

Article

Overcoming Low Germination and Low Quality of Flax Seeds (*Linum usitatissimum* L.) in Unfavorable Storage Using Static Magnetic Fields

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Abstract: Flax seeds stored in unfavorable conditions were exposed to static magnetic fields (SMFs) of 50–350 mT for 20–120 min to overcome low germination and quality. Seed germination increased slightly with increasing strength of SMF and duration of treatment. Seed germination from 89% to 100% was achieved in SMF treatments of 150 mT (120 min), 200 mT (80–120 mT), 250 mT (60–120 min), 300 mT (40–120 min), and 350 mT (40–120 min). In these treatments, germination was 2.78- to 3.12-fold higher than in the control after 10 days. Treatments with 350 mT for 100 and 120 min showed the best results in germination (100%), as well as a 26.81-fold increase in vigor I, 28.69-fold increase in vigor II, 1-fold increase in chlorophyll *a*, 0.84-fold in chlorophyll *b*, 0.46-fold increase in carotenoid content, and 2.63-fold increase in catalase activity compared to the control after 10 days. Also, SMF treatment of 350 mT (20–120 min) reduced cell leakage and electrical conductivity by 1-fold compared to the control. SMF is a healthy, biologically safe, and environmentally friendly treatment and can be a tool for overcoming problems of low germination and quality of seeds stored under unfavorable conditions.



Citation: Čalić, D.; Ristić-Djurović, J.L.; Ćirković, S.; Milojević, J.; Belić, M.; Stanišić, M.; Zdravković-Korać, S. Overcoming Low Germination and Low Quality of Flax Seeds (*Linum usitatissimum* L.) in Unfavorable Storage Using Static Magnetic Fields. *Agriculture* **2023**, *13*, 2120. <https://doi.org/10.3390/agriculture13112120>

Academic Editors: Agnieszka Piotrowicz-Cieślak and Dariusz J. Michalczyk

Received: 12 October 2023
Revised: 3 November 2023
Accepted: 5 November 2023
Published: 9 November 2023



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Keywords: flax seed; development; vigor indices; seed leachate conductivity; chlorophyll and carotenoid content; peroxidase and catalase activity

1. Introduction

The effect of static magnetic fields (SMFs) on seeds has been studied for over 90 years [1]. Applied SMF treatments were lower and higher than Earth’s geomagnetic field (30–60 μ T) [1,2]. Many positive effects of SMF seed treatments on seed germination rate, root, and embryonic shoot growth, vigor indices, yield, and photosynthetic pigment content have been noticed in many agricultural crops: wheat [3], maize [4], barley [5], sunflower [6], the legumes soybean [7–9], broad bean [10], as well as the vegetable species potato [11], tomato [12], cucumber [13], onion [14], etc. Some research determined that SMF treatments can improve the tolerance of plants to biotic [15] or abiotic stress [16] as a result of the activation of their antioxidant response [13].

Consequently, SMF treatments have the potential to minimize the adverse effects of drought, humidity, soil salinity, low or high temperatures, flood, pollution, radiation, or diseases on crop productivity [15]. As a result, special interest is devoted to understanding the physiological, biochemical, and molecular mechanisms underlying the improvement in the growth of plants treated with SMFs [4]. Many studies have pointed out the need for more research to understand the molecular mechanisms involved in seed germination, seedling vigor, and the plant’s photosynthetic capacity exposed to SMFs [15]. In addition to the numerous beneficial properties, the SMF treatments enable the plant to respond and adapt to stressful environmental conditions [7,8,17,18]. SMF treatments can be affected

at multiple levels, from morpho-structural changes to changes in gene expression and proteins or metabolite accumulation [14,19]. These impact plants depending on the SMF (type, dose, and time of exposure) and plant characteristics (e.g., species, cultivar, age, ploidy, and complexity of the target organ or tissue) [1].

The mechanisms of action of SMFs on seed germination included the combined effect of biochemical, metabolic, and physiological changes. SMF treatment changed physiological processes, such as respiration, photosynthesis, nutrient uptake, water relations, and biochemical attributes, including genes involved in ROS (reactive oxygen species), antioxidants, enzymes, proteins, and secondary metabolites. Several studies [9,10] reported the effect of SMFs on the production of ROS, the triggering of oxidative stress, the activity of enzymatic antioxidants, or the expression of their genes [13,18,20]. SMFs influence DNA and RNA synthesis and cell proliferation and cause changes in cellular metabolism and functions [8]. SMFs activate the cellular stress response as a protective mechanism that induces gene expression in the stress response. Treatment with SMFs induced the expression of the Adh/GUS transgene in *Arabidopsis* roots and leaves. Microarray analyses of 8000 genes showed 114 genes differentially expressed about 2.5-fold compared with the control [21]. SMF treatment has become popular in agriculture because it prevents oxidative stress and oxidative damage in plants and thus makes plants more resistant to disease [1,5,22,23].

The flax plant has been cultivated for seed and fiber production since ancient times [24]. The seeds have numerous health benefits based on their high content of α -linolenic acid, lignans, proteins, fats, vitamins, minerals, α -tocopherol (vitamin E), ascorbic acid (vitamin C), carotene, and B-group vitamins (thiamine, B1, and riboflavin, B2) [25,26]. α -Linolenic acid has been beneficial in metabolic syndrome, cancer, inflammation, obesity, neuropathy, and regulation of intestinal flora [27]. Flax and flax seed oil were used as medicine in ancient times and currently as a modern functional food [28]. Fibers are widely used for textile production, especially with biopolymers for biocomposites [29]. Despite the increasing demand for the fibers, flax production has declined in recent decades. The inadequate fiber quality, which is affected by the extreme environmental conditions and improper processing of the straw, makes it necessary to improve the fiber quality [30,31]. Ground flax seeds have much higher nutritional value than whole seeds [32]. Flax seed is harvested and stored at a moisture content of <10% for long-term storage for the 6 weeks required by the crop to attain dormancy [33]. During the non-dormant period, high levels of metabolic activity led to low seed quality, resulting in spoilage. Stored seed quality is affected by biotic and abiotic parameters [33].

Also, SMF treatment of seeds offers advantages over conventional chemical treatment, since it is an eco-friendly, cheap, and non-invasive technique that improves germination and seedling vigor [15,34].

Seed vigor and vitality are lost in storage due to deterioration, which ultimately results in the loss of expensive seed material. Keeping this drawback in mind, applying MF treatments may give a viable alternative for improving seed germination and vigor [35].

There is a lack of research on the impact of SMFs on flax seeds, their germination, and quality.

Therefore, this study aimed to investigate the effects of different SMF treatments on the improvement in germination and quality of flax seed stored in unfavorable conditions.

2. Materials and Methods

2.1. Plant Material and Magnetic Field Characteristics

Mature flax (*Linum usitatissimum* L. cv. Lirina) seeds were collected from storekeepers of health food stores. Flax seeds packed in 10 kg bags were imported from Ukraine. From each bag, 100 g of seeds was taken using a random sampling method. For the experiment, whole, mechanical undamaged seeds were used. The experiment was carried out in March, and the seeds came from the previous year's harvest.

Due to unfavorable storage conditions, the seeds had 13% moisture. Seed drying was induced by MFS treatments. After the MFS treatments, the moisture level dropped to 8–9%. For determination of seed moisture, flax seeds of 100 g were dried in an oven at 120 °C for 4 h, then cooled in a desiccator and reweighed. Total loss of moisture was calculated based on wet or dry weights. Seed mass was measured by the electronic analytic Chyo JL-200 Balance (0.0001 g to 200 g).

Eppendorf tubes (Sigma-Adrich, St. Louis, MO, USA) of 1.5 mL with 100 dry seeds were exposed to a controlled magnetic field (SMF). An electromagnet had been specially designed at the Institute of Physics, University of Belgrade, Serbia. The homogeneous SMF was generated between two poles of a custom-made electromagnet with water-cooled coils (Figure 1).

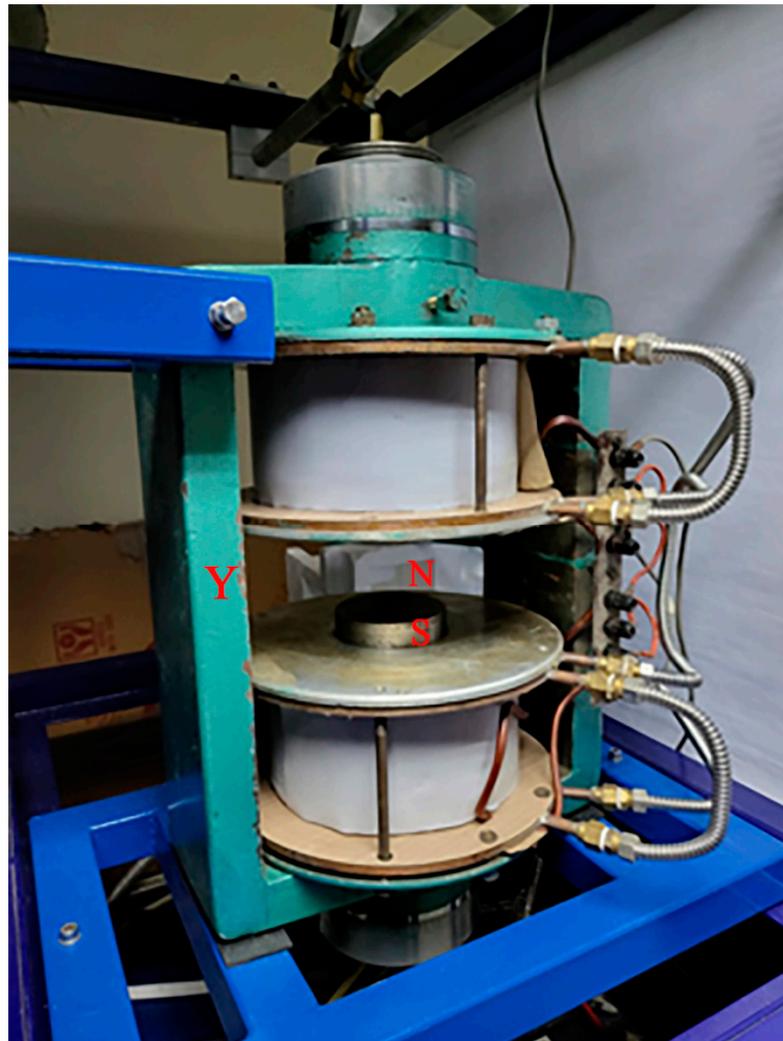


Figure 1. Electromagnet. The ferromagnetic structure of the custom-made electromagnet consists of the frame–yoke (Y) and two cylindrical poles (N, north; S, south) with a diameter of 100 mm whose vertical distance is adjustable, and the distance between the poles can vary between 10 and 100 mm. The poles are wrapped with copper coils, electrically connected in series, and powered by the DC Delta Power Supplies SM 120-25 D.

The coils supplied with the Power Supplies SM 120-25 D (Delta Elektronika BV, Zierikzee, The Netherlands) with a stable output voltage or current of 0.00002–0.0001. The ferromagnetic cylindrical poles have a diameter of 100 mm (Figure 1). At the same time, 12 tubes were put on the cylindrical poles. The distance between the poles was adjustable (a smaller distance provides better homogeneity of the field). The samples were exposed

to seven magnetic field strengths, namely, 350, 300, 250, 200, 150, 100 and 50 mT. The air gap between the poles of the electromagnet was 20 mm. For 350 mT, direct current and voltage were 3.6 A and 8.7 V, respectively, and the power was 31.32 W. The field strength is adjusted along the upper branch of the hysteresis loop of an electromagnet. Magnetic induction values were measured with the MPT-141 Hall probe of the Digital Teslameter (DTM-151, Group3 Technology, Auckland, New Zealand) with an accuracy of ± 0.01 per reading. Exposure durations were 20, 40, 60, 80, 100, and 120 min, each combined with the seven field strengths (42 treatments). The control group was not exposed to the SMF of the electromagnet. Each treatment was performed in three repetitions, with 100 seeds per repetition.

2.2. Seed Germination

Germination tests were performed under laboratory conditions according to the guidelines issued by the International Seed Testing Association [36] with slight modifications. SMF-treated seeds from Eppendorf tubes were transferred into Petri dishes of 90 mm diameter (Spektar, Čačak, Serbia). Twenty seeds per Petri dish were covered with two sheets of Whatman grade 181 seed filter paper soaked in 5 mL of water. For seed germination in dark conditions, each Petri dish was wrapped with opaque aluminum foil. Also, each Petri dish was filled after each 3 days with 5 mL of distilled water to the extent that the filter papers remained moist. A germination test was performed on 100 seeds per treatment, a summary of 5 petri dishes with 20 flax seeds. Flax seeds were grown in the dark at 23 ± 2 °C. The number of germinated seeds was recorded at 3, 7, and 10 days. The seeds were considered germinated when their radicle protruded at least 1 mm.

2.3. Seedling Growth and Vigor Indices

The objective of that test was to evaluate the effect of SMF seed treatments on seedling length and mass at 3, 7, and 10 days after sowing. Seedlings were grown in Petri dishes under laboratory conditions in the dark at a room temperature of 23 ± 2 °C. Seedling mass per Petri dish was determined on days 3, 7, and 10. Root length, shoot length, and vigor indices were measured on day 10.

Data are presented as mean \pm standard error of three replicates ($n = 3$) with 20 seedlings per replicate for early seedling growth traits (root and shoot length, seedling dry mass, and vigor index I and II).

Seedling vigor indices were calculated according to the formula of Abdul-Baki and Anderson [37] as follows:

$$\text{Vigor index I} = \text{Germination \%} \times \text{Total seedling length (root + shoot)}$$

$$\text{Vigor index II} = \text{Germination \%} \times \text{Total seedling dry mass (root + shoot)}$$

For vigor index I, the seedling was scanned with a Canon LIDE 300 scanner, and root and shoot length measurements were performed with ImageJ 1.53e measuring software. Seedling dry mass for vigor index II was measured by electronic analytic balance Chyo JL-200 Balance (0.0001 g to 200 g).

2.4. Seed Leachate Conductivity

The conductivity leachate of flax seed for seed quality assessment was measured immediately after SMF treatments (50–350 mT for 20, 40, 60, 80, 100, and 120 min) in glass jars containing 25 mL of double-distilled water at room temperature (23 ± 2 °C) of 25 flax seeds. A control was double-distilled water and double-distilled water with untreated seeds. The conductivity of the solution was measured after 24 h of incubation at room temperature (23 ± 2 °C) using an ECScan conductivity meter (Lovibond, Germany). The experiment had four replicates.

2.5. Chlorophyll and Carotenoid Extractions

Chlorophyll and carotenoid content were determined in seedlings grown in a growth room at 23 ± 2 °C in light conditions. The content of chlorophyll (*a*) and (*b*), as well as the total content of carotenoids, was studied in seed leaves of 10-day-old seedlings. These photosynthetic pigments were extracted with dimethyl sulfoxide (DMSO, VWR Chemicals, Rosny-sous-Bois cedex, France). The content of chlorophylls and total carotenoids was measured according to the modified method of Minocha et al. [38]. Two hundred milligrams of fresh seed leaves were placed in a 2 mL Eppendorf tube and 1.5 mL of DMSO was added. The seed leaves were then incubated at 65 °C for 6 h. After cooling the samples to room temperature, 200 µL of the supernatant was added to a microplate (Sarstedt 96/F wells) for spectrophotometric measurement, each sample in 3 replicates. Absorbance values were measured at 480, 649, and 665 nm using a UV-vis spectrophotometer (Multiskan Spectrum v1.2, SkanIt Software 2.2).

The following relationships were used to determine chlorophyll (Chl (*a*) and (*b*)) and total carotenoid (C_x + c) content:

$$\text{Chl } a = 12.47A_{665} - 3.62A_{649}$$

$$\text{Chl } b = 25.06A_{649} - 6.5A_{665}$$

$$C_x + c = (1000A_{480} - 1.29C_a - 53.78C_b) / 220$$

The Chl *a*/Chl *b* ratio was calculated from the quotient of Chl *a* and Chl *b* (C_a/C_b), whereas the Chl/carotenoid ratio was calculated from the quotient of their total values ($C(a+b)/C(x+c)$). The determination of photosynthetic pigments was performed in three biological samples of all SMF treatments. In addition, the absorbance of the supernatant was measured three times for each sample. The concentrations of all photosynthetic pigments analyzed are expressed as milligrams per gram fresh mass of the sample (FW).

2.6. Enzymatic Assays

2.6.1. Total Protein Extraction

Samples were 10-day-old flax seedlings derived from SMF-treated seeds (350 mT) for 20, 60, or 120 min. Pooled seedlings (approximately 1000 mg) were immediately frozen in liquid nitrogen and stored at -80 °C until use. Total protein extraction was performed from frozen plant material ground to a fine powder in liquid nitrogen and mixed with the extraction buffer in a 1:2 ratio. The extraction buffer consisted of 0.5 M Tris-HCl buffer (pH 7.6), 0.1 M EDTA (pH 8.0), 10% glycerol, 1.8 mM phenylmethanesulfonyl fluoride (PMSF), and 5% insoluble polyvinylpyrrolidone (PVP) (*w/v*).

After two repeated centrifugations at $10,000 \times g$ for 10 min at 4 °C, trace-free supernatants were obtained. Protein concentrations were determined by Bradford's [39] protein assay using bovine serum albumin (BSA) as standard. Then, 5 µL of each protein sample or BSA standard was mixed with 195 µL of commercial Bradford protein assay reagent (Coomassie Brilliant Blue G-250 dye; Cepham Life Sciences, Inc. Fulton, MI, USA) and absorbance was measured at 595 nm on a microplate reader (Multiskan FC, Thermo Scientific™, Waltham, MA, USA). Protein concentrations in samples were calculated from a standard curve equation.

2.6.2. Spectrophotometric Determination of Catalase (CAT) and Peroxidase (POD) Activities

CAT activity (EC 1.11.1.6) was determined according to the method of Aebi [40]. The reaction mixture (1.5 mL) consisted of 50 mM potassium phosphate buffer (pH 7.0) with an H₂O₂ concentration that allowed an absorbance of 0.85 ± 0.02 . After the addition of 10 µL of protein sample, a decrease in absorbance at 240 nm due to H₂O₂ degradation was measured during the 180 s time interval using a spectrophotometer (Agilent 8453, Santa Clara, CA,

USA). The specific activity of CAT was defined as the concentration of enzyme required to cleave 1 $\mu\text{mol H}_2\text{O}_2$ per min at 25 °C and calculated using the following equation:

$$\text{Volume activity (U/mL)} = (\Delta A \times V_q) / (0.0436 \times V_s)$$

ΔA = decrease in absorbance at 240 nm per min at 25 °C

V_q = reaction volume into cuvette (in mL)

0.0436 = millimolar extinction coefficient (ϵ) of H_2O_2 at 240 nm ($\text{mM}^{-1}\text{cm}^{-1}$)

V_s = volume (mL) of sample

The specific activity of CAT was expressed in units (U) per milligram of total protein (U/mg prot) according to the following equation:

$$\text{CAT activity (U/mg prot)} = \text{Volume activity (U/mL)} / \text{Protein sample concentration (mg prot/mL)} \quad (1)$$

POD activity (EC 1.11.1.7) was measured at 430 nm by the formation of purpurogallin from pyrogallol in the presence of H_2O_2 with a modified method of Flatmark [41]. The reaction mixture (1.5 mL) contained 30 μL of protein sample, 3 mM pyrogallol, and 3 mM H_2O_2 in 50 mM phosphate buffer (pH 6.5). The increase in absorbance at 430 nm was measured during a 180 s time interval using a spectrophotometer (Agilent 8453, Santa Clara, CA, USA). The specific activity of POD was defined as the amount of enzyme that oxidizes 1 μmol of substrate per minute at 25 °C and was calculated using the following equation:

$$\text{Volume activity (U/mL)} = (\Delta A \times V_q) / (2.47 \times V_s)$$

ΔA = increase in absorbance at 430 nm per min at 25 °C

V_q = reaction volume into cuvette (in mL)

2.47 = extinction coefficient (ϵ) of purpurogallin at 430 nm ($\text{mM}^{-1}\text{cm}^{-1}$)

V_s = volume (in mL) of sample

The specific activity of POD was expressed in units (U) per milligram of total protein (U/mg prot) according to the following equation:

$$\text{POD activity (U/mg prot)} = \text{Volume activity (U/mL)} / \text{Protein sample concentration (mg prot/mL)} \quad (2)$$

2.7. Statistical Analyses

The measurements of the seed germination, seedling length, seedling dry mass, vigor indices, leachate electrical conductivity, photosynthetic pigment content, peroxidase, and catalase activity were taken on 300 randomly chosen flax seeds (in three repetitions, each with 100 seeds) per SMF treatment.

To analyze the main effects and interaction effects strength (50–350 mT and duration 20–120 min), the data were subjected to analysis of variance (two-way ANOVA), and the means \pm SE were separated using Fisher's LSD (least significant difference) test according to an ad hoc test at a confidence level of $p \leq 0.05$.

3. Results

3.1. Seed Germination

All SMF treatments with different strengths (50–350 mT) and duration (20–120 min) significantly affected the germination of flax seeds. The germination test showed only 7% germination in control after 3 days. However, seed germination in SMF treatments of 50–350 mT ranged 12–32% at 20 min, 14–36% at 40 min, 15–42% at 60 min, 17–46% at 80 min, 18–50% at 100 min, and 20–55% at 120 min. Depending on SMF strength (50–350 mT), germination ranged from 0.71- to 3.57-fold for 20 min and 1.86- to 6.86-fold for 120 min (Figure 2).

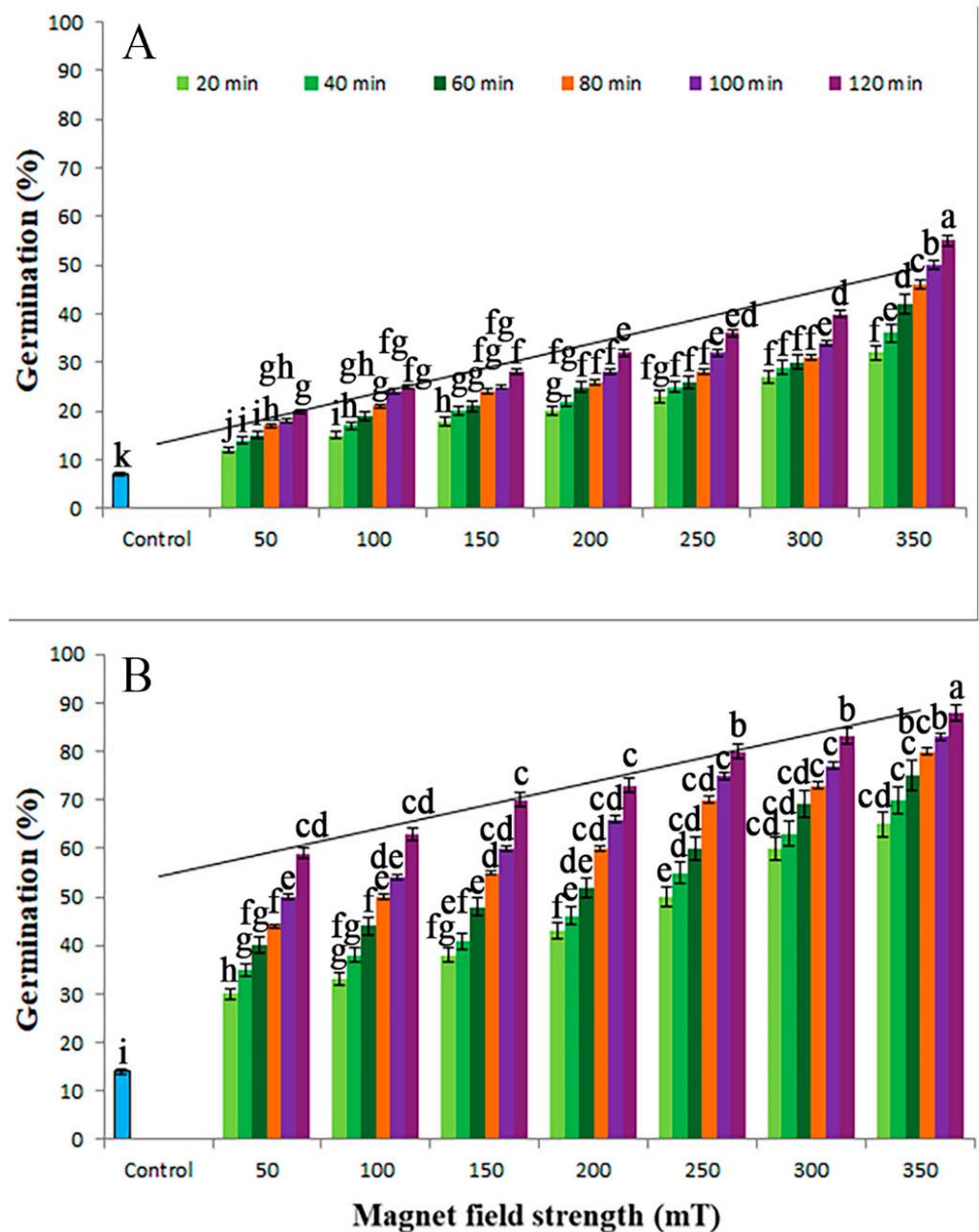


Figure 2. Germination percentage of flax seeds at 3 (A) and 7 (B) days after exposure to SMF of 50–350 mT for 20, 40, 60, 80, 100 or 120 min. All treatments were performed in three replicates of 100 seeds each. Data represent the means \pm standard errors. Values followed by different letters are significantly different at $p \leq 0.05$ on Fisher’s least significant difference (LSD) test.

After 7 days, the germination test showed only 14% germination in control. Nevertheless, SMF treatments of 50–350 mT showed significantly higher germination at 20 min (30–65%), 40 min (35–70%), 60 min (40–75%), 80 min (44–80%), 100 min (50–83%), and 120 min (59–88%). Also, depending on SMF strength (50–350 mT), germination ranged from 1.14- to 3.64-fold for 20 min and 3.21- to 6.86-fold for 120 min (Figure 2).

After ten days, the germination test showed only 32% germination in control. Flax seed germination in SMF treatments of 50–350 mT was significantly higher at 20 min (55–78%), 40 min (60–89%), 60 min (65–97%), 80 min (69–98%), 100 min (74–99%) and 120 min (78–100%). On these SMF treatments, germination ranged from 0.72- to 1.44-fold for 20 min and 1.44- 3.12-fold for 120 min (Table 1).

Table 1. Effect of various SMF treatments on flax seed germination and seedlings' shoot and root length, dry mass, and vigor indices, after 10 days.

SMF (mT)	Duration (min)	Germination %	Embryonic Shoot Length (cm)	Root Length (cm)	Dry Mass (g)	Vigor I	Vigor II	Anova p-Value
Control	control	32 ± 3.4 e	2.3 ± 0.2 g	2.0 ± 0.3 f	0.02 ± 0.002 h	137.60 ± 13.9 l	0.64 ± 0.08 h	≤0.05
	20	55 ± 5.9 d	4.4 ± 0.4 f	3.8 ± 0.4 ef	0.04 ± 0.004 g	451.00 ± 46.04 k	2.20 ± 0.23 g	≤0.01
	40	60 ± 6.2 c	4.6 ± 0.4 f	4.1 ± 0.4 ef	0.04 ± 0.005 g	522.00 ± 53.27 j	2.40 ± 0.25 g	≤0.01
50	60	65 ± 6.5 c	4.9 ± 0.5 f	4.5 ± 0.4 ef	0.05 ± 0.005 fg	611.00 ± 62.88 i	3.25 ± 3.33 fg	≤0.01
	80	69 ± 6.9 bc	5.2 ± 0.5 g	4.8 ± 0.5 e	0.05 ± 0.005 fg	690.00 ± 70.04 h	3.45 ± 0.35 f	≤0.01
	100	74 ± 7.2 b	5.6 ± 0.6 ef	5.1 ± 0.5 e	0.05 ± 0.006 fg	791.80 ± 79.06 g	3.70 ± 0.38 f	≤0.01
	120	78 ± 7.9 b	5.9 ± 0.6 ef	5.4 ± 0.6 e	0.06 ± 0.006 f	881.40 ± 89.05 fg	4.68 ± 0.48 ef	≤0.01
	20	60 ± 6.2 c	6.3 ± 0.6 ef	5.7 ± 0.6 e	0.06 ± 0.006 f	720.00 ± 73.55 h	3.60 ± 0.37 f	≤0.01
	40	65 ± 6.7 c	6.6 ± 0.7 ef	6.0 ± 0.7 e	0.06 ± 0.006 f	819.00 ± 82.11 g	3.90 ± 0.40 f	≤0.01
100	60	70 ± 7.1 bc	6.9 ± 0.7 e	6.2 ± 0.7 de	0.07 ± 0.007 f	917.00 ± 92.32 f	4.90 ± 0.50 ef	≤0.01
	80	74 ± 7.5 b	7.3 ± 0.7 e	6.6 ± 0.7 de	0.07 ± 0.007 f	1028.60 ± 104.03 ef	5.18 ± 0.51 ef	≤0.01
	100	79 ± 8.0 ab	7.6 ± 0.7 e	6.9 ± 0.7 de	0.07 ± 0.007 f	1145.50 ± 116.26 ef	5.53 ± 0.56 e	≤0.01
	120	83 ± 8.4 b	7.9 ± 0.8 de	7.2 ± 0.8 de	0.08 ± 0.008 ef	1253.30 ± 120.65 e	6.00 ± 0.68 de	≤0.01
	20	63 ± 6.5 c	8.2 ± 0.8 de	7.5 ± 0.8 d	0.08 ± 0.008 ef	989.10 ± 99.33 f	5.04 ± 0.54 ef	≤0.01
150	40	70 ± 7.2 bc	8.5 ± 0.8 de	7.9 ± 0.8 d	0.08 ± 0.008 ef	1148.00 ± 117.45 ef	5.60 ± 0.57 e	≤0.01
	60	75 ± 7.6 b	8.7 ± 0.9 d	8.2 ± 0.8 d	0.08 ± 0.008 ef	1267.50 ± 131.11 e	6.00 ± 0.62 e	≤0.01
	80	77 ± 7.9 b	8.9 ± 0.9 d	8.5 ± 0.9 d	0.09 ± 0.009 e	1339.80 ± 142.22 e	6.93 ± 0.69 de	≤0.01
	100	86 ± 8.8 b	9.3 ± 0.9 d	8.9 ± 0.9 d	0.09 ± 0.01 e	1565.20 ± 161.34 d	7.74 ± 0.76 d	≤0.01
	120	89 ± 9.0 a	9.6 ± 1.0 d	9.1 ± 0.9 d	0.09 ± 0.01 e	1664.30 ± 168.98 d	8.01 ± 0.82 d	≤0.01
	20	66 ± 6.8 c	10.1 ± 1.1 d	9.5 ± 0.9 cd	0.10 ± 0.01 de	1293.60 ± 131.54 e	6.60 ± 0.67 e	≤0.01
200	40	76 ± 7.7 b	10.4 ± 1.1 d	9.8 ± 1.0 cd	0.10 ± 0.01 de	1535.20 ± 155.76 d	7.60 ± 0.77 d	≤0.01
	60	79 ± 8.0 ab	10.7 ± 1.2 d	10.1 ± 1.0 cd	0.10 ± 0.01 de	1643.20 ± 172.55 d	7.90 ± 0.79 d	≤0.01
	80	85 ± 8.7 a	11.1 ± 1.2 d	10.5 ± 1.1 c	0.11 ± 0.01 d	1836.00 ± 189.16 cd	9.35 ± 0.96 c	≤0.01
	100	91 ± 9.3 a	11.6 ± 1.2 c	10.8 ± 1.1 c	0.11 ± 0.01 cd	2038.40 ± 200.88 c	10.01 ± 1.13 c	≤0.01
	120	95 ± 9.5 a	12.1 ± 1.3 c	11.2 ± 1.1 c	0.12 ± 0.01 cd	2213.50 ± 229.44 c	11.40 ± 1.15 b	≤0.01
	20	70 ± 7.1 b	12.6 ± 1.3 c	11.5 ± 1.2 c	0.12 ± 0.01 cd	1687.00 ± 170.82 d	8.40 ± 0.89 d	≤0.01
250	40	80 ± 8.2 ab	12.9 ± 1.3 bc	11.8 ± 1.2 c	0.13 ± 0.01 c	1976.00 ± 200.03 c	10.40 ± 1.05 c	≤0.01
	60	86 ± 8.8 a	13.1 ± 1.3 bc	12.4 ± 1.2 b	0.13 ± 0.01 c	2193.00 ± 220.37 c	11.18 ± 1.19 bc	≤0.01
	80	89 ± 9.1 a	13.5 ± 1.4 bc	12.8 ± 1.3 b	0.13 ± 0.01 c	2340.70 ± 238.91 bc	11.57 ± 1.75 b	≤0.01
	100	96 ± 9.7 a	13.8 ± 1.4 bc	13.2 ± 1.3 b	0.13 ± 0.01 c	2592.00 ± 256.26 b	12.48 ± 1.26 b	≤0.01
	120	98 ± 9.9 a	14.2 ± 1.4 bc	13.6 ± 1.4 b	0.14 ± 0.01 bc	2724.40 ± 279.98 ab	13.72 ± 1.39 ab	≤0.001
	20	73 ± 7.2 b	14.6 ± 1.5 b	13.9 ± 1.4 b	0.14 ± 0.01 bc	2085.50 ± 210.54 c	10.22 ± 1.04 c	≤0.01
300	40	86 ± 8.7 a	14.9 ± 1.5 b	14.1 ± 1.4 b	0.14 ± 0.01 bc	2494.00 ± 253.12 b	12.04 ± 1.22 b	≤0.01
	60	90 ± 9.1 a	15.3 ± 1.5 b	14.4 ± 1.5 b	0.15 ± 0.01 b	2673.00 ± 272.31 b	13.50 ± 1.37 ab	≤0.001
	80	95 ± 9.5 a	15.6 ± 1.6 b	14.7 ± 1.5 ab	0.15 ± 0.01 b	2878.50 ± 290.16 ab	14.25 ± 1.43 ab	≤0.001

Table 1. Cont.

SMF (mT)	Duration (min)	Germination %	Embryonic Shoot Length (cm)	Root Length (cm)	Dry Mass (g)	Vigor I	Vigor II	Anova <i>p</i> -Value	
350	100	98 ± 9.9 a	15.8 ± 1.6 b	15.2 ± 1.5 ab	0.15 ± 0.01 b	3038.00 ± 310.35 ab	14.70 ± 1.49 ab	≤0.001	
	120	99 ± 10.1 a	16.1 ± 1.6 b	15.5 ± 1.6 ab	0.16 ± 0.01 ab	3128.40 ± 328.33 ab	15.84 ± 1.59 ab	≤0.001	
	20	78 ± 7.9 b	16.4 ± 1.6 b	15.7 ± 1.6 ab	0.16 ± 0.01 ab	2503.80 ± 261.11 b	12.48 ± 1.27 b	≤0.01	
	40	89 ± 8.8 a	16.8 ± 1.7 b	15.9 ± 1.6 ab	0.17 ± 0.01 ab	2910.30 ± 300.34 ab	15.13 ± 1.54 ab	≤0.001	
	60	97 ± 9.8 a	17.1 ± 1.7 ab	16.5 ± 1.7 ab	0.17 ± 0.01 ab	3259.20 ± 327.25 ab	16.49 ± 1.68 ab	≤0.001	
	80	98 ± 9.7 a	17.4 ± 1.7 ab	16.9 ± 1.7 a	0.17 ± 0.01 ab	3361.40 ± 340.16 ab	16.66 ± 1.69 ab	≤0.001	
	100	99 ± 9.9 a	18.1 ± 1.8 a	17.4 ± 1.8 a	0.18 ± 0.01 a	3514.50 ± 366.22 a	17.82 ± 1.79 a	≤0.0001	
	120	100 a	19.5 ± 1.9 a	18.6 ± 1.9 a	0.19 ± 0.01 a	3810.00 ± 386.77 a	19.00 ± 1.92 a	≤0.0001	
	Anova <i>p</i> -value		≤0.0001	≤0.001	≤0.001	≤0.001	≤0.001	≤0.001	

Data represent the mean values ± standard error. Means followed by the same lowercase letters in a column are not significantly different according to the LSD test ($p \leq 0.05$).

The treatments at SMF also accelerated seed germination, as exposure of seeds to 350 mT for 120 min resulted in germination of approximately 55% of seeds within only 3 days (Figure 2A), whereas similar total germination for seeds exposed to 50 mT for 120 min was achieved after 7 days (Figure 2B) and in seeds exposed to 50 mT for 20 min after 10 days, while control seeds achieved only 32% germination after 10 days (Table 2).

Table 2. Two-way ANOVA showing the effect of strength (50–350 mT) and duration (20–120 min) of SMF treatments on flax seed germination and seedling fresh mass.

Source of Variation	df	Seed Germination		Seedling Fresh Mass	
		F-Value	p	F-Value	p
Strength of SMF (A)	6	7785	≤0.0001	777	≤0.0001
Duration of SMF (B)	5	760	≤0.0001	489	≤0.0001
A × B	30	104	≤0.0001	81	≤0.0001

SMF treatments showed the strongest effect on germination percentage after 3 days compared to 7 and 10 days. The most pronounced effect was for SMF treatment at 350 mT for 120 min. On this treatment, seed germination increased by 6.86-, 5.2-, and 2.13-fold compared to the control at 3, 7, and 10 days, respectively (Figure 2).

On treatments with 150 mT (120 min), 200 mT (80–120 min), 250 mT (60–120 min), 300 mT (40–120 min), and 350 mT (40–120 min), the best seed germination was achieved after 10 days. With increasing strength of the SMF (150–350), the required exposure time was shortened (from 120 to 40 min) to achieve maximum germination. On these SMF treatments, germination of 89% to 100% was induced, which is from 2.78- to 3.12-fold higher than the control (32%) (Table 1). The higher the SMF strength required, the shorter the time to achieve a germination percentage (Table 1; Figure 2). Even a short 20 min SMF treatment with 50 mT significantly increased seed germination compared to the untreated control (55% vs. 32%) after ten days. With increasing SMF, strength from 100 to 200 mT, germinated seeds doubled (60–66%) and reached 78% at 350 mT for 20 min (Table 1). Our results showed that germination increased steadily and significantly ($p \leq 0.0001$) with the strength and duration of SMF treatments (Table 2).

3.2. Seedling Growth

SMF treatment significantly improved seedling characteristics, such as embryonic shoot and root length and fresh mass ($p \leq 0.001$; Tables 1 and 2) after 3, 7, and 10 days (Figure 3). The mean values of seedling fresh mass per Petri dish were 11 ± 0.01 g, 20 ± 0.02 g, and 28 ± 0.03 g (350 mT, 120 min), while the mass of control seedlings was only 0.57 ± 0.06 g, 0.9 ± 0.09 g, and 1.9 ± 0.01 g at 3, 7, and 10 days, respectively (Figure 3). Increasing SMF strength and duration increased the fresh and dry mass of the seedlings. As such, all SMF treatments showed better results than the control (Figure 3).

Seedlings derived from SMF-treated seeds grew significantly faster than control seedlings as a function of SMF strength and treatment duration. A 350 mT treatment for 120 min produced seedlings with about 10 g mass per Petri dish in only 3 days, while seedlings from a 50 mT treatment (120 min) took 10 days to reach about the same value and control seedlings had only 1.9 g per Petri dish in 10 days (Figure 3). Control seedlings had significantly lower dry mass than seedlings grown from seeds treated with SMF (Table 1). Also, the SMF treatment at 350 mT for 120 min resulted in the seedlings with the highest dry mass (Table 1).

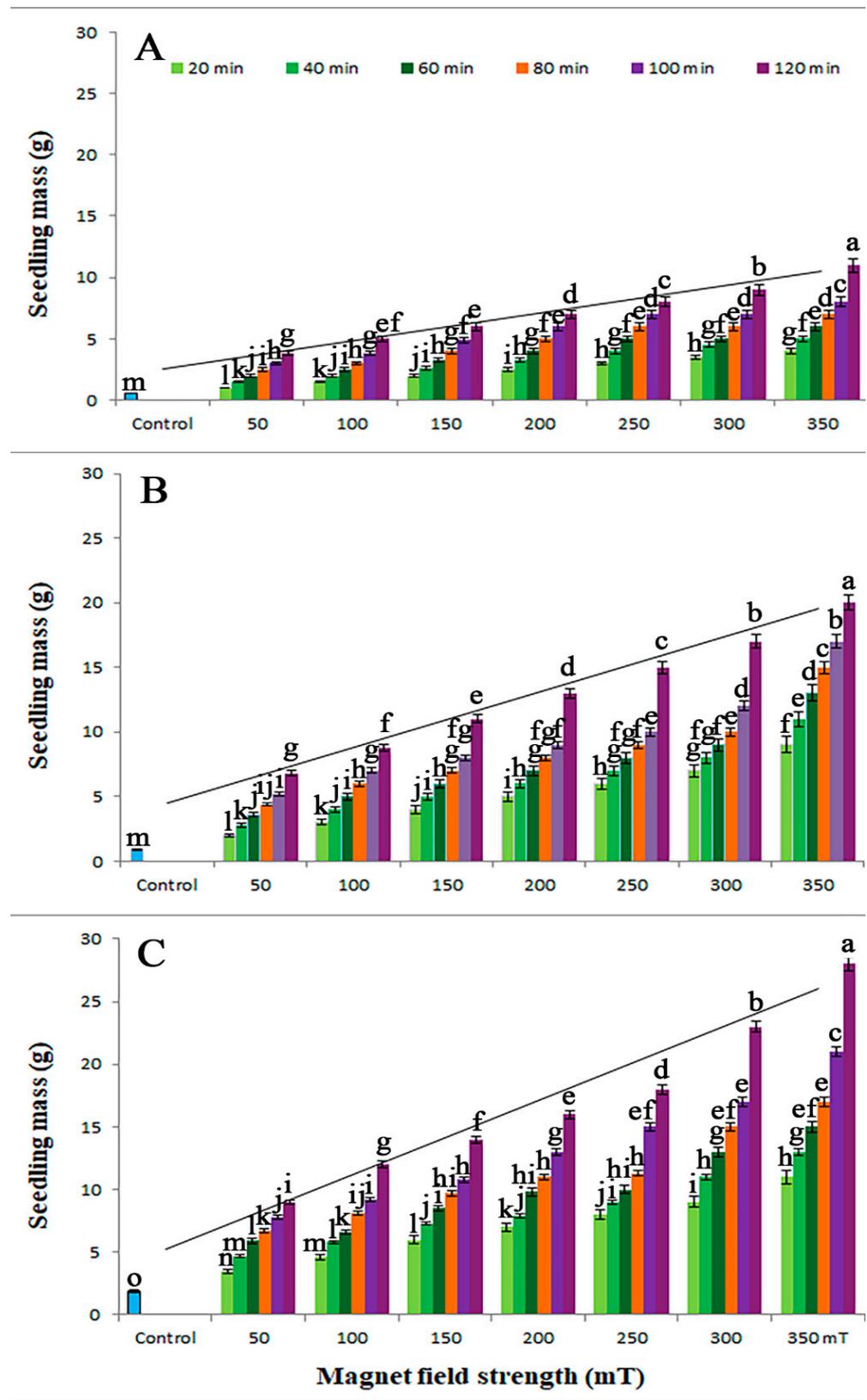


Figure 3. Mass of flax seedlings 3 (A), 7 (B), and 10 days (C) after exposure to SMF of 50–350 mT for 20, 40, 60, 80, 100, or 120 min. All treatments were performed in three replicates with 20 seedlings each. Data represent the means \pm standard errors. Values followed by different letters are significantly different at $p \leq 0.05$ on Fisher’s least significant difference (LSD) test.

The mass of seedlings derived from seed treatment of 350 mT 120 min increased by 21.22-fold after 7 days, with a slight decrease to 13.7-fold after 10 days. Both embryonic shoot and root length showed significant differences between the SMF treatments. While control seedlings averaged only 2.3 cm in length 10 days after sowing, seedlings developed from SMF-treated seeds had significantly more length: even seedlings from the mildest treatment (50 mT for 20 min) almost doubled their lengths (4.4 ± 0.4 cm) for the same period (Table 1). The length of the embryonic shoots continued to increase with the SMF strength, so that seeds exposed to 200 mT were more than 10 cm long, while the longest embryonic shoots (19.5 ± 1.9 cm) were obtained from 350 mT treatment for 120 min (Table 1). Similar results were obtained for the root length, except that the values of root lengths obtained in the mildest SMF treatment of 50 mT for 20–60 min were not significantly different from those of the control (Table 1). Root length increased steadily with MS strength and treatment duration, reaching the highest value with treatment at 350 mT for 120 min (18.6 ± 1.9 cm). SMF treatments with 350 mT (100 and 120 min) showed the best results for seedling length (Table 1).

The embryonic shoot length increased 6.87- to 7.48-fold, while root length increased 7.7- to 8.3-fold after 10 days under SMF treatment of 350 mT for 100 and 120 min.

In conclusion, exposure of flax seed to SMF of 350 mT for 100 and 120 min had a significant growth-promoting effect on the seedlings. Also, results showed that seedling fresh mass increased steadily and significantly ($p \leq 0.0001$) with the strength and duration of SMF treatments (Table 2).

3.3. Vigor Indices

The seed response to the treatments of SMF showed a positive effect on the vigor indices. Growth vigor indices I and II increased significantly with increasing strength (50/350 mT) and duration (20/120 min) of SMF treatment ($p \leq 0.001$, respectively; Table 1). Among all treatments, the 350 mT treatment (100 and 120 min) showed the best improvement in germination parameters. Seed treatment with 350 mT (120 min) showed a 26.81- and 28.69-fold increase in vigor index I and II, respectively, compared with the control (Table 1).

3.4. Seed Leachate Conductivity

Flax seed leachate conductivity in SMF untreated control was $0.577 \mu\text{S cm}^{-1}$. All SMF treatments significantly improved seed coat integrity and reduced cell leakage and electrical conductivity compared to the control. Seed leachate conductivity in SMF treatments of 50–350 mT was significantly lower at 20 min (0.460 – $0.315 \mu\text{S cm}^{-1}$), 40 min (0.436 – $0.310 \mu\text{S cm}^{-1}$), 60 min (0.452 – $0.303 \mu\text{S cm}^{-1}$), 80 min (0.450 – $0.294 \mu\text{S cm}^{-1}$), 100 min (0.447 – $0.289 \mu\text{S cm}^{-1}$) and 120 min (0.445 – $0.284 \mu\text{S cm}^{-1}$). On these SMF treatments of 50–350 mT, seed leachate conductivity ranged from 0.25- to 0.30-fold for 20 min and 0.83 to 1.03 for 120 min (Table 3). SMF treatment at 350 mT for 20–120 min showed the best results (Table 3). The strength of the SMF had a significant effect ($p \leq 0.0001$) on leachate conductivity (Table 3).

Table 3. Effect of various SMF treatments on seed leachate electrical conductivity ($\mu\text{S cm}^{-1}$) of flax.

Magnetic Field (mT)	Time (min)					
	20	40	60	80	100	120
Control	0.577 ± 0.041 a/A	0.577 ± 0.037 a/A	0.577 ± 0.034 a/A	0.577 ± 0.027 a/A	0.577 ± 0.034 a/A	0.577 ± 0.043 a/A
50	0.460 ± 0.036 b/B	0.456 ± 0.045 b/B	0.452 ± 0.053 b/B	0.450 ± 0.044 b/B	0.447 ± 0.036 b/B	0.445 ± 0.042 b/B
100	0.435 ± 0.040 b/B	0.430 ± 0.034 b/B	0.425 ± 0.044 b/B	0.421 ± 0.038 b/B	0.418 ± 0.041 b/B	0.413 ± 0.045 b/B
150	0.408 ± 0.032 b/B	0.404 ± 0.042 b/B	0.398 ± 0.033 b/B	0.394 ± 0.032 b/B	0.390 ± 0.042 b/B	0.385 ± 0.038 b/B
200	0.385 ± 0.027 b/B	0.381 ± 0.035 b/B	0.377 ± 0.037 b/B	0.372 ± 0.043 b/B	0.369 ± 0.034 b/B	0.363 ± 0.039 b/B
250	0.371 ± 0.051 bc/BC	0.368 ± 0.053 bc/BC	0.364 ± 0.023 bc/BC	0.360 ± 0.033 bc/BC	0.355 ± 0.030 bc/BC	0.350 ± 0.026 bc/BC

Table 3. Cont.

Magnetic Field (mT)	Time (min)					
	20	40	60	80	100	120
300	0.344 ± 0.033 bc/BC	0.340 ± 0.025 bc/BC	0.335 ± 0.036 bc/BC	0.330 ± 0.028 bc/BC	0.326 ± 0.024 bc/BC	0.322 ± 0.027 bc/BC
350	0.315 ± 0.029 c/C	0.310 ± 0.023 c/C	0.303 ± 0.041 c/C	0.294 ± 0.022 c/C	0.289 ± 0.027 c/C	0.284 ± 0.021 c/C

Data represent the mean values ± standard error. Means followed by the same lowercase letters in a column and capital letters in a row are not significantly different according to the LSD test ($p \leq 0.05$).

3.5. Chlorophyll and Carotenoids Content

In the present study, chlorophyll *a* and *b* content increased slightly ($p \leq 0.001$) in the SMF treatments compared with the control (Table 4). Chlorophyll *a* in flax seed leaves derived from SMF treatment of 50 mT for 20, 60, and 120 min and 100 mT for 20 and 60 min had similar values to the control. Also, chlorophyll *b* in seed leaves derived from SMF treatment of 50 mT for 20, 60, and 120 min showed control values. Chlorophyll values increased with increasing SMF treatment from 100 to 350 mT. Chlorophyll contents were about 1-fold and 0.84-fold higher under 350 mT treatment than control for 100 and 120 min (Table 4). Chlorophyll *b* was significantly higher in seedlings derived from all SMF treatments. Similar values of chlorophyll *a/b* ratio (Table 3) showed the stability of chlorophyll *a* in all SMF treatments. Total carotenoid content in seed leaves slightly increased on SMF treatments from 50 to 350 mT at 20 to 120 min durations ($p \leq 0.001$; Table 4). The best result of total carotenoid content detected in seed leaves derived from seed treated with 350 mT for 120 min. Carotenoid content in that treatment was 0.46-fold higher than the control.

Table 4. Effect of various SMF treatments on chlorophyll *a*, *b*, and carotenoid content (mg/g FW) in flax seed leaves.

Magnetic Field Strength (mT)	Duration (min)	Chlorophyll <i>a</i> (mg/g FW)	Chlorophyll <i>b</i> (mg/g FW)	Chlorophyll <i>a/b</i>	Carotenoids (mg/g FW)	Carotenoids/Chlorophyll <i>a + b</i>	ANOVA <i>p</i> -Value
Control		1.40 ± 0.18 b	1.15 ± 0.11 c	1.21 ± 0.12 b	0.70 ± 0.08 d	0.26 ± 0.02 b	≤0.01
50 mT	20	1.68 ± 0.20 b	1.21 ± 0.13 c	1.39 ± 0.14 a	0.90 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
	60	1.71 ± 0.19 b	1.23 ± 0.12 c	1.39 ± 0.14 a	0.92 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
	120	1.74 ± 0.17 b	1.26 ± 0.14 c	1.38 ± 0.13 a	0.94 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
100 mT	20	1.75 ± 0.18 b	1.27 ± 0.13 bc	1.38 ± 0.14 a	0.95 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
	60	1.77 ± 0.21 b	1.28 ± 0.14 bc	1.38 ± 0.14 a	0.96 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
	120	1.81 ± 0.20 ab	1.32 ± 0.14 bc	1.37 ± 0.15 a	0.98 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
150	20	1.83 ± 0.22 ab	1.33 ± 0.15 bc	1.38 ± 0.14 a	0.99 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
	60	1.86 ± 0.23 ab	1.36 ± 0.14 b	1.36 ± 0.13 a	1.00 ± 0.09 c	0.31 ± 0.03 ab	≤0.01
	120	1.89 ± 0.21 ab	1.39 ± 0.15 b	1.36 ± 0.14 a	1.05 ± 0.09 bc	0.32 ± 0.03 ab	≤0.01
200	20	1.91 ± 0.20 ab	1.38 ± 0.14 b	1.38 ± 0.15 a	1.06 ± 0.09 bc	0.32 ± 0.03 ab	≤0.01
	60	1.94 ± 0.21 ab	1.41 ± 0.15 b	1.38 ± 0.14 a	1.08 ± 0.10 bc	0.32 ± 0.03 ab	≤0.01
	120	1.98 ± 0.19 ab	1.44 ± 0.16 ab	1.37 ± 0.14 a	1.10 ± 0.11 bc	0.32 ± 0.03 ab	≤0.01
250	20	2.04 ± 0.20 ab	1.48 ± 0.15 ab	1.38 ± 0.14 a	1.12 ± 0.11 bc	0.32 ± 0.03 ab	≤0.01
	60	2.11 ± 0.21 ab	1.54 ± 0.17 ab	1.37 ± 0.14 a	1.17 ± 0.12 bc	0.32 ± 0.03 ab	≤0.01
	120	2.18 ± 0.22 ab	1.57 ± 0.16 ab	1.39 ± 0.13 a	1.22 ± 0.12 bc	0.32 ± 0.03 ab	≤0.01
300	20	2.22 ± 0.23 ab	1.64 ± 0.17 ab	1.35 ± 0.14 a	1.30 ± 0.13 b	0.33 ± 0.03 ab	≤0.01
	60	2.27 ± 0.24 ab	1.65 ± 0.16 ab	1.37 ± 0.14 a	1.31 ± 0.13 b	0.33 ± 0.03 ab	≤0.01
	120	2.29 ± 0.23 ab	1.64 ± 0.17 ab	1.39 ± 0.14 a	1.32 ± 0.13 b	0.33 ± 0.03 ab	≤0.01
350	20	2.42 ± 0.23 a	1.71 ± 0.18 a	1.40 ± 0.14 a	1.50 ± 0.14 ab	0.36 ± 0.04 a	≤0.001
	60	2.66 ± 0.24 a	1.87 ± 0.19 a	1.42 ± 0.14 a	1.64 ± 0.15 ab	0.36 ± 0.04 a	≤0.001
	120	2.84 ± 0.25 a	1.97 ± 0.20 a	1.44 ± 0.15 a	1.84 ± 0.17 a	0.38 ± 0.04 a	≤0.001
Anova <i>p</i> -value		≤0.001	≤0.001		≤0.001		

Data represent the mean values ± standard error. Means followed by the same lowercase letters in a column are not significantly different on LSD test ($p \leq 0.05$).

3.6. Catalase and Peroxidase Activity

Catalase activity increased by 0.53-fold (48,090 U/mg Prot), 1.46-fold (77,091 U/mg Prot), and 2.63-fold (113,789 U/mg Prot) in seedlings from seeds exposed to 350 mT treatment for 20, 60, and 120 min, respectively, compared with the control (31,316 U/mg Prot; Figure 4).

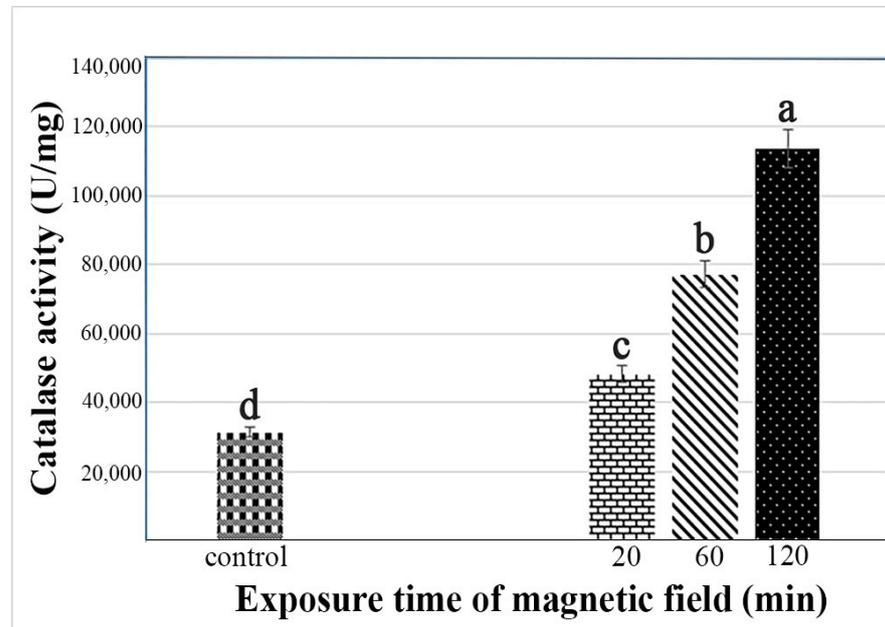


Figure 4. Catalase activity in flax seedlings derived from seeds treated SMF with 350 mT for 20, 60, or 120 min compared to control. Values followed by different letters are significantly different at $p \leq 0.05$ on Fisher's least significant difference (LSD) test.

In contrast to catalase activity, POD activities were generally lower in seedlings developed from SMF-treated seeds. The shortest 350 mT treatment of 20 min (6069 U/mg prot) resulted in no significant POD change compared with the control (5964 U/mg prot), but 60 min (5171 U/mg prot) and 120 min treatments (3314 U/mg prot) caused a significant reduction of 0.13-fold and 0.44-fold of the control, respectively (Figure 5).

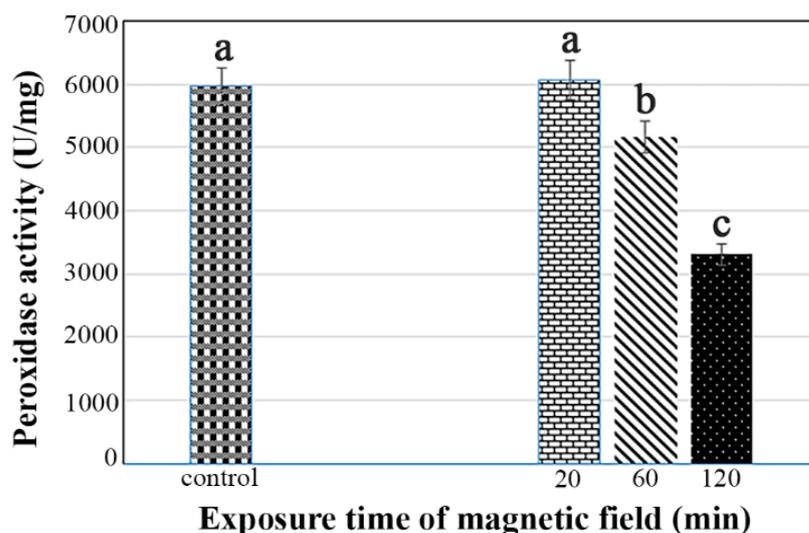


Figure 5. Peroxidase activity in flax seedlings derived from seeds treated SMF with 350 mT for 20, 60, or 120 min compared to control. Values followed by different letters are significantly different at $p \leq 0.05$ on Fisher's least significant difference (LSD) test.

4. Discussion

Physiological seed quality characteristics included germination and vigor. It is known that seeds with low germination due to unfavorable storage conditions produce weaker seedlings with reduced yield potential [42]. The non-residual and non-toxic stimulating effect of SMF on seed germination has also been described by numerous researchers [43]. Hence, the strength and duration of SMF must be optimized for each species [1,2,20]. It is known that seeds exposed to SMF absorb more moisture [44] and improve germination and seedling vigor in some plant species, such as rice [45], barley [46], and wheat [47], including seeds with low viability [48]. The present results on flax seed are consistent with germination data of wheat [3], barley [5], and sunflower seeds exposed to SMF [6,46].

Our finding that SMF treatments of 150 mT (120 min), 200 mT (80–120 mT), 250 mT (60–120 min), 300 mT (40–120 min), and 350 mT (40–120 min) provoked the highest enhancement of flax seeds germination and seedling vigor are in agreement with results on SMF-treated barley seeds [8]. Flax seeds responded to SMF more efficiently than barley seeds. The flax seeds exposed to 300 mT SMF improved their germination percentage threefold after 10 days, while the optimal SMF treatment of barley seeds brought only a 15% increase compared to the control [8]. Flax seed vigor tested by the ISTA [36] standard showed high potential for seed germination in a wide range of environments. Flax seed vigor described several characteristics of seed, such as rate and uniformity of seed germination and seedling growth, emergence ability of seeds under unfavorable environmental conditions, and performance after storage, particularly retention of the ability to germinate [36].

In addition, flax seeds under 300 mT SMF showed rapid and uniform germination, which is desirable for producing quality seeds. In many cases, SMF treatment allowed synchronization of germination of seed lots with different germination abilities. As such, SMF treatment gives a better chance of that seed withstanding competition from weeds and being resistant to pathogens and pests [44]. The better germination rate of SMF-treated flax seed indicates that oxidative stress was reduced and antioxidant mechanisms were enhanced, which Kumar et al. [45] showed on maize.

In the present study, the reduced electrolyte leakage of flax seeds showed a positive effect of SMF treatment on membrane stability. This is in agreement with Vashist and Nagarajan [46] and Poinapen et al. [12] who observed reduced electrolyte leakage rate and consequently increased membrane stability in SMF-treated seeds of maize and tomato. Increased electrolyte leakage is an indicator of oxidative damage to membrane lipids and macromolecules such as proteins, DNA, and RNA [46].

Our results showed that SMF treatments improved assimilatory pigment content (chlorophyll *a* and *b* and carotenoids) in flax, which is in correlation with results in other crop species, such as barley [5], soybean [47], maize [48], and wheat [49]. SMFs in the range of 10–100 mT and exposures for 30–360 min significantly increased photosynthetic pigments (chlorophyll, carotenoids).

The higher chlorophyll content is most likely the result of an increase in leaf thickness and palisade parenchyma, leading to a greater photosynthetic capacity and thus a better physiological state and faster growth of the whole plant [50]. Chlorophyll and carotenoid concentrations and their ratios are clear and sensitive indicators of stress recognition and tolerance [51].

Carotenoids can protect the photosynthetic machinery from excess light by inhibiting ROS [52]. Moreover, as antioxidants, carotenoids have specific physiological activities: they scavenge various ROS, prevent lipid peroxidation, and ultimately alleviate oxidative stress [53]. Only one study was found that looked at gene expression analyses to uncover the molecular mechanism of how electric currents can increase carotenoids in tomatoes [54].

In chloroplasts, H₂O₂ is formed in the Mehler reaction and also in the superoxide dismutase-catalyzed disproportionation of the superoxide radical anion [55]. Nevertheless, under non-stressful environmental conditions, plant cells mainly produce ROS in chloroplasts because they also play an important role in cell signaling. They are produced by the

direct delivery of excitation energy or electrons to oxygen from the photosynthetic electron transport chain [55].

In our study, the SMF treatment with 350 mT increased about 2.63-fold the catalase activity in the flax seedlings, which contributes to the protection against the harmful effect of ROS. The present results showed that SMF treatment caused increased catalase activity but decreased peroxidase activity, which is consistent with the results of Payez et al. [56].

Higher catalase activity in flax seedlings correlated with rapid germination and early seedling vigor. Also, Bhardwaj et al. [13] found that CAT activities had a 0.83-fold increase in cucumber seeds exposed to SMF compared to the control. SMF increased the relative expression of the CAT and Fe transporter gene, which resulted in an increase in iron content in the plants compared with the control. Moreover, greater expression of the ferritin gene, which is involved in protection against oxidative stress and catalase as the main scavenger of H₂O₂, was shown. SMF influence on gene expression depended on its intensity and application time. Also, a higher expression of the α -amylase gene under the influence of SMFs resulted in increased seed germination and vigor of seedlings [56].

SMFs' intensity of 6 mT showed a high impact on cell shape and the structure of the cell membrane, thus increasing their permeability. Root meristem cells showed significant changes in the density and size of the root meristem cells, ultrastructural changes in the organization of some organelles, and a decrease in the volume of the granular component of the nucleus [57].

SMFs increase the content of auxins and the activity of enzymes that regulate the elongation of the plant cell wall. They lead to an increase in CAT and POD, the stimulation of ROS, and changes in the activity of amylase and nitrate reductase in seeds [58].

Also, SMF affects the membranes and Ca²⁺ signaling in plant cells, and many magnetic effects in living organisms are probably due to the alterations in membrane-associated Ca²⁺ flux. Na channels are less affected than Ca²⁺ channels due to the changes in Ca²⁺ channels reduced in SMF-treated plants. SMF treatment in seeds induces changes in their protein and lipid profiles [59].

The results indicated that SMF treatment significantly improved the physiological flax seed quality of flax seed stored in inadequate conditions.

5. Conclusions

SMF treatments of 150 mT (120 min), 200 mT (80–120 min), 250 mT (60–120 min), 300 mT (40–120 min), and 350 mT (40–120 min) can be a tool for overcoming problems of low seed germination of seeds stored under unfavorable humid conditions.

The increase in intensity and the extension of SMF treatment duration helped improve seed vitality, enhancing seedling stress resistance and photosynthetic capacity.

Flax seed vitality was restored with 350 mT SMF treatment for 100 min and 120 min and had the best effect on seedling yield, quality, and seedling production.

Author Contributions: D.Ć. and J.L.R.-D., conceptualization, methodology, validation, and supervision; D.Ć., J.L.R.-D., S.Ć., J.M., M.B., M.S. and S.Z.-K., investigation; J.M., M.B., D.Ć., M.S. and S.Z.-K., formal analysis and analysis of data; D.Ć., writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant number 451-03-47/2023-01/200007. The APC was funded by the University of Belgrade, Institute for Biological Research “Siniša Stanković”—National Institute of the Republic of Serbia.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data will be made available on request.

Conflicts of Interest: The authors declare no conflict of interest.

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