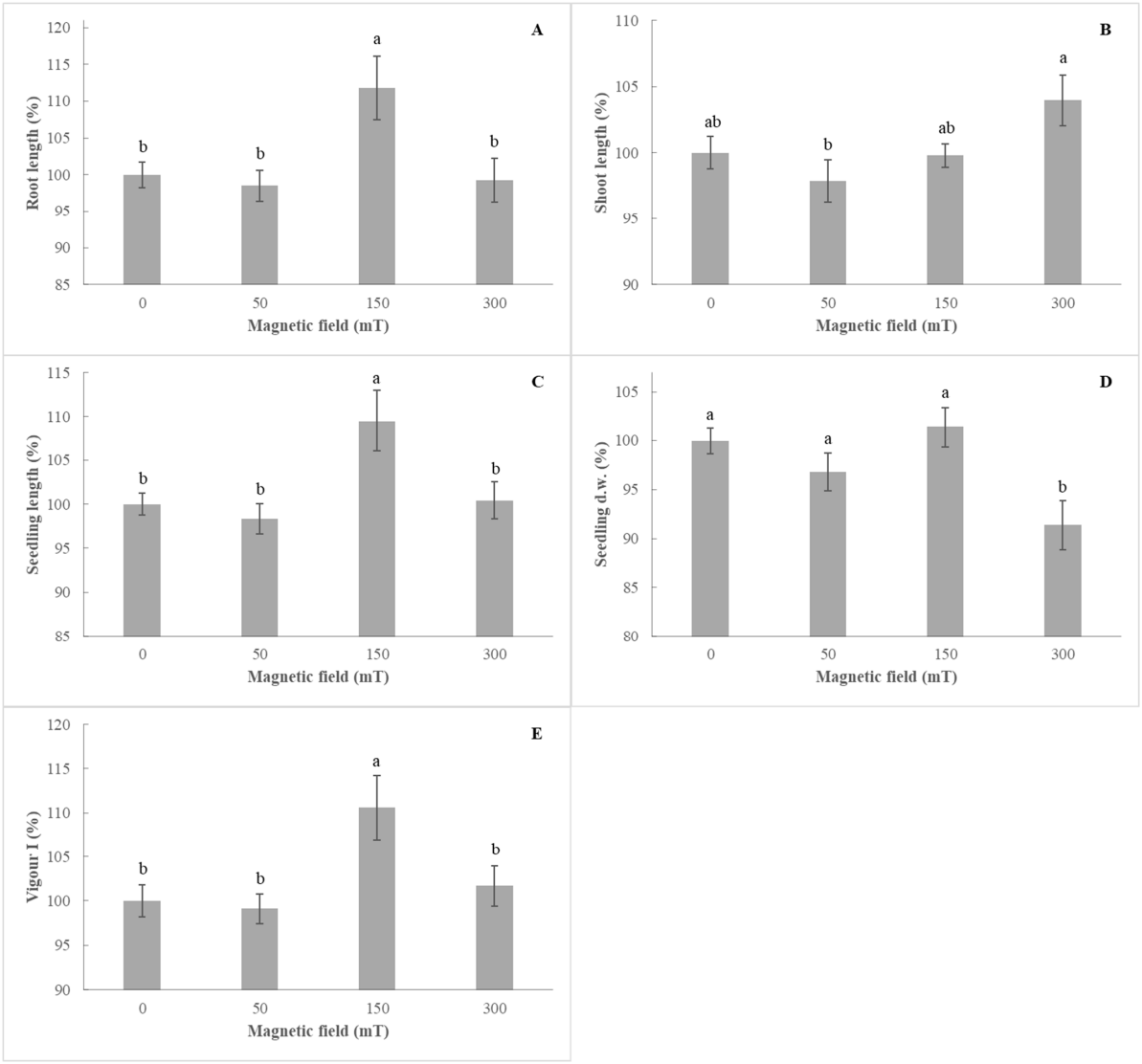


Figure 6. Germination characteristics ((**A**) root length, (**B**) shoot length, (**C**) seedling length, (**D**) seedling d.w., (**E**) Vigour Index I) of rocket seeds exposed to different levels of a magnetic field. Each value is the mean of four replicates ± S.E. Different letters at each column denote significant differences between means in the same parameter (Duncan's multiple range test, $p < 0.05$). The control average value of each parameter was considered as 100 and all raw data were accordingly expressed.

Table 3. Analysis of variance for germination, speed of germination, root length, shoot length, seedling length, seedling fresh weight, seedling dry weight, Vigour index I, and Vigour index II of lettuce seeds treated with different doses of magnetic field (0, 45, 150, and 300 mT) and various exposure times (0, 30, 60, 90, and 120 min) before sowing.

Source of Variance	DF [Z]	Germination	Speed of Germination	Root Length	Shoot Length	Seedling Length	Seedling f. w.	Seedling d. w.	Vigour I	Vigour II
Magnetic field (A)	3	***	*	*	NS	*	NS	NS	NS	NS
Exposure time (B)	4	NS	NS	NS	NS	NS	NS	NS	NS	NS
A × B	12	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error	60									

[Z] Degree of Freedom; *, *** Significant effect at the 0.005, 0.01, and 0.001 levels, respectively; NS not significant.

Germination percentage and speed of germination were significantly higher than control exposure for the seeds exposed to a 300 mT magnetic field (Figure 7A,B). On the other hand, a magnetic flux density of 45 mT was more effective in increasing root and seedling length compared to control exposure (Figure 7C,D).

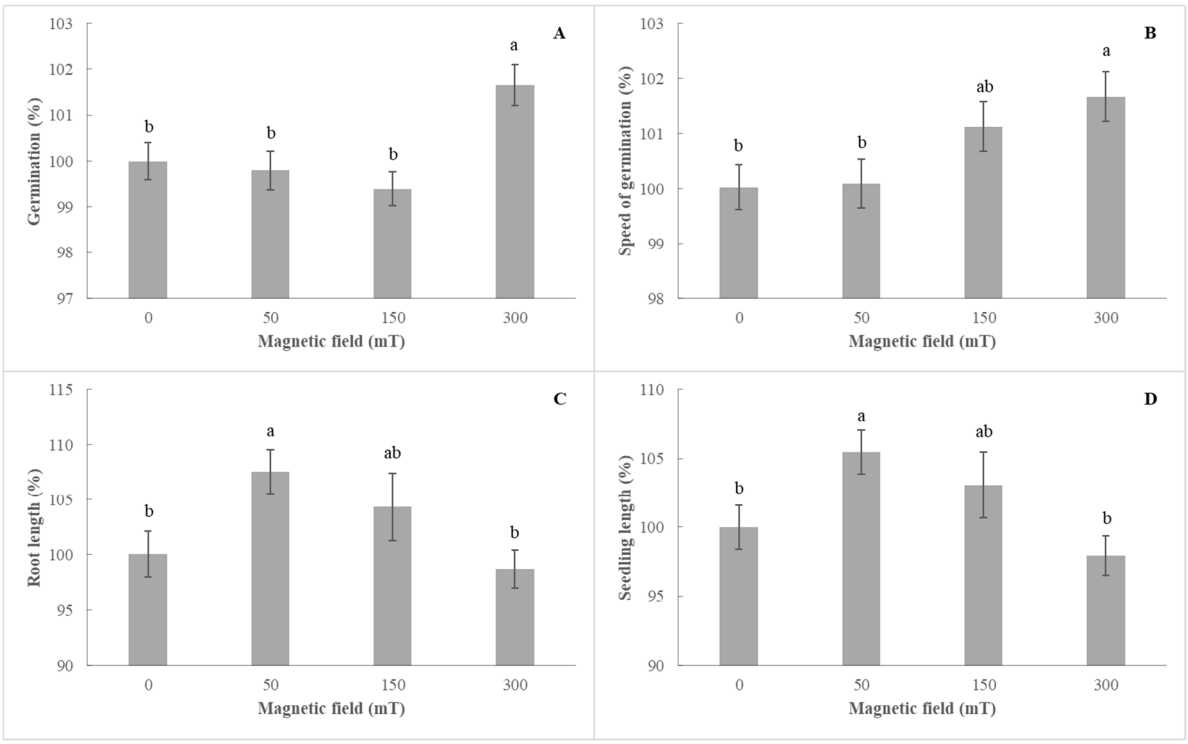


Figure 7. Germination characteristics ((**A**) percentage. (**B**) speed, (**C**) root length, (**D**) seedling length) of lettuce seeds exposed to different levels of a magnetic field. Each value is the mean of four replicates ± S.E. Different letters at each column denote significant differences between means in the same parameter (Duncan's multiple range test, $p < 0.05$). The control average value of each parameter was considered as 100 and all raw data were accordingly expressed.

4. Discussion

There are several studies in the literature on the effects of the pre-sowing treatment of seeds with magnetic fields [7,11], since is considered as a promising alternative method for seed treatment [12]. A small number of these studies have investigated the magnetic field exposure of tomato seeds before sowing. Unfortunately, the exposure parameters in these studies are highly variable. The examined growth parameters are also disparate. Moon et al. [21] have used an AC magnetic field of 0.3 to 100 mT at 60 Hz but for very short exposures of 15 to 60 s. After a week of incubation, the percentage of germination was 10 to 30% higher for the treated groups compared to the control group. De Souza et al. [23] used a 60 Hz full-rectified sinusoidal current to treat the tomato seeds in two different ways, either at 90 mT peak for 10 min or at 154 mT peak for 3 min. The total dry weight of the plants 120 days after their transplantation was between 14 and 29% larger compared to the control group. It appeared that the effect was greater for the higher field–shorter exposure treatment. In our study, although we did not examine fully grown plants but only seedlings, the dry weight peaked at 90 min of exposure for 150 mT. In another study [20], the effects of both short- and long-term exposure of tomato seeds were reported. The authors treated the seeds with ring permanent magnets with nominal magnetic field flux densities of 125 and 250 mT, although no measurements were actually reported. The duration of treatment varied between 1 min to more than 24 h. At the lower magnetic field, the mean germination time (MGT) was the smallest, for an exposure of 20 min, whereas at the higher magnetic field, the MGT became smaller with increasing exposure duration. Compared to the control

group, exposure of the seeds to either magnetic flux density level for 20 min or 60 min resulted in a decrease of 3 to 7% in the MGT. This value is in agreement with the increase in the speed of germination that we report here for treatment with 45 mT. Efthimiadou et al. [22] have recently treated tomato seeds before sowing with a pulsed magnetic field of 12.5 mT flux density amplitude and 3 Hz repetition frequency. They found an increase in the plants' dry weight of 13% for both the 5 and 10 min treatment duration, which corresponds well to the results presented by De Souza et al. [23]. As mentioned above, it is not possible to directly compare the effect of magnetic field treatment on dry weight in the current study with previous ones, because we have evaluated this effect on seedlings, not on fully grown plants.

It was not possible to identify any study on the effects of magnetic field treatment of salad rocket seeds. On the other hand, there has been some research work performed on lettuce seeds. Garcìa Reina et al. [27] have shown that water uptake by seeds treated with a static magnetic field increased with the magnitude of magnetic flux density (values used ranged from 0 to 10 mT). An improvement in the growth of lettuce plants was reported elsewhere [28]. In the latter study, the authors treated the seeds with a magnetic field of either 109 mT for 3 min,145 mT for 1 min, or 145 mT for 5 min. The values for the magnetic flux density refer to the peak of a full-rectified signal at 60 Hz. In all cases, the shoot height and root length of the exposed seeds were more than 20% and 17% larger, respectively, compared to the control group of seeds. These values considerably exceed the here-reported 6% and 8% increase obtained in the seedling and root length, respectively, achieved with exposure at 45 mT magnetic flux density.

Similar studies were conducted with various vegetables and cereals. In a study involving spinach, 150 mT for 1 h enhanced the seed germination and subsequent plant growth in spring compared to 300 mT or/and longer exposure times (i.e., 24 h) [29]. Soybean seeds exhibited higher germination and chlorophyll content in seedlings upon treatment with 400 mT static magnetic field for 3 min including the application of an algal extract, or with an alternating magnetic field with a frequency of 50 Hz and an induction of 30 mT for 2.5 min [30]. On the other hand, sunflower seeds did not show significant germination and growth responses after being treated with a constant magnetic field of 0, 5, 25, or 120 mT for 7 days including a solution with Fe_3O_4 nanoparticles [31]. A study conducted with wheat seeds demonstrated that certain magnetic treatments (10 mT for 10 min, 10 mT for 15 min, and 15 mT for 15 min) showed a high germination rate, improved the plant growth, and enhanced its yield and other relevant parameters due to increased nutrient absorption [32].

Apart from the different impact of static magnetic field strength intensity on the three vegetable seeds, obviously, due to the differences among the species, we observed that for all tested vegetable seeds mainly the magnetic field strength affected germination and young seedlings parameters and did not affect exposure time. This is in agreement with Poinapen et al. [33], who reported that magnetic field strength was a more critical factor than exposure time for tomato seed performance. The exact mechanism of action in the tested species should be clarified and probably could explain the above observation. However, currently, the understanding of how plants perceive and respond to magnetic fields is not fully clear. Some studies suggest that blue light photoreceptors, called cryptochromes, might be implicated [34], but further investigation is needed to explore this aspect of magnetobiology and, in particular, to uncover the molecular mechanisms involved in enhancing seed germination, seedling vigor, and photosynthetic capacity in magneto-primed plants. Moreover, the combined effect of magnetic fields with environmental factors, such as light, humidity, and temperature, may play a crucial role in influencing horticultural and seed performance, as concluded in [35] for the role of relative humidity. Therefore, more experimental studies, carefully designed, are necessary for developing optimized magneto-priming protocols adapted to specific seeds.

5. Conclusions

In this current work, we have presented the design and the full characterization of an exposure setup that allows the simultaneous treatment of seeds using static magnetic fields. The effect of the static magnetic field and exposure time on the germination parameters of tomato, rocket, and lettuce is mixed, with some crops being affected positively (tomato, rocket) and the lettuce not. The results have shown that tomato seedlings significantly benefit from a pre-sowing treatment with a magnetic flux density of 45 mT for 90 min. To improve the growth of salad rocket seedlings, the magnetic field had to be 150 mT, whereas the results for lettuce seeds were a bit inconsistent. Further experiments are required to understand the mechanism of action, especially in species with restricted studies until now, such as lettuce and rocket.

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