

## Article

# Effect of Pre-Sowing Magnetic Field Treatment on Enzymes and Phytohormones in Pea (*Pisum sativum* L.) Seeds and Seedlings

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**Abstract:** The aim of the presented studies was to evaluate the magnetic field (MF) effect on changes in some enzymes and phytohormones that takes place in the process of seed germination and growth of seedlings. Studies were led in the climatic chambers HERAEUS, on Petri dishes during six consecutive days. Pea seeds were divided into three groups from which one was the control (without stimulation) and two were treated with different doses of magnetic field (30 and 85 mT, respectively). Contents of amylolytic enzymes (AE) and phytohormones were determined at seven terms (0; 24; 48; 72; 96; 120 and 144 h) after placing them on the dishes. A favorable effect of seed stimulation with MF was found on the biochemical processes in the germinating seeds and pea seedlings. The size of changes in enzyme concentration was dependent mainly on the advancement of germination process (i.e., on length of time in which the seeds were on the dishes) and the MF treatment. The use of MF also had an effect on the increase in hormone content in the seeds and organs of seedlings, but values from objects treated with different doses did not differ significantly.

**Keywords:** pea; magnetic field; stimulation of seeds; enzyme activity; phytohormones; trans-zeatin; trans-zeatin-riboside



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## 1. Introduction

One of the most important factors determining the plant yield is the seed material quality, which determines the emergence course, and density of plants. Seedlings grown from high-quality seeds are characterized by greater vigor and resistance in unfavorable factors of environment, especially with water shortage in soil and infection by pathogens [1]. However, the course of germination and seedling growth can be improved by pre-sowing seed preparation [2]. The most effective and the most common methods used in practice are chemical, which are related to seed treatment with preparations containing various active substances, sometimes hazardous to the environment [3,4]. Many of them can penetrate inside the seeds, modify their chemical composition, and also pollute the soil environment. Therefore, other methods of pre-sowing seed preparation are increasingly being sought for use in agricultural practice such as physical factors like, among others, stimulation of seeds with laser light [5–8] or magnetic field (MF) [9–11]. These factors are considered as safe for the environment and plants because an effect of their use is that seeds are stimulated to germinate, which favors better dynamic of emergence and good growth of seedlings. For this reason, this kind of crop's seed treatment has become very popular in the agricultural sector. For instance, pre-sowing treatment of seeds with a magnetic field is a non-destructive and dry seed priming treatment that has been reported to increase the germination rate and vigor of seedlings of many crops [12–16]. MF also promoted the germination ratios of bean seeds (*Phaseolus* sp.), and plants grown from these seeds had a

faster dynamic of growth than the control [17]. The influence of MF treatment in field pea has also proved favorable on the emergence, growth, development, and seed yield [18]. A pre-sowing MF treatment of chickpea seeds (*Cicer arietinum*) enhanced their germination and early growth characteristics [19]. The same effect was observed in beans (*Phaseolus* sp.), wheat (*Triticum aestivum* L.) [17], soybean (*Glycine max* L.) [12], and maize (*Zea mays* L.) [20].

In the literature, there is little information concerning the explanation of MF impact on the changes in seeds, especially with regard to phytohormones (auxins, gibberellins and cytokinins), which affect the growth and development of plants. Observations of these changes may considerably improve the state of knowledge and contribute to the partial explanation of the mechanism of the magnetic field effect on seed germination and further development of plants. As is widely known you know plant hormones play an important role in breaking of seed dormancy and in the initiation of their germination. According to Jankiewicz [20], indolyl-3-acetic acid (IAA) stimulates both  $\alpha$ -amylase activity and the starch decomposition into simple sugars. In pea (*Pisum sativum*), auxin is necessary to maintain proper gibberellic acid ( $GA_1$ ) concentration in elongating internodes [21]. While GA regulates the metabolism of seed storage compounds, gibberellins have a significant influence on the whole plant ontogenesis [22].

In the presented paper are shown the results of research with pea, whose cultivation in Poland is characterized by rather low and variable seed yield over the years. Therefore, farmers are not interested in cultivating this valuable crop. Our previous research with pea shows that the germination decides the further growth of plants [23]. An important and new element of these studies is the determination of cytokinin (i.e., trans-zeatin and trans-zeatin riboside) concentration, which as a group of growth regulators are especially necessary in the early stage of plant growth and development [24].

The aim of this study was to determine the effect of pre-sowing seed treatment with magnetic field (MF) on some AE activity and phytohormone content in seeds and seedlings of pea and what impact it has on the course of germination and further growth of plants.

## 2. Materials and Methods

### Experimental Conditions

The experiment was carried out at the Institute of Soil Science and Plant Cultivation—State Research Institute in Puławy [51°24′59″ N, 21°58′09″ E], Poland. The study was performed on Petri dishes placed in climatic chambers (HERAEUS Vötsch, HPS 2000 type, Hanau, Germany). Each plate (diameter 150 mm) lined with filter paper had 80 seeds of pea Piast cultivar. The germination capacity of seeds was 92%, thousand seed weight amounted to 624 g, and humidity was 14%. During the whole experiment (144 h) the paper in each Petri dish was sprayed with distilled water. In this period, we used 170 mL of water. Air humidity in the climatic chamber was 90%, air temperature was 24 °C, and light intensity was 10,000 lx.

### 3. Experimental Factors

The experimental factors were three doses of MF intensity: D<sub>0</sub>-no stimulation (control), D<sub>1</sub>-30 mT, t = 15 s, and D<sub>2</sub>-85 mT, t = 15 s. Magnetic stimulation of seeds was performed at the Department of Physics, Life Science University in Lublin, using a device for the pre-sowing treatment of seeds with a MF, which was equipped with an electromagnet powered at 50 Hz with a smooth regulation of magnetic induction [25].

#### 3.1. Amylolytic Enzymes Determination

The seeds previously subjected to magnetic stimulation were placed on Petri dishes and then analyzed in the following hours after MF stimulation: 0, 24, 48, 72, 96, 120, and 144. Enzyme activity was determined based on the amount of glucose released from starch by the complex of enzymes contained in the supernatant. This supernatant was obtained by the addition of 5 mL of acetate buffer with pH 4.8, containing 20  $\mu$ mol CaCl<sub>2</sub> to 0.5 g of dried plant material and then centrifuged within 10 min at 3000 rpm. The

amount of 0.5 mL starch solution with a concentration of 0.5% was added to 0.5 mL of the supernatant and hydrolyzed at 37 °C for 10 min. The glucose content was determined by the modified Somogy–Nelson method [26], which is most often used in biochemical tests for determining the content of reducing sugars. This method uses the color reduction of the arsenic-molybdenum reagent with cuprous ions formed in the solution as a result of the reaction of the copper reagent with reducing sugars. The intensity of the color was measured by a colorimeter at the wavelength  $\lambda = 520$  nm, while the content of reducing sugars was determined on the base of a calibration curve made for glucose solutions.

### 3.2. IAA and GA<sub>3</sub> Determination

IAA content was determined by HPLC and the modified Hardin and Stutte method [27] while the content of GA<sub>3</sub> was determined by the high-pressure HPLC (high-performance liquid chromatography) method [28,29]. The analytical procedure is described in detail by Podleśna et al. [30] and Podleśny [31].

### 3.3. Trans-Zeatin and Trans-Zeatin-Riboside Determination

A total of 25 g of plant material (aboveground part and roots) was fragmented in a mortar before pouring 30 mL of methanol and mixing to obtain a homogeneous mass. The whole material was filtered by a filter funnel with sinter and the obtained amount of filtrate was filled up to 50 mL. Two 10 mL portions were collected from this solution, and to the second portion was added known amounts of trans-zeatin and trans-zeatin-riboside. Ten mL of solution was evaporated on a vacuum evaporator to a volume of 0.1–0.2 mL, and to this liquid was added 0.6 mL of water and 1 mL 1M Na<sub>2</sub>HPO<sub>4</sub>. Next, everything was mixed and in the next step, 3 mL of butanol and ethyl acetate (1:1) mixture was added. Everything was mixed again, transferred to 5 mL tubes and shaken. After centrifugation, 3 mL of solvent was collected and evaporated in vacuum to dryness, then to it was added 1.5 mL of water with methanol (1:1). Next, 30 mL of solution was fed to the chromatograph dispenser. Composition of the mobile phase was as follows: 865 mL of water, 5.1 g of sodium citrate, 1.82 g citric acid, 0.5 mL of acetic acid, 0.15 mL of triethyl amine, 35 mL of methanol, and 100 mL of acetonitrile. Detection was at 254 nm, column NUKLEOSIL C 18 AB 300 mm, and flow 1 mL/min. Values of trans-zeatin and trans-zeatin-riboside concentration were given at ng/g of plant mass.

### 3.4. Measurement of Pea Seedlings Organs

Measurement of the length of pea stems and roots was made with an electronic caliper (electronic digital caliper). Dry matter content of seedlings was determined by the gravimetric method [32] after drying of plant material at 105 °C.

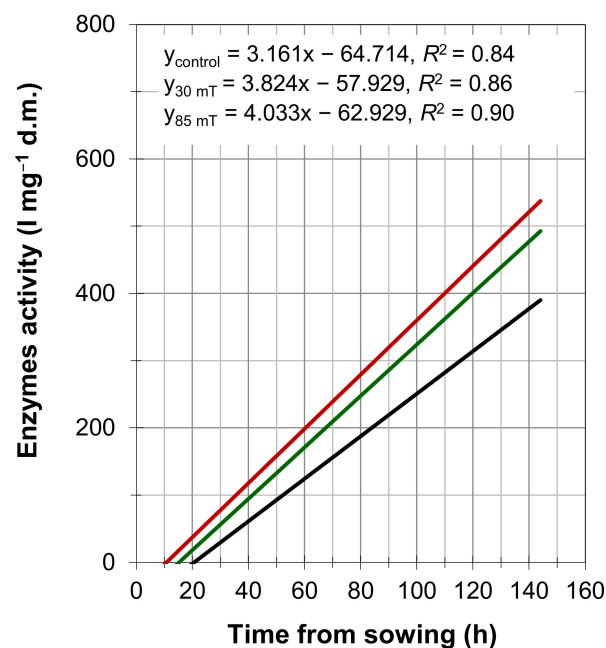
### 3.5. Statistics

Data were processed statistically in the Statistica v.13.1 application. The statistical calculations were undertaken using one-way ANOVA. Significance of differences among the averages were evaluated using Tukey's HSD test ( $p \leq 0.05$ ). To showing the course of AE activity and changes in dry matter of seedlings after the magnetic stimulation of seeds, linear regression was used linear regression. Results are the means from four replications.

## 4. Results

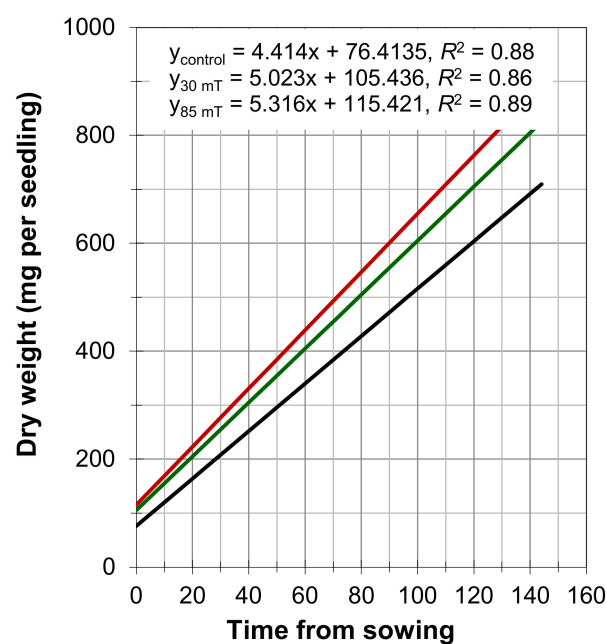
Treating the seeds with MF caused the increase in AE activity in seeds and seedlings of pea. The changes in the activity of enzymes under the influence of MF took place in the whole period after the seeds were laid out in the Petri dishes (Figure 1) reaching the highest value after 144 h from sowing. The greatest variation in enzymatic activity between the control and stimulated seeds was found 96 h after sowing. Both doses of MF induction caused a similar effect, although the higher dose (85 mT) had a slightly stronger effect on the germination rate of pea seeds than the lower one (30 mT). This increase, presented as the mean for both doses of stimulation, was as follows: amounted: 24.0, 30.0, 46.2, 37.5,

25.5, and 14.4% respectively for 24, 48, 72, 96, 120 and 144 h, respectively, after the sowing of seeds on the Petri dishes.



**Figure 1.** The course of changes in the activity of amylolytic enzymes in pea seeds and seedlings treated and not treated magnetic field (MF). Untreated control—black line, 30 mT—green line, 85 mT—red line.

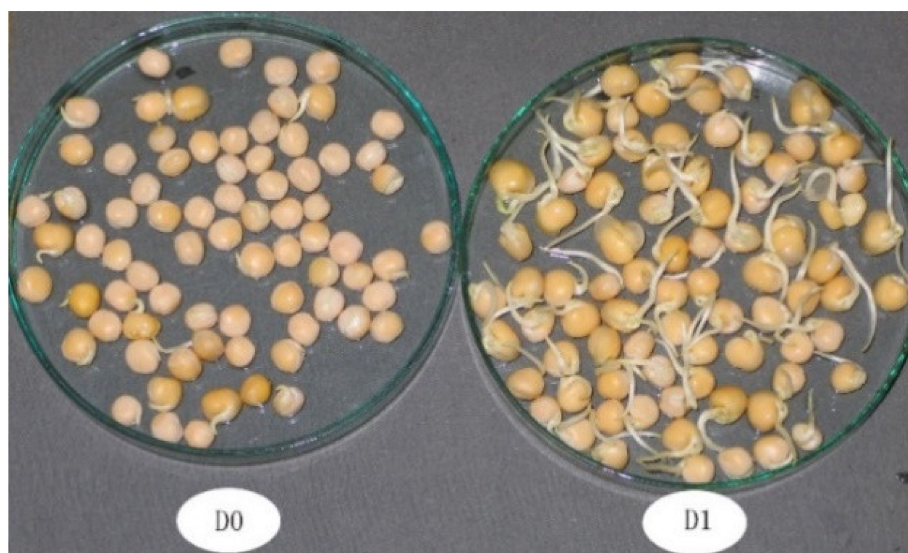
Pre-sowing stimulation of seeds with MF significantly affected the rate of water absorption and the mass of swelling seeds (Figure 2). Seeds previously treated with a MF significantly increased their mass compared to untreated seeds.



**Figure 2.** The course of changes in dry weight of seeds and seedlings of pea depending on the dose of the MF. Untreated control—black line, 30 mT—green line, 85 mT—red line.

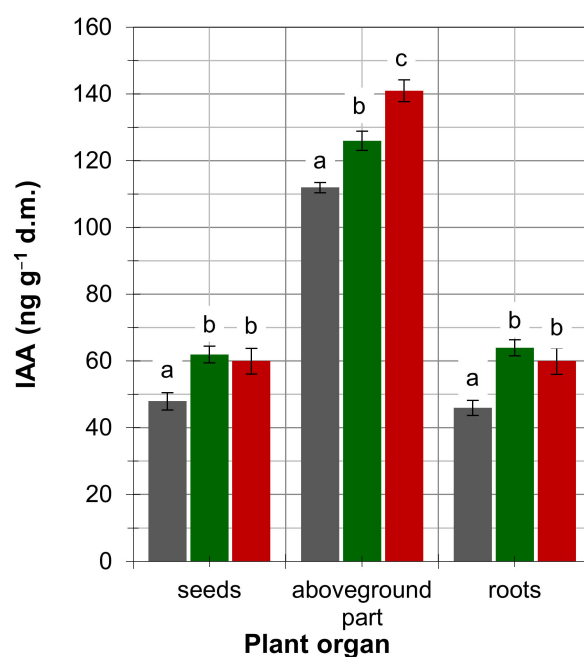
The average mass of one swelling control seed and a seedling during the period from sowing to germination was 0.44 g, while the average mass of a seed previously treated

with MF was 0.58 g for both its doses. Pre-sowing treatment of seeds with MF caused a faster increase in the mass of the swelling pea seeds mass depending on the MF dose were significant (Figure 2). The lower dose (30 mT) of field induction caused the increase in the swelling pea seeds' mass by 20.5%, and the higher dose (85 mT) was by 23.1 % compared with untreated seeds. This, of course, had consequences on their faster germination (Figure 3).

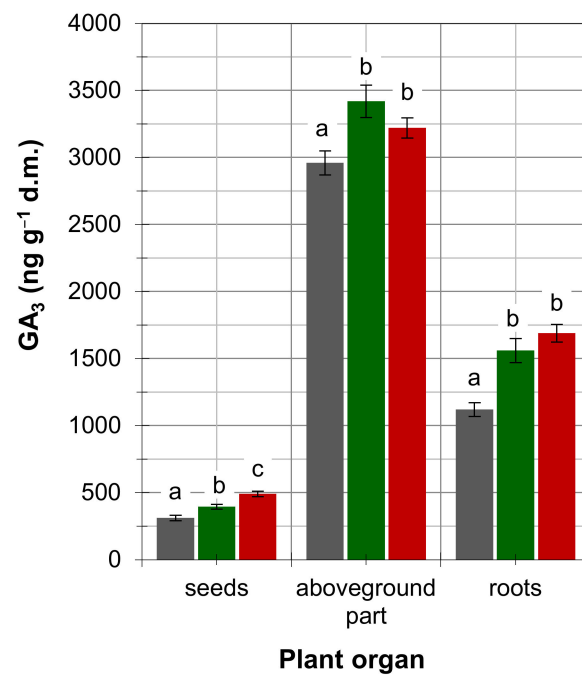


**Figure 3.** Germination of pea seeds treated with MF and control seeds (three days after sowing); D0—control seedlings, D1—treated seedlings.

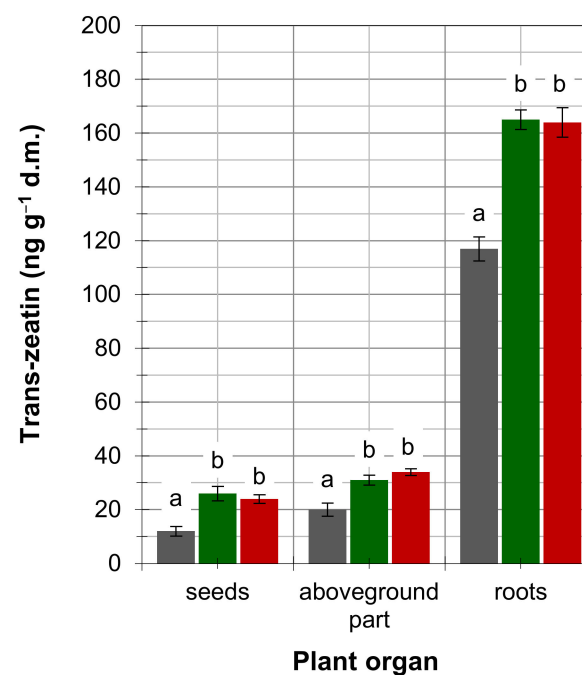
Treating the seeds with MF considerably increased the amount of IAA, GA<sub>3</sub>, trans-zeatin, and trans-riboside-zeatin during germination as well as in the aboveground parts and roots of young seedlings of pea in comparison to the control (untreated seeds and plants) (Figures 4–7).



**Figure 4.** The content of IAA in pea organs depending on the dose of MF. Untreated control—black bars, 30 mT—green bars, 85 mT—red bars. Values that are marked with different letters within the same plant organ differed significantly at  $p \leq 0.05$ . Means  $\pm$  SE.

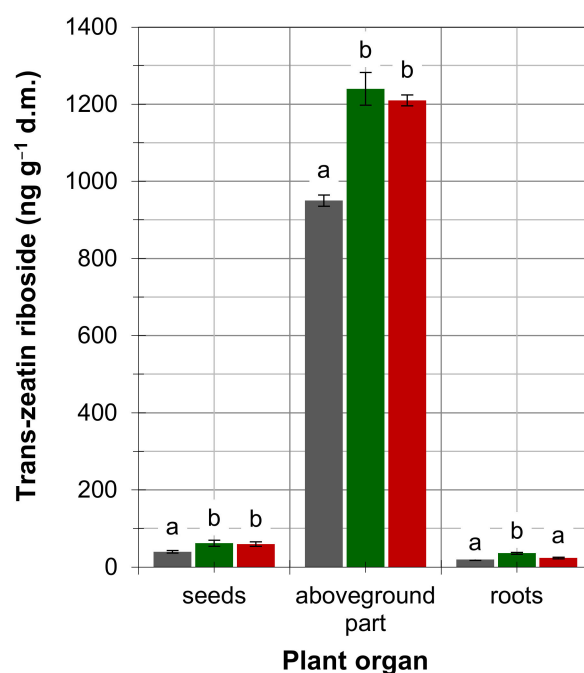


**Figure 5.** The content of GA<sub>3</sub> in the pea organs depending on the dose of the MF. Untreated control—black bars, 30 mT—green bars, 85 mT—red bars. Values that are marked with different letters within the same plant organ differed significantly at  $p \leq 0.05$ . Means  $\pm$  SE.



**Figure 6.** The content of trans-zeatin in pea organs depending on the dose of the MF. Untreated control—black bars, 30 mT—green bars, 85 mT—red bars. Values that are marked with different letters within the same pea organ differed significantly at  $p \leq 0.05$ . Means  $\pm$  SE.



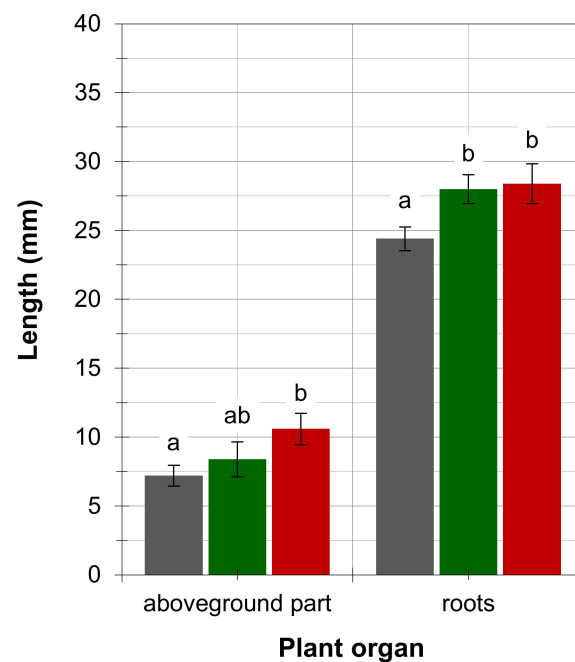


**Figure 7.** The content of trans-zeatin-riboside in pea organs depending on the dose of the MF. Untreated control—black bars, 30 mT—green bars, 85 mT—red bars. Values that are marked with different letters within the same pea organ differed significantly at  $p \leq 0.05$ . Means  $\pm$  SE. The application of two various doses of MF (D1 = 30 mT and D2 = 85 mT) did not significantly differentiate (on the whole) the content of IAA, GA<sub>3</sub>, trans-zeatin-riboside and trans-zeatin in the seeds, aboveground parts, and roots of the pea plant. A significant effect of MF dose was found in the case of IAA in the aboveground part and GA<sub>3</sub> in the pea seeds. The highest differences in the content of these phytohormones in tested parts of plants occurred between the objects with use of seed biostimulation and control.

Both doses of used MF increased the level of IAA in seeds, aboveground parts, and roots, but statistically significant differences between doses were found only for the aboveground parts (Figure 3) whereas higher GA<sub>3</sub> content in the aboveground parts was found in seedlings grown from seeds treated with a lower dose of MF (30 mT) and in the seeds and roots as the result of the impact of its higher dose (85 mT) (Figure 4). Differences between used MF doses were statistically significant only for seeds.

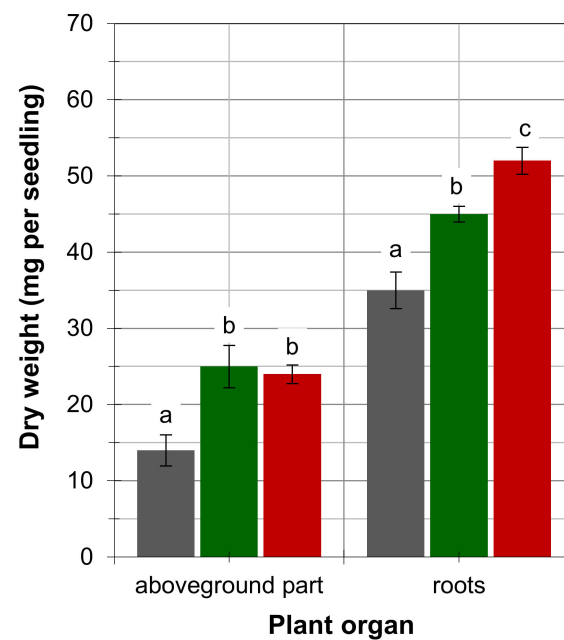
Irrespective of the MF treatment, the highest content of IAA, GA<sub>3</sub>, and trans-zeatin-riboside (Figure 6) was found in the aboveground parts of young pea plants and trans-zeatin in their roots (Figure 5). The differences between the concentration of trans-zeatin and trans-zeatin-riboside in the effect of seed stimulation with the MF doses were not statistically significant.

Although both MF doses significantly influenced the increase in the length of roots and stems, better results were obtained by treating seeds with a higher dose (Figure 8). On average, both MF doses caused an increase in the length of the hypocotyl and roots of pea seedlings by 31.9 and 15.6%, respectively.



**Figure 8.** The length of stem and roots of pea plant in dependency on the dose of MF measured 144 h after sowing. Untreated control—black bars, 30 mT—green bars, 85 mT—red bars. Values that are marked with different letters within the same pea organ differ significantly at  $p \leq 0.05$ . Means  $\pm$  SE.

Stimulation of seeds with MF affected the initial growth and further development of seedlings, which was observed as an increase in their roots and stem mass (Figure 9). Six days after sowing seeds on the dishes, it was found that both MF doses caused an average increase in the roots and stem weight by 14.6 and 28.5%, respectively, in relation to seedlings from the control object.



**Figure 9.** The dry matter of pea plant organs in dependency on the dose of MF measured 144 h after sowing. Untreated control—black bars, 30 mT—green bars, 85 mT—red bars. Values that are marked with different letters within the same pea organ differed significantly at  $p \leq 0.05$ . Means  $\pm$  SE.



## 5. Discussion

The most well-known MF in the world is the Earth's magnetic field. Studies on its effect on crops were conducted in the last century in Canada [32]. Pittman [32] showed that in spite of the low value of Earth's magnetic field induction, there existed a close dependency of the cereal germination dynamic on the direction of seed positioning relative to the lines of the magnetic field energy. Seeds positioned with a longer axis of symmetry along the line of this field energy germinated earlier and more uniform than seeds arranged perpendicularly. Values of MF induction used in our studies were several hundred times bigger than the Earth's MF, with value amounts for Poland of about 0.05 mT.

The presented studies show that pre-sowing treatment with MF had a significant effect on the increase in amylolytic enzyme activity in pea seeds. As a consequence, we observed changes in germination dynamics and in the growth of pea seedlings like in the studies with faba bean [30]. MF caused an increase in the content of IAA, GA<sub>3</sub>, trans-zeatin, and trans-zeatin-riboside in germinating seeds as well as in the aboveground parts and roots of young seedlings. According to Galland and Pazur [33], MF affects not only chemical properties in the plant, but also changes in various physical properties of solutes inside the plant cell such as cytoplasm and outside it like the growth medium and the water to irrigation [33]. Moreover, some scientists have indicated that MF had significant effects on seed germination, growth, yield, enzyme activity, water relations, and chemical components of some plants [34–37]. Iqbal et al. [38] reported that MF caused a significant increase in the germination rate of pea seeds.

Radhakrishnan and Kumari [36] examined the effect of the stimulation of soybean with magnetic field compared to the control and showed an increase in plant morphology elements as well as the fresh and dry mass of plants. Carbonell et al. [39] also proved that pre-sowing stimulation with MF affected the morphological features of seedlings. Podleśny and Sowiński [40] and Podleśny and Pietruszewski [41] showed an increase in the dry matter yield of stems, seeds, and roots as well as an increase in pea seed yield under the influence of MF. Selim et al. [42] recorded that the application of magnetic treatments in wheat plants resulted in a significant increase in grain number and weight; grain and straw yield, 1000 grains weight, and harvest index compared with the control.

Our studies showed an increase in enzyme activity in pea seeds as an effect of this treatment. Other authors [43] have also found an increase in enzyme activity of  $\beta$ -amylase, acid phosphatase, polyphenol oxidase, and catalase, with a simultaneous decrease in the content of  $\alpha$ -amylase, alkaline phosphatase, and protease after MF stimulation.

According to Dhawi [44], enzymatic activity and other biochemical reactions occurring in seeds under the influence of MF may be affected by an increase in the number of hydrogen bonds in the molecules as well as by an increase in cell permeability and active energy in cell electrolyte solutions, which affects physiological processes in plants, consequently accelerating seed germination. Furthermore, according to Atak et al. [45], MF affects cell reproduction and cellular metabolism. De Souza et al. [46] and Selim et al. [42] considered that the magnetic field promoted IAA, cytokinins, and GA syntheses, and this may bring about the promotion of cell division and plant enlargement. Studies by Selim et al. [43] showed that MF had a positive effect on growth promoters (IAA, GA, and cytokinins), while Davies [47] claims that plant phytohormones play important roles in all aspects of plant growth and development. However, it should be underlined that one of the new elements of our studies was the determination of cytokinin concentration in the seeds and seedlings. It was found that MF increased their content in stimulated seeds and young plants. This phenomenon is very important for all plants, especially in the case of legumes and their ability in the biological nitrogen fixation (BNF) process. According to Jarzyniak [25], cytokinins play the key role in the modulation of symbiotic interactions and regulation of roots and nodule formation. Therefore, an increase in the hormone content in young pea plants as an effect of MF stimulation is favorable for further nitrogen fixation as well as for its growth and yield.

## 6. Conclusions

The activity of amylolytic enzymes increased together with the experiment duration, reaching the highest value after 144 hours from the sowing of seeds. It also increased the effect of the stimulation of seeds with MF, wherein a higher dose had a greater effect on this activity than a lower one. Treatment of seeds with MF enlarged the content of tested phytohormones in the germinated seeds as well as in the aboveground parts and roots of pea seedlings. The significant effect of MF was found in the case of both doses and all tested parameters. Seed stimulation affected the growth of the stem and roots of pea seedlings. The higher dose of magnetic induction caused greater effects in relation to the length of pea stem, whereas no significant difference was found in the roots between the tested doses of MF. An effect of seed stimulation was also the increase in the mass of the pea seedlings. A greater dose of MF more favorably affected this feature than the lower one.

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