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Pulp or Potential? Magneto-Priming as a Seed Priming Technique Has Little Effect on Seedling Performance but Results in Significant Alteration of *trans*-Zeatin and *cis*-Zeatin in Soybean (*Glycine max*)

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Abstract: Magneto-priming (MP) has been reported as a sustainable method to enhance crop yield and resistance to adverse environmental conditions; however, any physiochemical basis for these findings remains unavailable. In the present study, soybean (*Glycine max*) seeds (n = 232) were exposed to a static magnetic field (SMF) of 150–205 mT for 60 min. The effect of SMF exposure on seedling mass, hypocotyl length, radicleemergence rate, total seedling height, leaf area, chlorophyl content, and physiological attributes was evaluated. Differential analysis of the cytokinin (CK) profile of seedling tissues was achieved using ultra-high-performance liquid chromatography coupled with electrospray-ionization high-resolution tandem mass spectrometry (UHPLC-(ESI)-HRMS/MS). Results indicate that MP seedlings achieved radicle emergence earlier; however, no other statistically significant differences could be established. The absence in significant improvement in the agronomic and physiological attributes measured is in stark contrast to the results reported in other studies. Nonetheless, significant differences were found in the concentrations of *trans*-Zeatin (tZ) and *cis*-Zeatin (cZ) between control and MP plants. These results are incongruent as it would be assumed that insignificant differences in agronomic and physiological properties would align with phytohormone profiles. Future work should be performed to determine if there are any meaningful bioeffects that can be assigned to MP-generated hormone changes.

Keywords: magneto-priming; static magnetic field; soybean; cytokinins; seedlings

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1. Introduction

Popular methods for enhancing crop productivity include nutrient fortification and the selection of high-yielding crop varieties (HYVs). Aside from the energy and environmental costs from producing them, mineral fertilizers are associated with eutrophication of aquatic ecosystems, soil acidification, diminished microorganism biodiversity, and soil nutrient imbalances [1–4]. Though necessary to accommodate the high demand for agricultural output, HYV monocultures reduce genetic diversity across crop fields, making them more susceptible to disease, in addition to requiring extra external input, such as chemical fertilizers, to sustain their rapid growth [1,2,5]. While their drawbacks present a negative connotation, crop enhancing methods are necessary to maintain the high agricultural productivity required to sustain the growing population. Hence, much research has been dedicated to developing sustainable alternatives, such as seed priming.

Seed priming refers to the broad constellation of methods used to invigorate seeds before they are planted and includes chemical priming (soaking seeds in organic or inorganic solutions), halo priming (soaking seeds in isotonic solutions), and hydropriming (soaking

seeds in hypertonic solutions). Collectively, these methods attempt to initiate biochemical and metabolic processes necessary for earlier germination [6]. Intended biochemical influences include enzyme activation, deoxyribonucleic acid (DNA) damage repair, and the enhanced breakdown of germination inhibitors [6]. These attributes are purported to produce rapid early growth, higher yields, and enhanced tolerance to environmental stressors such as temperature, drought, salinity, and ultraviolet (UV) light [6]. Though effective in vitro, success with various liquid seed priming techniques rarely translates to the field.

A subclass of seed priming, known as magneto-priming (MP), involves treating seeds with static or pulsed magnetic fields (SMF and PMF, respectively) before they are planted. MP has received considerable attention as a method for enhancing the germination and vegetative success of several economically important crops, including soybean (*G. max*), maize (*Z. mays*), wheat (*Triticum aestivum*), tomato (*Lycopersicon esculentum*), carrot (*Daucus carota*), rice (*O. sativa*), and chickpea (*Cicer arietinum*) [7–14]. MP has repeatedly been reported to enhance crop health including seedling vigor, yield, overall chlorophyl content, total biomass accumulation, growth uniformity, and photosynthetic performance [8,15–18]. During seed germination, seed pre-treatment with MP has been reported to reduce seed dormancy, accelerate germination, and increase overall seedling standing, albeit in controlled laboratory conditions [6,15,16,19,20]. However, the type and magnitude of effects observed in relation to MP remain inconsistent throughout the available literature, with some studies reporting null or even inhibitory effects [17,18,20].

MP is not analogous to other crop enhancement methods which constantly expose seedlings throughout their development. This is because the magnetic fields utilized for constant exposure are magnitudes lower than those typically used in MP investigations, making their ability to influence physiological and metabolic processes drastically different [7,20]. Furthermore, the plethora of other physiological processes that can be influenced during seedling development, such as electron transport in the thylakoid lumen, inhibits the extrapolation of outcomes from one technique to the next [20].

Attempts to elucidate the possible effects of MP on the embryonic cells of seeds are numerous. Importantly, no one hypothesis has prevailed within the scientific community, likely contributing to the wide variability in published outcomes due to differences in experimental methodology. Given the propensity for studies with null hypotheses to go unpublished, it is likely that the small numbers of studies available concerning MP represent a much larger collection of studies which would have inconclusive or negative results. These inconsistencies raise concern as to whether MP is a reliable technique; especially absent is any examination of phytochemical responses to treatment.

Due to their ubiquitous role in regulating fundamental processes in plant growth and development, the evaluation of phytohormone fluctuations in seedlings pre-treated with MP could provide a biochemical basis as to whether a significant bioeffect is achieved. As a subclass of phytohormones, cytokinins (CKs) are a diverse family of N^6-substituted adenine derivatives which influence plant development, including directing processes that control cell division, tissue differentiation, morphogenesis, and the activity and maintenance of the meristem [21–23]. CKs are also responsible for directing responses to abiotic stressors and exert considerable influence on the uptake and distribution of essential macronutrients [22,24].

The concentrations of CKs within a plant can provide indications of the physiological activity of the seedling and its yield potential [25]. Importantly, monitoring changes in their abundance in response to MP treatment could provide key insight into the biophysical mechanism of action of MP. In addition to being an economically important crop, the CK profile of soybean (*G. max*) has been extensively studied [26–29]. In soybean (*G. max*), *trans*-Zeatin (tZ) and isopentenyl adenine (iP) have been established as the dominant active forms responsible for carrying out a majority of the functions associated with CK activity including cell division, cell differentiation, seed set and filling, apical dominance, shoot initiation and growth, and inhibition of senescence [21,30–32].

It is the objective of this investigation to develop and use a strictly controlled set of MP conditions to test a suite of phenotypic, physiological, and biochemical changes to discern whether MP induces a significant bioeffect in treated soybean (*G. max*) seeds. Performance measures included seedling mass, hypocotyl length, radicle emergence rate, total seedling height, leaf area, and chlorophyl content, photosynthetic performance, transpiration, and conductance of seedling tissues. On a more sensitive level, CK profiles were analyzed using ultra-high-performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS/MS). This study represents the most thorough measurements of biochemical, physiological, and morphological attributes of a large number of seedlings grown in a rigidly controlled environment. It also represents the first comprehensive examination of the effects of MP on phytohormone dynamics in soybean (*G. max*) using UHPLC-HRMS/MS.

2. Materials and Methods

2.1. Plant Material

White hilum food-grade soybean (*G. max*) seeds (ACC MANDOR) were obtained from the Eastern Cereals and Oilseed Research Centre, Agriculture and Agrifood Canada (Government of Canada, Ottawa, ON, Canada). Germination tests performed on a previously obtained batch found seed germination was greater than 85%.

2.2. MP Parameters, Seed Selection, and Pre-Treatment

The electromagnet (EM) used to pre-treat the soybean (*G. max*) seeds was a dual-pole configuration (Ernst Turner Electrical Instruments LTD.; SN: 81833; Electromagnet type C; High Wycombe, UK). The diameter of each iron core was 3.70 cm with a pole gap of 5.30 cm. Copper coils had a width of 4.70 cm and a diameter of 4.95 cm (inclusive of the iron core). Conductor thickness was 0.5 mm (23 gauge), and each pole contained approximately 9300 turns. A recirculating chiller (RC006-RC045; Lytron Inc.; Woburn, MA, USA) provided cooling water (5 °C) through the internal channels of the EM to prevent overheating. An infrared (IR) thermometer was used to monitor EM coil surface temperatures, ensuring it did not exceed 25 °C. The maximum inter-pole magnetic field was 679 ± 13 mT at 2.5 A, excited using DC current, and verified with the use of a handheld gaussmeter (YFYIQI, Model: AMST-1). The magnet was pre-chilled for 30 min prior to conducting each experiment.

In the relevant literature, exposure to an SMF of 200 mT for 1 h was most effective in reducing germination time and increasing the rate of imbibition [7]. Studies investigating MP effects on soybean (*G. max*) have since continued to use this treatment duration and intensity [33–37]. To remain consistent with previous work, an exposure of 150–205 mT (±4) mT for 1 h was selected. However, due to the smaller size of the EM utilized, the decreased homogeneity of the inter-pole of magnetic flux density (MFD) was compensated for by treating a smaller number of seeds compared to other reports.

Three soybean (*G. max*) seeds were treated with MP at once, selected randomly. Seeds with visible defects or discoloration were rejected. Seeds weighing between 190 and 230 mg (Mettler Toledo© PB303-S analytical balance; Toledo, OH, USA) were used to prevent surface area bias. Size-exclusion criteria were not implemented for early vegetative growth experiments. Candidate seeds were placed in two clean glass cylinders (12 × 75 mm) in a bespoke 3D-printed sample holder (Figure S1). Control seeds were placed on a non-conductive surface outside the magnetic field range for the duration of the treatment.

2.3. Seedling Experiments

Circular pieces of blotting paper (Cat. No. 28298-020, VWR International, Radnor, PA, USA) were cut and added to the bottom of sterilized 9 cm petri dishes (90 × 1.5 mm, Fisher Scientific; Pittsburgh, PA, USA). Cotton was used to ensure that there was water exposure all around the seed to limit dehydration. An additional petri dish was prepared without seeds as a hydration control to check for water loss during the incubation period.

After MP treatment was completed, primed and control seeds were placed into their respective beakers containing 15 mL of 1% sodium hypochlorite. The seeds were left to soak for 3 min and subsequently rinsed with 10 mL of sterile deionized water and dried on paper towel for 5 min. Initial masses of seeds were recorded before being placed inside the petri dishes. A 2 mL measure of 5 μM sediment filtered water was dispensed onto each of the 12 cotton rolls per dish. Petri dishes were sealed using parafilm and placed in an environmental chamber (27 °C) protected from light. At six separate intervals (0.5, 1.5, 2, 24, 48, and 72 h), the dishes were opened and the seeds were removed, blotted, weighed, and inspected for radicle emergence.

At 72 h, seeds were placed vertically between two pieces of paper towel and rolled into a cylinder. A 5 mL measure of 30 μM filtered water was pipetted onto the paper towel over the seedling using a graduated cylinder and Pasteur pipette. The MP and control cohort of seedlings were rolled into their own cylinders using Cut-Rite® wax paper. The ends were folded and taped shut to ensure moisture was retained. After both the MP and control seedlings were wrapped in the wax paper, the rolls were hung inside a cardboard box in the environmental chamber with the radicle tip facing downwards. Every day, the seedling rolls were taken out of the wax paper and an additional 5 mL of 30 μM filtered water was pipetted onto the seedlings. At 4 and 8 days post-initiation, the seedlings were removed from the paper towel rolls and had their mass and length measured using an analytical balance and calipers, respectively. The germination percentage was calculated for seedlings that had reached a hypocotyl length of ≥1 cm by 3 days of growth using the equation below:

$$\text{Germination percentage} = \frac{\text{Number of seedlings with hypocotyls } \geq \text{ 1 cm on day 3 of growth}}{\text{Number of total seedlings}} \times 100 \quad (1)$$

2.4. Early Vegetative Growth Evaluation

MP and control seeds were planted in nursery trays (16 well, 125 cm^3) prepared with Miracle Grow Potting Mix® (0.21-0.11-0.16 NPK, 30% organic matter). Seeds were planted to uniform depth 0.75″ using a planting gauge. Seed sterilization was omitted in the early vegetative growth experiments as the natural microenvironment of the soil was maintained.

Nursery trays were maintained in an environmental chamber with supplemental lighting (Sunblaster T5HO 54W 6400K Light Reflector with NanoTech T5 Reflection, Langley, BC, Canada) with high full-spectrum photosynthetic flux density (PPFD) of 900 μmol s^{-1} m^{-2}, a 12/12 photoperiod, and steady temperature control at 27 °C and 60% RH, and watered daily with 15 mL of 30 μM filtered water per well. Each well was inspected daily, vegetative emergence (V_E) was considered achieved when the hypocotyl could be observed through the soil, and cotyledon emergence (V_C) was achieved when the cotyledon leaves were fully unfolded. Once over 80% of the MP and control seedlings had successfully reached V_C, the experiment was terminated. Both the MP and control trays were removed from the environmental chamber and the height, leaf length, and leaf width of the different seedlings were measured. The height was measured from the start of lateral root structures to the tip of the epicotyl, the leaf width was measured across the midpoint of the largest V_C leaf, and the leaf length was measured along the mid-rib. Leaf area was calculated by finding the product of the leaf width and the leaf length. The chlorophyl content of the leaves was analyzed using a SPAD meter, with the measurement being taken in the bottom proximal corner of the leaf. A seedling was considered to successfully germinate if it had reached the V_E developmental stage. The germination was calculated for seedlings that had reached a stem length of ≥7 cm at termination according to the following equation:

$$\text{Germination percentage} = \frac{\text{Number of seedlings reaching VE with a stem length } \geq \text{ 7 cm}}{\text{Number of total seedlings}} \times 100 \quad (2)$$

2.5. Physiology Measurements

Physiological performance was analyzed using the LI-COR® 6400 portable gas analyzer (LI-6400; LI-COR Biosciences Inc.; Lincoln, NE, USA). Parameters measured included photosynthetic rate (μmol CO_2 m^{-2} s^{-1}), transpiration rate (mmol H_2O m^{-2} s^{-1}), stomatal conductance rate (mol H_2O m^{-2} s^{-1}), and the intracellular CO_2 concentration (μmol CO_2 mol^{-1}). The method developed for the measurement of mature soybean (*G. max*) V_C leaves assumed stomatal density of 250 mm^{-2} and sampling area of 36 cm^2. The block temperature of the sensor head and light intensity (PPFD) were set to 27 °C and 900 μmol s^{-1} m^{-2}, respectively. The gas flow rate was static at 500 μmol s^{-1}, with a CO_2 reference target of 400 μmol mol^{-1}.

Three seedlings from the MP and control cohorts were randomly selected for physiology measurements from 5 separate trials. In each plant, the V_C leaf with the largest surface area was selected and if both leaves were the same size, then the one with the highest chlorophyl content was used. After the leaf was clamped, the chamber was left to equilibrate for 3 min before the measurement was recorded. Two measurements, approximately 1 min apart, were taken for each of the leaves and the values averaged. This process was repeated for all thirty of the seedlings analyzed. For all trials, the MP seedlings were measured first followed by the controls.

2.6. Tissue Harvesting

After physiological measurements were taken, tissue samples were harvested from mature V_C leaf tissue (~12 days post planting). Using clean dissecting instruments, a portion of tissue equidistant between the apex and base of the leaf was resected transversely, weighing 100 ± 10 mg. Tissue samples were quickly coiled and inserted into labeled 2 mL microfuge tubes (Eppendorf™; Hamburg, Germany) and preserved at -80 °C overnight, then freeze-dried for 24 h (Labcono Model: 7753020 at −56 °C and 0.28 mBar).

2.7. Phytohormone Extraction

A CK extraction protocol used to minimize enzymatic activity and prevent dephosphorylation of CK nucleotides was modified for soybean (*G. max*) leaf samples [38]. Briefly, two zirconium oxide (5 mm) grinding beads were placed into microfuge tubes containing the freeze-dried tissue along with 1 mL of ice-cold 50% ACN. Internal standards (10 μL CK master mix, 1 ng/μL) (see ^{2} H standards in Table 1) were added, and samples were homogenized by grinding using a ball mill (MM 301 Mixer Mill; Retsch GmbH, Haan, Germany) in a cold room (4 °C) for 5 min at 27 Hz, twice. Homogenized samples were vortexed briefly, then centrifuged (4 °C, 10 min, 11,180× *g*; Eppendorf 5417C, Mississauga, ON, Canada) before being placed into a chilling block (−20 °C). A 1 mL measure of the supernatant from each of the samples was transferred to clean 2 mL microfuge tubes. A 500 μL measure of ice-cold 50% ACN was added to tubes containing the tissue pellet, vortexed, and centrifuged again for 10 min. A 500 measure μL of the supernatant was transferred to tubes containing bulk supernatant from the previous step. An aliquot of 700 μL was transferred from the bulk supernatant to a new set of clean 2 mL tubes and dried overnight using a vacuum concentrator (Model SPD111V; Thermo Scientific, Ottawa, ON, Canada). Dry residues were stored at −20 °C until further processing could occur.

The residue was protonated by dissolving in 1 mL of 1M formic acid (FA) and centrifuged (Thermo Scientific, Sorvall ST16; 5 min, 4696× *g*). Solid-phase extraction (SPE) was performed using mixed cation exchange (MCX) cartridges (SPE MCX Violet 200 mg/6 mL 40 μM) (Oasis MCX, Mississauga, ON, Canada). Columns were equilibrated using 5 mL MeOH and 5 mL 1M FA before the sample was applied, followed by 5 mL 1M FA and 5 mL MeOH to wash the column. Nucleotides (NTs) and free bases (FBs) were eluted using 5 mL of 0.35M NH_4OH and 5 mL of 0.35M NH_4OH in 60% MeOH, respectively. FB and NT samples were dried overnight by vacuum and stored at −20 °C until further processing.

Table 1. Cytokinins (CKs) scanned for using liquid chromatography–positive electrospray ionization tandem mass spectrometry in soybean (*G. max*) cotyledon leaf samples.

Cytokinin (CK)	Labeled CK Standard
Nucleotides (mono-di-and tri-phosphates; CK-NTs)	$[^2H_3]$DHZRMP
trans-Zeatin nucleotide (tZNT)	
cis-Zeatin nucleotide (cZNT)	
Dihydrozeatin nucleotide (DHZNT)	
Isopentenyladenine nucleotide (iPNT)	$[^2H_6]$iPRMP
Ribosides (CK-RBs)	
trans-Zeatin riboside (tZR)	$[^2H_5]$ZR
cis-Zeatin riboside (cZR)	
Dihydrozeatin riboside (DHZR)	$[^2H_3]$DHZR
Isopentenyladenosine (iPR)	$[^2H_6]$iPR
Free bases (CK-FBs)	
trans-Zeatin (tZ)	$[^2H_5]$Z
cis-Zeatin (cZ)	
Dihydrozeatin (DHZ)	$[^2H_3]$DHZ
Isopentenyladenine (iP)	$[^2H_6]$iP
Glucosides (CK-GLUCs)	
trans-Zeatin-O-glucoside (tZOG)	$[^2H_5]$ZOG
cis-Zeatin-O-glucoside (cZOG)	
Dihydrozeatin-O-glucoside (DHZOG)	$[^2H_7]$DHZOG
trans-Zeatin-O-glucoside riboside (tZROG)	$[^2H_5]$ZROG
cis-Zeatin-O-glucoside riboside (cZROG)	
Dihydrozeatin-O-glucoside (DHZROG)	$[^2H_7]$DHZROG
trans-Zeatin-9-glucoside (tZ9G)	$[^2H_5]$Z9G
cis-Zeatin-9-glucoside (cZ9G)	
Dihydrozeatin-9-glucoside (DHZ9G)	$[^2H_5]$DHZ9G
Methylthiols (2MeS-CKs)	
2-Mehtylthio-Zeatin (2MeSZ) *	$[^2H_5]$2MeStZ
2-Methylthio-Zeatin riboside (2MeSZR) *	$[^2H_5]$2MeStZR
2-Methylthio-N^6-isopentenyladenine (2MeSiP) *	$[^2H_6]$2MeSiP
2-Methylthio-N^6-isopentenyladenosine (2MeSiPR) *	$[^2H_6]$2MeSiPR

* The analytical procedure used in this study does not have the capacity to separate 2MeStZ and 2MeStZR from the corresponding cis-isomers 2MeScZ and 2MeScZR. The total levels of methylthiolated Z-type CKs were reported as total 2MeSZ and total 2MeSZR.

NT residues were redissolved in 1 mL of 0.1M ethanolamine (pH 10.4) and vortexed. A 12 µL measure of calf-intestinal alkaline phosphatase enzyme mix (950 µL of 10x dilution rCutSmart with 50 µL QuickClip enzyme) (New England Biolabs Ltd., Whitby, ON, Canada) was added to each of the tubes to dephosphorylate the unstable NTs into ribosides (RBs). The tubes containing the phosphatase enzyme were incubated overnight at 37 °C in a water bath and subsequently dried by vacuum the following day.

The newly converted RBs were reconstituted in 1.5 mL of B-pure water, vortexed, and centrifuged (Thermo Scientific, Sorvall ST16; 5 min, 4696× *g*). SPE was performed using C18 cartridges (SPE C18 Violet 200 mg/6 mL 40 µM; Oasis MCX, Mississauga, ON, Canada). Columns were equilibrated using 3 mL MeOH and 6 mL B-pure water before sample injection. The columns were washed with 3 mL of B-pure water before eluting RBs using 1.25 mL of 80% MeOH. Collected fractions were dried overnight by vacuum and stored at −20 °C until they could be transferred to vials.

FB and RB residues were redissolved in 300 µL 0.08% acetic acid in 5% acetonitrile (ACN) (SC solvent). FB tubes were centrifuged (Thermo Scientific, Sorvall ST16; 5 min, 4696× *g*) before being transferred to 300 µL MS glass vials with attached 1000 µL inserts. The same process was followed for the 2 mL NT tubes. A 1.5 mL measure of SC solvent was aliquoted into 5 MS vials with no inserts, in addition to 1 vial being prepared with 990 µL of SC solvent and 10 µL of CK master mix.

2.8. UHPLC-(ESI)-HRMS/MS Analysis

CK concentrations were measured using high-performance liquid chromatography–electrospray ionization–tandem mass spectrometry (HPLC-(ESI)-HRMS/MS; QExactive Orbitrap mass spectrometer; ABI Sciex Concord, ON, Canada; Agilent 1100 series HPLC; Agilent, Mississauga, ON, Canada). A 25 µL aliquot was injected into a Luna C18 reversed-phase column (3 µm, 150 × 2.0 mm; Phenomenex, Torrance, ON, Canada). The conditions used for heated electrospray ionization (HESI) probe were as follows: capillary temperature, 350 °C; sheath gas, 30 arbitrary units; auxiliary gas, 8 arbitrary units; probe heater temperature, 450 °C; S-lens rf level, 60%; and capillary voltage, 3.9 kV. Bottle filters (0.2 µm, PVDF, Fisher Brand) were used to remove particulate matter from HPLC solvents. Ultrapure water (18.2 MΩ-cm) was obtained from a Milli-Q system for the preparation of the aqueous solvent (component A), and HPLC acetonitrile for the organic (component B). Both components were spiked with 0.08% acetic acid (CH_3CO_2H). The chromatographic method (0.5 mL min^{-1}) included initial conditions 5% A and 95% B for 0.5 min before changing linearly to 95% A and 5% B over 8 min. The ratio remained constant for 5 min before immediately returning to initial conditions for 18 min of equilibration.

Target analytes were detected using positive ionization mode at 35,000 resolution. CKs were identified based on their analyte-specific retention times, precursor mass, and MS^2 product ions. Concentrations were determined according to isotope dilution analysis upon direct comparison of the endogenous analyte peak area against the recovered internal standards [39]. Levels of the *cis* isomer of zeatin-type CKs were determined relative to the recovery of labeled tZ-types and retention time of unlabeled cZ standards.

2.9. Data Analysis and Statistics

Two-sample variance F-tests were conducted using Microsoft Excel®. Data were considered significantly different if $p < 0.05$. Seedling experiments were performed in 17 replicates with $n = 6$ samples per replicate. Samples where the growth was inhibited (length $\geq$ 1 cm by day 3 of growth) or infection was present were removed from further statistical analysis. Early vegetative growth experiments were performed in 20 replicates with $n = 16$ samples per replicate. Trials for which the average height for both the control and MP replicates remained below 7 cm were excluded from further statistical analysis. The experiments included for evaluation utilizing the LI-6400 gas analyzer were performed in triplicate for each of the five trials included. The three plants to be sampled from each of the trials were selected using a random number generator for the seedlings that had reached the V_C maturity stage. The cotyledon leaf selected was determined based on previous parameters from the early vegetative growth experiments, being the size of the leaf and its pigmentation. Duplicate measurements were taken for each of the V_C leaves analyzed and the values were averaged for further statistical analysis. For samples where duplicate measurements of photosynthetic rate scaled greater than 3 µmol CO_2 m^{-2} s^{-1}, only the highest value was taken on the assumption of human error in measurement of the duplicate. Experiments evaluating CK production by MP soybean (*G. max*) were performed in 5 biological replicates ($n = 3$ plants per replicate). The data were analyzed using Analyst (v 1.6.2) software (AB Sciex, Framingham, MA, USA), to calculate peak area. Quantification was achieved through isotope dilution analysis based on recovery of ^{2}H-labeled internal standards.

3. Results

3.1. Agronomic and Morphological Attributes

The day of radicle emergence was significantly decreased by 9.7% ($p = 0.03$) in the MP seeds relative to controls in seedlings experiments, with the average day being 1.6 for MP seedlings, compared to 1.8 days for controls ($n = 84$ and 95, respectively) (Figure 1a). There was no difference in the time to reach the V_E or V_C stages between the MP and control seedlings in the early vegetative growth experiments ($n = 232$) (Figure 1a). MP seedlings

took an average of 5.3 and 10.8 days to reach V_E and V_C, respectively, compared to controls which took an average of 5.3 and 10.9, respectively.

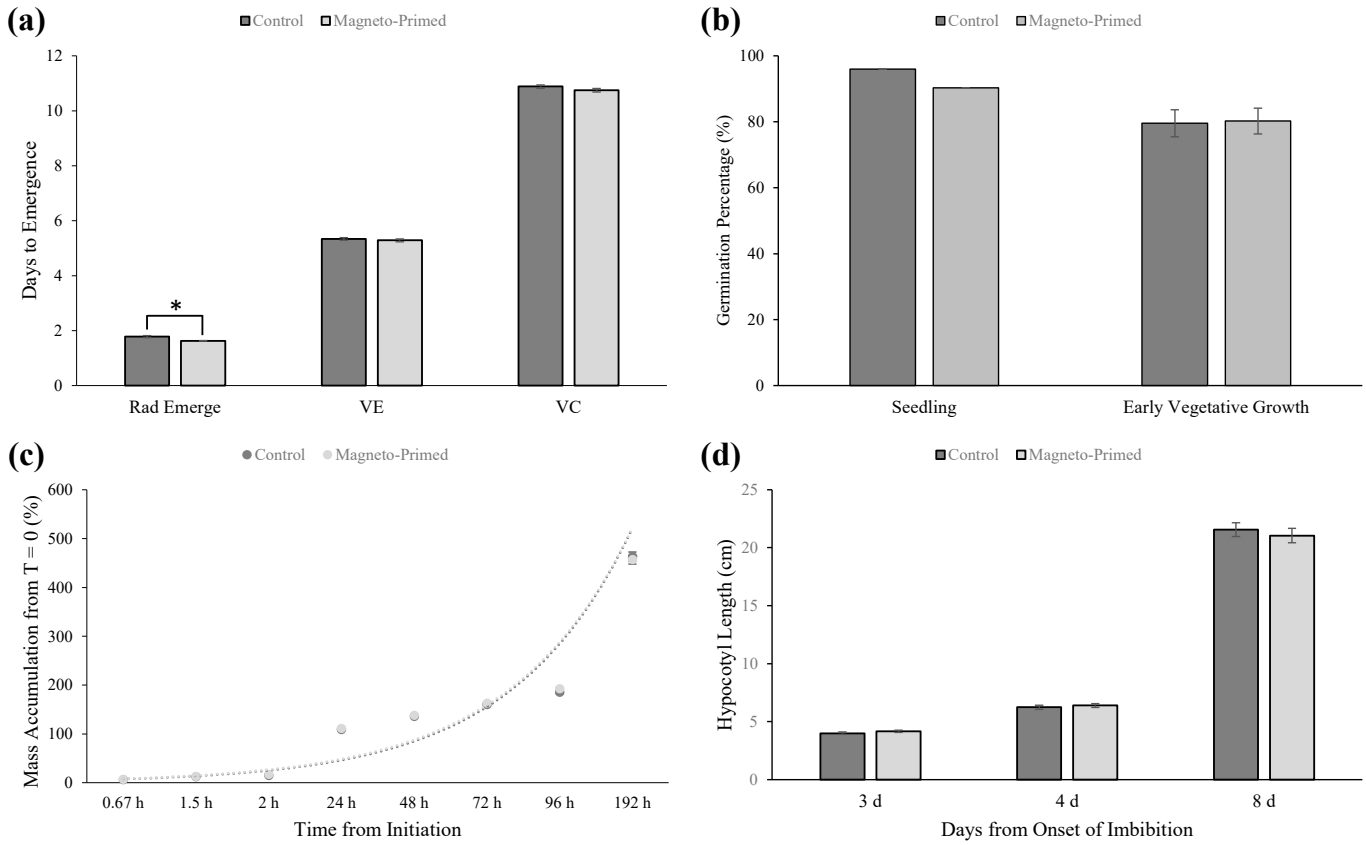


Figure 1. (**a**) Average number of days for magneto-primed and control seedlings to reach radicle emergence (n = 84 and 95, respectively), the vegetative emergence (V_E) developmental stage (n = 232), and the cotyledon emergence developmental stage (V_C) (n = 232). (**b**) Germination percentages of magneto-primed and control seedlings in seedling experiments (n = 93 and 99, respectively) and early vegetative growth experiments (n = 232). (**c**) Percent mass accumulation from T = 0 for magneto-primed (n = 77 on day 8) and control seedlings (n = 85 on day 8) over 8 days. Data plotted using exponential trend line function in Excel® (**d**) Average hypocotyl lengths up to day 8 between magneto-primed (n = 77 on day 8) and control seedlings (n = 85 on day 8). Notably, a * signifies that a significant difference ($p < 0.05$) had been achieved.

No significant variation was found for the germination percentage in the seedling experiments, with MP and control seedlings exhibiting germination percentages of 90.3% and 96.0%, respectively (n = 93 and 99, respectively) (Figure 1b). Similarly, no difference was found in the early vegetative growth experiments, where MP and control seedlings exhibited nearly identical germination percentages of 80.2 ± 3.9% and 79.5 ± 4.1%, respectively (n = 232) (Figure 1b).

Pre-treatment with MP had no effect on imbibition, or subsequent biomass accumulation in seeds (n = 77 and 85 for MP and controls on day 8, respectively). The points depicting the average percent difference in mass gain nearly overlapped one another up to 3 days post-initiation (Figure 1c). Upon day 4, MP seedlings had a minor increase in mass (3.8%) of 192.3 ± 2.1% compared to 185.2 ± 2.7% for controls. However, the control seedlings had surpassed the MP by day 8, gaining 463.0 ± 9.5% compared to 456.8 ± 9.1%, an increase of 1.4%.

A similar trend was witnessed in measured hypocotyl lengths. Importantly, no variations in hypocotyl length were observed by the end of the experiment (n = 77 and 85 for MP and controls on day 8, respectively) (Figure 1d). On days 3 and 4, MP seedlings had a

slightly larger hypocotyl length of 4.16 ± 0.1 cm and 6.39 ± 0.2 cm compared to controls with an average of 4.00 ± 0.1 cm and 6.24 ± 0.2 cm, an increase of 3.9% and 2.4%, respectively. However, the control seedlings surpassed the MP by day 8, exhibiting an average hypocotyl length of 21.55 ± 0.6 cm against the MP with an average of 21.16 ± 0.6 cm, an increase of 1.9%.

The final height was larger in control seedlings (2.0%) compared to MP, with values of 11.85 ± 0.2 cm and 11.62 ± 0.2 cm, respectively (n = 232) (Table 2). The final leaf areas were nearly identical to one another, with MP seedlings having an area of 11.40 ± 0.3 cm^2 and 11.39 ± 0.2 cm^2 for the control seedlings, a difference of only 0.09% (n = 232) (Table 2). The same trend was observed when the chlorophyl content was measured for the MP (33.81 ± 0.1) and controls (34.01 ± 0.2), a difference of 0.6% (n = 232) (Table 2).

Table 2. Values obtained from early vegetative growth experiments for plant height, leaf area, and the total chlorophyl content for magneto-primed (n = 232) and control seedlings (n = 232).

Treatment Group	Plant Height (cm)	Leaf Area (cm^2)	Chlorophyl (SPAD)
Magneto-Primed	11.62 ± 0.2	11.40 ± 0.3	33.81 ± 0.1
Control	11.85 ± 0.2	11.39 ± 0.2	34.01 ± 0.2

3.2. Physiological Attributes

The photosynthetic rate was higher in the MP seedlings compared to controls (4.7%), with values of 11.43 ± 0.40 µmol CO_2 m^{-2} s^{-1} and 10.91 ± 0.36 µmol CO_2 m^{-2} s^{-1}, respectively (n = 15) (Table 3). In four of the five trials analyzed, MP seedlings had no improvements, with the largest difference being 15.0% on day 12 of growth (Figure S3a). The transpiration rate showed no variation (1.5%), with MP seedlings having an average value of 2.48 ± 0.1 mmol H_2O m^{-2} s^{-1}, compared to 2.45 ± 0.1 mmol H_2O m^{-2} s^{-1} for controls (n = 15) (Table 3). Notably, between trials, the largest difference was 29.8% on day 10 of growth in favor of controls (Figure S3b). Following similar trends, MP seedlings exhibited no significant improvement (3.6%) in the stomatal conductance rate, with a value of 0.11 ± 0.0 mol H_2O m^{-2} s^{-1}, compared to 0.10 ± 0.0 mol H_2O m^{-2} s^{-1} for controls (n = 15) (Table 3). Unlike the transpiration rate, the largest difference found within the trials was on day 10 of growth, with MP seedlings exhibiting a 29.7% increase (Figure S3c). Intracellular CO_2 concentrations did not exhibit any differences between the MP and control trials (n = 15). Controls had slightly higher intracellular CO_2 concentration (3.5%) of 195.67 ± 4.2 µmol CO_2 mol^{-1} against MP seedlings with a value of 189.05 ± 5.5 µmol CO_2 mol^{-1} (Table 3). When concentrations were evaluated within the trials, it was observed that the control seedlings had a significantly higher (p = 0.04) intracellular CO_2 concentration on day 13 of growth, with a value of 216.39 ± 6.2 µmol CO_2 mol^{-1}, compared to the MP value of 173.52 ± 8.7 µmol CO_2 mol^{-1}, an increase of 24.7% (Figure S3d). However, no significant differences were found on any of the other days of growth, with MP seedlings having larger values on days 11 and 12 of growth (9.4% and 6.5%, respectively) and controls on day 10 (20.5%) (Figure S3d).

Table 3. Values obtained from LI-COR analysis for photosynthetic rate, transpiration rate, stomatal conductance rate, and intracellular CO_2 concentrations for magneto-primed (n = 15) and control seedlings (n = 15).

Treatment Group	Photosynthetic Rate (µmol CO_2 m^{-2} s^{-1})	Transpiration Rate (mmol H_2O m^{-2} s^{-1})	Conductance Rate (mol H_2O m^{-2} s^{-1})	Intracellular CO_2 Concentration (µmol CO_2 mol^{-1})
Magneto-Primed	11.43 ± 0.4	2.48 ± 0.1	0.11 ± 0.0	189.05 ± 5.5
Control	10.91 ± 0.4	2.45 ± 0.1	0.10 ± 0.0	195.67 ± 4.2

3.3. Cytokinin Profile

No variations were found between the MP and control samples for isopentenyl riboside (iPR) concentrations (n = 9 and 6, respectively). MP seedlings had an insignificantly higher (p = 0.65) concentration of 46.56 ± 7.06 pmol/mg against controls with a value of 41.02 ± 10.21 pmol/mg, a difference of 13.5% (Figure 2a). Due to the small number of samples that had detectable iPR levels, intertrial variability could not be assessed.

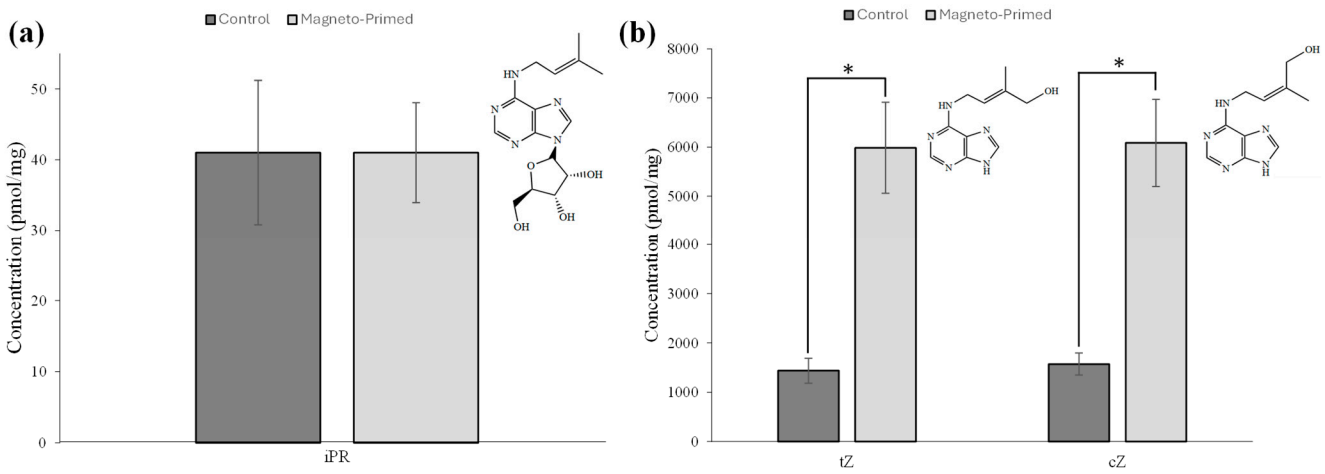


Figure 2. (**a**) Isopentenyl adenosine riboside (iPR) concentrations (pmol/mg) of magneto-primed (n = 9) and control (n = 6) samples. (**b**) *trans*-Zeatin (tZ) and *cis*-Zeatin (cZ) freebase concentrations (pmol/mg) of magneto-primed (n = 13) and control (n = 8) samples. Functional groups of cytokinin species depicted to the right of their respective graphs. Notably, a * represents a significant difference ($p < 0.05$).

trans-Zeatin (tZ) and *cis*-Zeatin (cZ) exhibited large and statistically significant increases (p = 0.00033 and 0.00025, respectively) in concentration for MP cotyledon samples compared to controls (n = 13 and 8 for MP and controls, respectively). MP seedlings had average increases of 200.0% to 483.6% and 189.5% to 417.8% for tZ and cZ, respectively (Figure 2b).

4. Discussion

Numerous studies have reported significant enhancements in plant health associated with MP of seeds including improvements in physiological activity and resistance to adverse conditions [7,14,39–41]. However, the extent to which improvements were achieved remains inconsistent throughout the literature, raising concerns about repeatability and reliability of MP impacts. Our work has provided a rigorous re-examination of the effects of MP on soybean (*G. max*), including the most comprehensive analysis of MP-induced phytohormone dynamics through HPLC-HRMS/MS. Results indicate that no statistically relevant physiological or morphological benefits were conferred to soybeans (*G. max*). These results represent a notable departure from many other investigators despite the use of identical, if not enhanced, experimental techniques. Interestingly, however, we do find and report the first important connection between MP pre-treatment and endogenous phytohormone dynamics in soybean (*G. max*) seedlings.

4.1. Magneto-Priming Effects on Imbibition

In previous MP investigations with potato (*Solanum tuberosum*), soybean (*G. max*), grass pea (*L. sativus*), tomato (*L. esculentum*), and lentil (*Lens culinaris*), the germination percentage was enhanced [39,42–44]. No significant perturbations were observed in the current investigation, though differences in the rate of germination were found (Figure 1a,b). While our work indicates some improvement to the rate of germination, the enhancement

has little value substantively (~2 h) and is negligible compared to other studies on MP of soybean which reported germination rates that were 42% faster than controls (Figure 1a) [7].

Interestingly, studies which report null effects on germination percentage have still reported improvements in other developmental metrics. For example, Shine et al. [7] documented improvements in seedling length and vigor index I for soybean (*G. max*) seedlings treated in 150 and 200 mT SMFs for 60 min. Similarly, Kataria et al. [14] reported that soybean (*G. max*) seeds treated with a 200 mT SMF for 60 min had significant improvements in seedling length (33%) and vigor indices I (25%) and II (25%), despite a relatively small difference of 5% in the germination percentage. Despite no observable changes in germination percentage, we report strikingly smaller, and non-significant improvements in the hypocotyl length (3.9%) relative to non-MP seeds (Figure 1d).

Shine et al. [7] soybean (*G. max*) seeds pre-treated with a 250 and 300 mT SMF for 40–60 min showed significantly faster rates of imbibition (~90%). Statistically relevant time-dependent responses were also repeatedly produced at a flux intensity of 200 mT SMF; however, no enhancement of imbibition rate could be established for pre-treatment of seeds at SMF densities of 150 mT or less [7]. Our work found no difference in the imbibition or biomass accumulation rate of soybean (*G. max*) seeds pre-treated with MP (200 mT SMF) at any point over the 8-day incubation period (Figure 1c).

4.2. Magneto-Priming on Early Vegetative Growth

In the early vegetative growth experiments, the time to reach the key developmental stages emergence (V_E) and cotyledon maturation (V_C) exhibited no differences (Figure 1a). In other studies concerning MP and soybean (*G. max*), the exact days to reach key developmental stages were not tracked; however, the rates of post-emergence development in potato (*S. tuberosum*), lentil (*L. culinaris*), and grass pea (*L. sativus*) were decreased in MP-treated groups compared to controls [42]. Additionally, we find no variation in germination uniformity between MP and control cohorts, again deviating from the findings of previous investigations [19].

Results obtained from the examination of morphological attributes in soybean (*G. max*) pre-treated with MP contradict the reported findings from previous MP investigation studies on soybean (*G. max*) and other crops including corn (*Z. mays*), pea (*Pisum sativum*), celery (*Anathallis graveolens*), chickpea (*C. arietinum*), wheat (*T. aestivum*), tomato (*S. lycopersicum*), and carrot (*D. carota*) [10–12,15,19]. Shine et al. [7] reported that soybean (*G. max*) seeds pre-treated with 150 and 200 mT SMF for 60 min showed significant improvements in the shoot length (33% and 44%, respectively), shoot fresh weight (42.5% and 69%, respectively), and leaf area (51% and 138%, respectively) in 1-month-old plants. These results, however, pale in comparison to a study by Baghel et al. [41], which documented increased plant height (138%), leaf area (131%), total biomass (205%), and total chlorophyl content (126%) in soybeans (*G. max*) pre-treated with MP (SMF at 200 mT for 1 h) before planting. Our investigation could not substantiate these findings despite a large sample size (n = 232), with control seedlings exhibiting an improved seedling height (2.0%) and chlorophyl content (0.6%) compared to MP plants (Table 2).

Previous investigations also found improvements in physiological measures in response to MP pre-treatment. For example, Kataria et al. [33] reported significant increases in photosynthesis and stomatal conductance for trifoliated soybean (*G. max*) leaves pre-treated with MP and subsequently exposed to UV stress (32% and 41%, respectively) or maintained under optimal growth conditions (40% and 50%, respectively). Physiological improvements have also been reported longitudinally in mature soybean (*G. max*) plants pre-treated with MP which exhibit higher transpiration and stomatal conductance rates (30% and 21%, respectively) relative to untreated controls after 45 days [45]. Interestingly, these reports are not limited to soybean (*G. max*), and include wheat (*T. aestivum*), corn (*Z. mays*), and chickpea (*C. arietinum*) [40,46,47]. Our work shows no statistically significant improvements to photosynthetic rate (4.7%), transpiration rate (1.5%), nor stomatal conductance rate (3.6%) (Table 3).

4.3. tZ, cZ, and iPR CK Differential Profile and the Radicle Pair Mechanism

Of the 14 CK forms scanned for, we consistently detected three: iPR, cZ, and tZ. iPR is considered to be a predominant transport form, while both cZ and tZ are active free bases [48,49]. Interestingly, tZ and cZ FBs exhibited significantly higher concentrations in the MP seedlings of $5.98 \times 10^3 \pm 9.25 \times 10^2$ pmol/mg and $6.08 \times 10^3 \pm 8.87 \times 10^2$ pmol/mg compared to $1.44 \times 10^3 \pm 2.53 \times 10^2$ pmol/mg and $1.57 \times 10^3 \pm 2.24 \times 10^2$ pmol/mg in the control seedlings—differences of 316.5% and 287.3%, respectively (Figure 2b). It is important to note that concentrations of tZ and cZ were nearly identical, although tZ has been reported to be the main active CK in soybean (*G. max*) [50]. However, higher activities of cZ have also been reported in other legume species, including pea (*P. sativum*) [51,52]. The absence of any noticeable differences in iPR concentrations may reflect their different roles from the free base CKs, since nucleotide and riboside forms are considered inactive precursors and transport forms, respectively. In fact, they are claimed by some to exhibit little to no biological activity associated with CKs [49,53].

Due to their ubiquitous role in regulating growth and developmental processes including meristem maintenance and activity, cell division, and tissue morphogenesis, differences in morphological and physiological properties would have been presumed to occur in conjunction with higher CK concentrations [21–23]. However, CK activity is not restricted to growth and development, being fundamental in abiotic and biotic stress resistance, seed filling, macronutrient acquisition, and senescence [22,24]. These activities would have been undetectable in the current investigation due to the early stage of development in which experiments were terminated, and the controlled growing conditions to which seeds were exposed. What remains unapparent is why perturbations were reported for morphological and physiological properties at and before the V_C stage in previous studies, whereas none were observed here.

Soybean (*G. max*) seedlings were grown in ideal temperature and humidity conditions, being watered daily and supplied with an excess of macronutrients, isolated from competitors. These tightly controlled, non-stressful conditions seedlings were exposed to inhibited analyses of resistance responses to abiotic stressors between the MP and control cohorts. Though it is unknown whether introducing unfavorable conditions would have led to a more pronounced effect, the benefits reported from previous MP investigations were maximized when seedlings were exposed to abiotic stressors, suggesting an active role of phytohormones in ameliorating the negative impacts imposed onto the plant [33–36,40,41,53]. Confounding variables may have resulted in acute stress exposure to the plants in previous work, leading to more exacerbated differences between cohorts due to MP's presumed ameliorative effect on external stressors. In fact, our work with lentils indicated that increased CK had more pronounced positive effects on plant performance when there was a significant growing condition stress that had to be overcome [54]. Notably, while changes in plant hormone concentrations in response to MP may be detectable by highly sensitive analytic methods, the induced changes may remain below the threshold required to trigger morphologic alteration (enhanced growth or germination) or be otherwise buffered or counteracted by the activity of other hormones and metabolites.

Imbibing seeds in isolation resulted in the omission of any biochemical recognition of other individuals within their proximity, diminishing the need for accelerated growth to acquire limited resources. This effect has been shown in an investigation on Chinese plantain (*Plantago asiatica*), where seeds underwent faster germination when grown in the presence of sibling and non-sibling seeds [55]. As seeds were imbibed together in previous MP studies, the acknowledgement of other individuals could have served as a factor for the enhanced rate of growth during early developmental stages [7,8,11,14]. Rather than aimlessly expend resources on growth, which would impart no benefit on the fitness of the organism, resources would be better reserved for when nutrient-limiting conditions are experienced or to enhance the nutrient content and yield of their progeny. Notably, CKs are associated with seed development and fatty acid biosynthesis in soybean (*G. max*), ultimately serving as a fundamental driver in seed yield [27,31].

Other CK forms including RBs and NTs could not be quantified; however, the presence of spiked deuterated CKs during analysis would suggest endogenous compounds were below the limit of detection for the instrument. CK forms and concentrations vary depending on the rate of growth and stage of development, with actively dividing tissues being predominated by the biologically active FBs [52,56,57]. Due to the early stage of growth in which tissue harvesting occurred, most of the CKs biosynthesized were likely converted to the active FB forms to sustain growth, or to ameliorate the effects of abiotic stressors imposed onto the seedling [56,58]. In a study analyzing the CK profile of kernels extracted from wheat (*T. aestivum*), the predominant CK forms during early kernel development included tZ and cZ FBs, with other forms (RBs and NTs) and types (iP and DZ) exhibiting higher concentrations at later growth stages [59]. Other possibilities for the absence of quantifiable RB and NT CK data could be due to signal suppression caused by matrix effects of already extremely low endogenous quantities [60].

Though this is the first study to analyze the CK profile of MP seedlings, other investigations have determined relative ratios of other phytohormones including gibberellic acid (GA) and abscisic acid (ABA) in plants other than soybean (*G. max*) [11,61]. For example, tomato (*L. esculentum*) seeds treated with an SMF of 100 mT for 30 min exhibited significantly higher GA-to-ABA ratios at 12 and 24 h of imbibition [11]. Biochemical analysis indicated an increase in the expression level of proteins ABA 8′-hydroxylase and GA3oxidase 1, responsible for catalyzing the breakdown of ABA and synthesis of GA, respectively [11]. These results indicate that one of the primary mechanisms by which MP induces its effects is through phytohormone regulation. Future work should be centralized on further analyzing relative ratios and quantities of phytohormones across different developmental stages in both stressed and non-stressed conditions.

5. Conclusions

MP provided no consistent enhancement in any of the agronomic and morphological parameters analyzed. However, CK analysis showed significant improvements in the active tZ and cZ, phytohormones critical for regulating growth, development, and environmental stress tolerance [53]. Based on the results from this investigation, it cannot be conclusively stated that MP provided no physiological benefit to the seedling. The lack of significance in the current investigation may be due to differences in the electromagnet used for seed pre-treatment; however, more likely it is due to more rigorous oversight in experimental design and control over possible confounding variables such as soil quality, temperature regulation, and solenoid cooling. Collectively, our study demonstrates that a more thorough evaluation on MP needs to be taken rather than continuing to declare MP as an effective priming technique while ignoring contradicting reports.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijpb15040089/s1, Figure S1: The environmental chamber where the seeds were exposed to the static magnetic field. The inductively coupled plasma-tandem mass spectrometry chiller (A) is located on the right, the potentiostat (B) regulating the current entering the electromagnet (C) on the far left of the table, and the Hall probe (D) used to measure the magnetic flux density throughout the runs. Figure S2: The electromagnetic field generator used in the study with the sample holder in the middle. In the figure above, A and B refer to the inflow (A) and outflow (B) ports attached to the inductively coupled plasma-tandem mass spectrometry chiller that actively cooled the electromagnet throughout the run. The symbol X refers to the two solenoids of the electromagnet where the current transverses to produce the magnetic field. N and S refer to the North and South poles of the electromagnet, respectively. Figure S3: (a) Photosynthetic rate of cotyledon leaves sampled from magneto-primed and control seedlings (n = 3 per trial for days 13, 12, 10 and n = 6 for day 11). (b) Transpiration rate of cotyledon leaves sampled from magneto-primed and control seedlings (n = 3 per trial for days 13, 12, 10 and n = 6 for day 11). (c) Intracellular CO_2 concentration of cotyledon leaves sampled from magneto-primed and control seedlings (n = 3 per trial for days 13, 12, 10 and n = 6 for day 11). (d) Intracellular CO_2 concentration of cotyledon leaves

sampled from magneto-primed and control seedlings (n = 3 per trial for days 13, 12, 10 and n = 6 for day 11).

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