Publication II

Plant Growth Regulation

Promotion of germination, morphological and physiological response of aged Triticum aestivum seeds after treatment with electromagnetic field (EMF)

--Manuscript Draft--

Manuscript Number:	
Full Title:	Promotion of germination, morphological and physiological response of aged Triticum aestivum seeds after treatment with electromagnetic field (EMF)
Article Type:	Original Research
Keywords:	electromagnetic field exposure; seed vigour; germination; priming; wheat; phytohormones
Corresponding Author:	Adriana Szmidt-Jaworska Nicolaus Copernicus University Torun, POLAND
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Nicolaus Copernicus University
Corresponding Author's Secondary Institution:	
First Author:	Daniele Cecchetti
First Author Secondary Information:	
Order of Authors:	Daniele Cecchetti
	Agnieszka Pawełek
	Joanna Wyszkowska
	Adriana Szmidt-Jaworska
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Seed storage is a necessity related to the seasonality of crops, for breeding programmes, and insurance against crop failure. Stored seeds may deteriorate over time, which is a natural phenomenon as seeds tend to lose viability and vigour even under optimal storage conditions. Recently, the use of physical factors such as electromagnetic fields (EMFs) to improve seed fitness has received more attention. The aim of this study was to analyse the priming effects of EMF treatment (50 Hz, 7 mT) on germination, emergence, and seedling growth of ageing seeds of wheat (Triticum aestivum) separated into groups of small and big seeds. Different biochemical changes in EMF-treated seeds and seedlings were examined, including membrane integrity, H_2O_2 levels, α-amylase activity, and changes in phytohormones (gibberellins, GAs; indole-3-acetic acid, IAA; abscisic acid, ABA; jasmonic acid, JA; and salicylic acid, SA) content. It was revealed that the chosen storage conditions mostly affect small wheat seeds' germination parameters while pre-sowing exposure to EMF stimulated most, the germination kinetics and seedling growth dynamics of small aged seeds. Moreover, significant changes in studied biochemical traits and phytohormones content were detected depending on EMF exposure, seeds size, and plant tissue type. The results of our study shed light on the mechanism controlling the observed effects of EMF treatment on plant growth processes and the possibility of using this priming factor in deteriorated seeds to improve their quality for sowing.

Dear Editor,

Hereby we submit our manuscript titled “Promotion of germination, morphological and physiological response of aged winter wheat (*Triticum aestivum* L.) seeds after treatment with electromagnetic field (EMF)” by Daniele Cecchetti, Agnieszka Pawełek, Joanna Wyszkowska, Adriana Szmidt-Jaworska for your consideration for publication as an original article in Plant Growth Regulation.

In the light of our scientific interest we undertook to investigate the changes occurring in aging seeds and the impact of the electromagnetic field on improving seed germination parameters. We shed light on the processes underlying plant responses to EMF. The obtained results indicate the close relationship between germination of winter wheat and seed aging process, seed size, phytohormones, α-amylase and H_2O_2 levels in control conditions and after pre-sowing seed treatment with EMFs (50 Hz, 7 mT). These opens a new perspective on understanding the reactions initiated by the electromagnetic field and indicate the possibility of using the electromagnetic field as a technique supporting the recovery of wheat seeds subjected to natural aging.

We believe our results will be of interest to the plant physiology and agronomy community. Therefore, we would be grateful if you would consider it for publication in your journal.

We declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere.

As Corresponding Author, I confirm that the manuscript has been read and approved for submission by all named authors.

Sincerely,

Adriana Szmidt-Jaworska

Promotion of germination, morphological and physiological response of aged *Triticum aestivum* seeds after treatment with electromagnetic field (EMF)

Daniele Cecchetti[1], Agnieszka Pawełek[1], Joanna Wyszkowska[2], Adriana Szmidt-Jaworska[1]*

1. Nicolaus Copernicus University in Toruń, Faculty of Biological and Veterinary Sciences, Department of Plant Physiology and Biotechnology, Lwowska 1 St. 87-100 Toruń, Poland
2. Nicolaus Copernicus University in Toruń, Faculty of Biological and Veterinary Sciences, Department of Animal Physiology and Neurobiology, Lwowska 1 St. 87-100 Toruń, Poland

***- corresponding author: asjawors@umk.pl**

Abstract

Seed storage is a necessity related to the seasonality of crops, for breeding programmes, and insurance against crop failure. Stored seeds may deteriorate over time, which is a natural phenomenon as seeds tend to lose viability and vigour even under optimal storage conditions. Recently, the use of physical factors such as electromagnetic fields (EMFs) to improve seed fitness has received more attention. The aim of this study was to analyse the priming effects of EMF treatment (50 Hz, 7 mT) on germination, emergence, and seedling growth of ageing seeds of wheat (*Triticum aestivum*) separated into groups of small and big seeds. Different biochemical changes in EMF-treated seeds and seedlings were examined, including membrane integrity, H_2O_2 levels, α-amylase activity, and changes in phytohormones (gibberellins, GAs; indole-3-acetic acid, IAA; abscisic acid, ABA; jasmonic acid, JA; and salicylic acid, SA) content. It was revealed that the chosen storage conditions mostly affect small wheat seeds' germination parameters while pre-sowing exposure to EMF stimulated most, the germination kinetics and seedling growth dynamics of small aged seeds. Moreover, significant changes in studied biochemical traits and phytohormones content were detected depending on EMF exposure, seeds size, and plant tissue type. The results of our study shed light on the mechanism controlling the observed effects of EMF treatment on plant growth processes and the possibility of using this priming factor in deteriorated seeds to improve their quality for sowing.

Keywords: electromagnetic field exposure, seed vigour, germination, priming, wheat, phytohormones

Introduction

Many crops are reproduced through seeds thus, requiring the production, storage, and transport of large quantities of seeds. Seeds are stored for one or more seasons in order to accommodate the seasonality of crops and the needs of breeding programmes and also insure against crop failure (McDonald 1999; Bewley et al. 2013). Naturally, stored seeds even under optimal storage conditions, may lose their quality, viability, and vigour over time (McDonald 1999). Adverse environmental factors may also contribute to the deterioration of seeds. Seed ageing during storage may cause retardation of field establishment, and may eventually result in seedling abnormalities or even failure of emergence (Bewley et al. 2013; Ziegler et al. 2021). Ellis (2022) reports of potentially reduced germination rate, lowered tolerance to suboptimal germination conditions, and reduced seedling growth due to ageing of stored seeds.

Storability of seeds has mainly a genetically regulated mechanism and is influenced by factors including seed quality at the time of storage, pre-storage history of seeds (environmental factors during pre- and post-harvest stages), seed moisture content, ambient relative humidity, temperature of storage environment, duration of storage, and biotic agents (Ziegler et al. 2021). Some seeds, referred to as orthodox, are able to tolerate desiccation and retain their viability for a long time in the dry state in contrast to their recalcitrant (syn. non-orthodox) counterparts (Roberts 1973).

The viability of orthodox seeds, such as wheat (*Triticum aestivum*), is relatively easily sustained by lowering their moisture contents and storage temperature, although, they deteriorate gradually and eventually die even under such suitable storage conditions. The mechanisms causing seed deterioration are slowly being understood. Deteriorated seeds are characterized by: (1) disruption of cellular membranes, which manifests itself e.g. by an increase in leakage cytoplasmic components; (2) a reduction of enzyme activities; (3) a decrease of respiration rate; (4) a reduction in efficiency of antioxidant systems; (5) the peroxidation of lipids; (6) impairment of protein synthesis systems; (7) depletion of food reserves; and (8) the damage of genetic integrity (Hendry, 1993; McDonald, 1999; Kibinza et al. 2006; Bewley et al. 2013; Gebeyehu, 2020). Since the physiological and biochemical parameters of seeds are important for successful seedling establishment (Corbineau, 2012), the cellular parameters could help evaluate and promote new approaches for identifying differences important for establishing the quality of stored seeds (Pritchard, 2020).

Damage from the seed ageing process can be delayed or stopped by adopting various treatments at different agronomic stages (pre-sowing, harvesting, and storage periods). Seed priming is a widely-used pre-sowing treatment to improve seed performance in terms of germination speed, final germination rate, seedling vigour, uniformity, and tolerance to both biotic and abiotic stress (Lutts et al. 2016, Rifna et al. 2019; Biswas et al. 2023, Kaya et al. 2024).

A range of priming treatments have been developed, but none has been established as being universally effective or beneficial for all crops (Farooq et al. 2019). They differ in their effectiveness depending on a complex interaction of factors including plant species or genotypes, water potential of priming agents, duration of treatment, and environmental features. Physical priming methods include treatment with magnetic fields,

which can be static (SMF) or alternating. Alternating magnetic fields are also referred to as electromagnetic fields (EMF), which are characterized by a dominant magnetic component. These fields/methods are considered non-invasive and environmentally friendly means of enhancing crop growth and development (Sarraf et al. 2020; Bernard et al. 2024). Exposure of seeds of *Hordeum vulgare* (Shabrangy et al. 2021) and *Triticum aestivum* (Cecchetti et al. 2022) to EMF (both 50 Hz, 7 mT) improved their germination parameters. Dry and wet seeds of *Valeriana officinalis* treated with EMF (60 Hz, 1 and 2 mT) showed improved growth parameters and activity of scavenging enzymes in their leaves (Farzpourmachiani et al. 2015). Dry and wet seeds of *Salvia nemorosa* treated with similar doses of EMF (50 Hz, 2, 4, and 6 mT) produced plants with higher contents of micronutrients and photosynthetic pigments (Ghaemi et al. 2020). Dziwulska-Hunek et al. (2023) have reported of the positive impact of EMF (30 mT for 60 sec) treatment of *Glycine max* seeds on their germination. Ali et al. (2024) have also discovered that corn (*Zea mays* L.) growth and grain yield can be directly increased by treatment with an EMF (from 60 to 180 mT) for a short time (3-6 min). Surprisingly, seedlings obtained from treated seeds are more resistant to unfavourable environmental conditions (Pietruszewski and Kania, 2010).

Subsequent studies have shown a beneficial effect of electromagnetic fields on the response of plants to biotic and abiotic stress factors (Sharraf et al. 2020). The application of EMF can reduce the detrimental effect of pathogenic microbes and increase the growth and yield of plants (Galland and Pazur 2005). For example, citrus plants intermittently exposed to 10 Hz EMF showed a substantial rise in fresh and dry leaf weight in healthy as well as *Phytoplasma aurantifolia* infected plants (Abdollahi et al. 2012). Study conducted on *Nicotiana tabacum* showed that treatments with SMF and EMF can be an effective application for increasing plant resistance to phytopathogen *Tobamovirus* (Trebbi et al. 2007). One of these positive effects was noted in the case of plants grown in salt, as reported for seeds of *Zea mays* treated with 200 mT SMF (Baghel et al. 2019), or in heavy metal stress conditions, as reported for EMF-primed seeds of *Triticum aestivum* L. cv. Xiaoyan (600 mT) (Chen et al. 2017) and *Vigna radiata* (600 mT) (Chen et al. 2011).

The use of physical technique including exposure to EMFs as priming methods has received much attention since these methods are considered as safer to apply, relatively cheaper, and environmentally friendly. Conducting well-planned research might allow us to use physical methods to improve seed quality of numerous crops. The aim of this study was to analyse the priming effects of EMF treatment (50 Hz, 7 mT) on the germination and growth parameters of ageing seeds of wheat (*Triticum aestivum*). Different biochemical changes in EMF-treated seeds and seedlings are examined, including membrane integrity, H_2O_2 levels, α-amylase activity, and changes in phytohormones (gibberellins, GAs; indole-3-acetic acid, IAA; abscisic acid, ABA; salicylic acid, SA; jasmonic acid, JA). The results of our study shed light on the mechanisms controlling the observed effects of EMF treatment on the germination process and the potential of using this method to improve growth parameters in deteriorated seeds.

2 Materials and Methods

2.1 Plant material

112 Seeds of winter wheat (*Triticum aestivum*) var. Owacja harvested in 2018 were received from the IHAR Group
113 (Poland). For all experiments, undamaged seeds were selected and divided into two groups based on their size
114 range (**Fig 1A**) of the three axial dimensions (length, width, and thickness) as was established by Cecchetti et
115 al. 2022. Those seeds will be mentioned as 'aged small' and 'aged big' seeds in the next parts of the paper.
116
117 **2.2 Monitoring of Seed Ageing Process**
118 The seeds were divided into two groups and stored in thick cardboard boxes at two different environmental
119 conditions: (1) temperature 24.5 ± 0.5°C and 60% of relative humidity (RH) (to promote ageing process) and
120 (2) temperature 10 ± 1°C and 40% of RH (to maintain seed viability). To determine the ageing process seeds
121 were sorted randomly every six months for germination control (**Table 1**). Germination tests were performed:
122 (1) for 5 days in Petri dishes (diameter of 9 cm), filled with filter paper, moistened with 3 mL of sterilized
123 deionized water (0.05 µS cm^{-1}); (2) for 8 days in plastic pot (upper diameter 8.5 cm, lower diameter 6.5 cm,
124 hight 7.5 cm), filled with a substrate mix of peat (Substral osmocote Warszawa, Poland), vermiculite, and sand;
125 40/40/10 (v/v/v). All germination tests were conducted in laboratory conditions at 24.5 ± 0.5°C, 60% RH under
126 long-day constituting 15 h of light and 9 h of darkness. The photosynthetic photon flux density (PPFD) was
127 30 µmol m^{-2} s^{-1}, provided using two OSRAM L 30 W/865 (LUMILUX – Cool Daylight) and one OSRAM L
128 30 W/77 (FLUORA) lamps kept 98 cm above where the pots and Petri dishes were placed. The seeds were
129 considered as germinated when roots were protruded from caryopses (test in Petri dish) or coleoptiles emerged
130 from the soil (test in pots).
131
132 **2.3 Exposure to electromagnetic field**
133 Before seeding, the seeds kept in falcon tubes were exposed for 24 h to the electromagnetic field, EMF (50
134 Hz, 7 mT). A detailed description of the set-up and environmental conditions has been published by Cecchetti
135 et al. (2022) and Bienkowski and Wyszkowska (2015). In the control variant, the seeds were placed in the
136 same external conditions without exposure to the EMF.
137
138 **2.4 Germination assay**
139 After the treatment was performed, the seeds were placed inside the Petri dishes (9 cm diameter and at the
140 bottom covered with filter paper). Then, sterilized deionized water (3 ml) was added and the Petri dishes were
141 sealed with sealing film (PARAFILM® M). Tests were carried out on three replicates for each single variant,
142 constituted by 50 seeds divided in 10 seeds for each Petri dish. The seeds were put in continuous darkness
143 conditions at 24 ± 0.5°C, with 60% RH.
144 The experiment last for 72 h after seeding (AS), with a total of nine time points (0, 4, 8, 12, 16, 20, 24, 48,
145 72 h) selected for germination parameters analysis. The germination process was analysed by observing the
146 two phenomena related to the germination process: (1) coleorhiza emergence, observed by 0 to 24 h AS; (2)
147 radicle emergence of at least 2 mm in length (complete germination), observed by 0 to 72 h AS. The evaluation
148 of coleorhiza and radicle emergences were ascertained through the use of the following germination

parameters: Mean Germination Time (MGT), Coefficient of Variability of germination time (CVt), Coefficient of velocity of germination (CVG), Germination index (GI), Median germination time (t50), and Germinability (G). Details for the calculation of those germination parameters have been presented in the published paper (Cecchetti et al. 2022).

The fresh and dry weight was estimated for the seedlings that germinated completely, at 72 h AS. The total fresh weights of the entire seedlings and the separated organs (roots and coleoptiles) were noted. Additionally, the length of roots and coleoptiles was measured. The dry weight of the seedlings and separated organs were estimated after drying in an oven for 48 h at 70°C (Cecchetti et al. 2022).

2.5 Root growth studies

For root growth studies, 3-day-old seedlings were used after growing in conditions described in "Germination Assay". The number and type (primary or seminal) of roots were assessed based on the growth direction with respect to the seeds tip (Huang et al. 1991; Nakamoto and Oyanagi, 1994). The classification for normal and abnormal seedlings was based on the absence or the presence of the primary root and the presence of coleoptile (**Fig 1B**). The affiliation of the roots to the appropriate type was confirmed through the use of ImageJ software (Schneider et al. 2012). Moreover, the abnormal seedlings were divided into three main classes: (1) 0 roots (0 primary, 0 seminal, 1 coleoptile; inhibited germination) (2) 1 root (0 primary, 1 seminal, 1 coleoptile), and (3) 2 roots (0 primary, 2 seminal, 1 coleoptiles). The normal seedlings were sorted into main classes based on the primary root as follows: (1) 3 roots (1 primary and 2 seminal), (2) 4 roots (1 primary and 3 seminal), and (3) 5 (1 primary and 4 seminal).

2.6 Seed water uptake and membrane integrity

For studies of water absorption of treated and non-treated seeds, seeds were weighed and placed in falcon tubes filled with 20 mL of deionized water and incubated at room temperature for 2, 4, 6, 8, 12, 16 and 24 h. Before weighing seeds were blotted dry with a paper towel to remove excess. For each variant (small and big seeds), three replicates of 30 seeds were prepared. The water uptake was calculated by the following formula:

Water uptake [%] = ((fresh weight of seed - dry weight of seed) x 100) / (dry weight of seed)

The membrane integrity was measured based on ion leakage following the procedure of Pawelek et al. (2022). The electrical conductance of the samples (mS cm^{-1}) was measured using a digital conductivity meter (Elmetron CX-105, Zabrze, Poland).

2.7 α-Amylase assay

The α-amylase activity was determined using a modified 3,5-dinitrosalicylic acid (DNS) method (Miller et al. 1959) with some modifications. Seeds germinating in Petri dishes (section "Germination Assay") were used for the analysis and were collected at nine time points (0, 4, 8, 12, 16, 20, 24, 48, 72 h) AS. Seed samples were subjected to extraction according to Pawełek et al. 2022. The amount of reducing sugar released was measured using a spectrophotometer (UV-160 1PC, Japan) at 540 nm with maltose as the reducing sugar standard. The

α-amylase activity was calculated from a standard curve and expressed as mg of maltose per mg of fresh weight.

2.8 Hydrogen peroxide

For hydrogen peroxide (H_2O_2) measurements seeds germinating in Petri dishes (section "Germination Assay") were collected at nine time points (0, 4, 8, 12, 16, 20, 24, 48, 72 h), ground in liquid nitrogen and subjected to extraction according to Pawełek et al. 2022. The content of H_2O_2 was determined from a standard curve by measuring the absorbance at 390 nm (UV-160 1PC, Shimadzu, Japan).

2.9 Determination of phytohormones

To examine the concentrations of selected endogenous phytohormones gibberellins (GA_1, GA_3, GA_4, GA_7), indol-3-acetic acid (IAA), abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) mass spectrometry combined with liquid chromatography (LC-MS/MS), and the QuEChERS-based extraction methods (Pu et al. 2018), with some modifications, were used. For this analysis, the roots, coleoptiles, embryos and whole small and big seeds were used. The tissue samples were collected at different time points in three biological replies as follows: (1) for the whole seeds at 0, 8, 16, 24, 48, and 72 h AS; (2) for the embryos at 8, 16, 24, and 48 h AS; (3) for the roots and coleoptiles at 72 h AS.

Sample extraction was performed according to Cecchetti et al. 2022 with the following modifications. Two different chilled extraction solutions were used depending on water content in samples: (1) for the whole seeds and embryos at 0 and 8 h AS a mixture of acetonitrile (ACN), formic acid (FA), and double-distilled water (60, 4, 36; (v/ v/ v)); (2) for the whole seeds and embryos at 16, 24, 48, and 72 h AS a mixture of acetonitrile (ACN), formic acid (FA), and double-distilled water (80, 4, 16 (v/ v/ v)). After homogenization 15 mg of butylhydroxytoluene (BHT) and deuterated internal standards (10 ng μL^{-1} D_2–GA_1; 10 ng μL^{-1} D_2–GA_3; 10 ng μL^{-1} D_2–GA_4; 10 ng μL^{-1} D_2–GA_7; 5 ng μL^{-1} D_2–IAA; 5 ng μL^{-1} D_6–ABA; 10 ng μL^{-1} D_6–SA; 10 ng μL^{-1} D_5–JA (OlChemim s.r.o, Olomouc, Czech Republic)) were added to the samples. After overnight incubation at 8°C with continuous shaking, 200 mg of sodium sulphate and 600 mg of NaCl/($MgSO_4$ x $7H_2O$) (1/3 (m/m)) were added to the solution and mixed. The samples were centrifuged for 10 min at 10,000 x *g*. The obtained supernatants were collected for the purification step performed according to Cecchetti et al, 2022. The total phytohormonal concentration was determined in triplicate using LC–MS/MS Nexera UHPLC and LCMS–8045 integrated system (Shimadzu Corporation, Kyoto, Japan) according to Cecchetti et al, 2022. The ionization source parameters were optimized in positive ESI mode using pure GA_1, GA_3, GA_4, GA_7, IAA, ABA, SA, and JA dissolved in HPLC-grade water (Sigma-Aldrich, Darmstadt, Germany).

2.10 Statistical analysis

The statistical analysis of the seed ageing process, germination parameters, root growth, seed water uptake and membrane integrity and phytohormones analysis were conducted using a two-tailed t-test. The seedling growth parameters, α-amylase activity, and hydrogen peroxide content were analysed with one-way ANOVA, followed

223 by the Tukey test and Levene's test. For all analyses, the PAST 4.0 program was used (Hammer and Harper,
224 2001) and the level of significance was set at $p < 0.05$.
225
226 **3 Results**
227
228 **3.1 Monitoring of seed ageing process**
229 The storage conditions of seeds for 2.5 years had a significant impact on the germination process (**Table 1**).
230 The analysis revealed that the final germination percentage was high in seeds just after harvesting (October
231 2019) and then decreased gradually during storage at 24.5 ± 0.5°C. After 30 months of storing at 24.5 ± 0.5°C
232 a significant reduction (17%) in final germination percentage compared to October 2019 appeared. Such a
233 situation was not observed when seeds were kept at 10 ± 1°C. Storage of seeds at 24.5 ± 0.5°C reduced
234 significantly by 12% also the final percentage of seedling emergence, compared to October 2019. However,
235 storage of seeds at 10 ± 1°C preserved seed viability, without a significant decline in the final percentage of
236 seedling emergence.
237
238 **3.2 Germination assay (aged seeds, EMF exposure)**
239 The wheat seeds subjected to a natural ageing process were used to analyse the potential impact of EMFs as a
240 factor on germination process. In the research coleorhiza emergence (0–24 h AS) and radicle emergence
241 (complete germination, 0–72 h AS) were the subject of observation (**Table 2**). When considering the emergence
242 of the coleorhiza the results showed that, in control conditions, small seeds germinate slower than big seeds.
243 The reduction of the coleorhiza emergence of small seeds was 100% at 8 h AS, 50% at 12 h AS, and 22% at
244 16 h AS, compared to control big seeds (**Table 2**). In the control samples, no significant differences in the
245 speed of radicle emergence were noted between big and small seeds (**Table 2**).
246 After EMF exposure, faster coleorhiza emergence was observed in both small and big seeds compared to
247 controls. The speed of coleorhiza emergence in big seeds increased by 89% at 8 h, 42% at 12 h, and 26% at 16
248 h AS, while in the small seeds increasement was 46% at 12 h, 25% at 16 h, and 4% at 24 h AS (**Table 2**). In
249 case of radicle emergence, the significant improvement by EMF treatment was noted both in big and small
250 seeds compared to the controls. However, the speed of radicle emergence in big seeds increased by 79% at 20
251 h AS, while for small seeds it increased by 63% at 20 h and 50% at 24 h AS **(Table 2**).
252 In this study, six different germination parameters were analysed for the coleorhiza and radicle emergence of
253 small and big seeds in control conditions and after exposure to EMF (**Fig 2**). Assessing the germination
254 parameters of seeds in control conditions confirmed the slower rate of germination of aged small seeds
255 compared to aged big seeds. Significant differences in the selected germination parameters between the aged
256 small and aged big seeds were detected only for coleorhiza emergence (**Fig 2A-E**). Under the control
257 conditions, the aged small seeds, compared to the aged big seeds, obtained the different germination parameters
258 during coleorhiza emergence: 17% higher MGT, 17% higher t50, 27% lower GI and 0.93% lower CVG. EMF
259 exposure had a significant effect on the selected germination parameters of the wheat seeds. During coleorhiza

emergence, significant stimulations were detected for both aged small (**Fig 2A and C-F**) and aged big seeds (**Fig 2A-E**). The aged treated small seeds compared to the controls were stimulated as follows: 6% reduction in MGT, 8% reduction in t50, 0.32% increase in CVG, 14% increase in GI, and 3% increase in G; meanwhile, the aged treated big seeds compared to the controls were stimulated as follows: 6% reduction in MGT, 8% reduction in t50, 0.42% increase in CVG, 10% increase in GI, and 4% increase in CVt.

In turn, in the case of the radicle emergence, significant stimulations after EMF exposure were detected for both aged small and big seeds (**Fig 2G, I, and J**). The analyses conducted on aged treated small seeds compared to controls show improvements for the chosen parameters: 19% reduction in MGT, 0.63% increase in CVG, and 20% increase in GI. For aged treated big seeds, compared to the controls, a 10% reduction in MGT, a 0.31% increase in CVG, and an 11% increase in GI were noted.

3.3 Growth parameters

In addition to the 72-h germination assay, measurements of the length of roots and coleoptiles (enclosing the first leaf), as well as the fresh and dry weight of individual organs, of the three-day-old wheat seedling were carried out in control conditions and after exposure to EMF. The results achieved are shown in **Table 3**.

The results from control samples show that aged small seeds present statistically significant differences in values compared to aged big seeds. In the control condition, the growth parameters of small seeds were higher compared to the aged big seeds for the following plant organs: 7% higher coleoptiles length, 81% higher roots dry weight, and 92% higher coleoptiles dry weight. On the other hand, compared to the controls, it was revealed that the final effect of EMF treatment is dependent on the seed size. For small seeds, the EMF pre-sowing treatment improved the root and coleoptile length by 17% and 11%, respectively, together with the increment of root dry weight and coleoptile fresh weight by 28% and 10%. Results obtained for aged big treated seeds show that the EMF treatment improves the coleoptile length (2%), root dry weight (57%), and seedling fresh weight (8%). Thus, the strongest stimulation of the early growth under the influence of the pre-sowing EMF treatment is related to the organs from the small seeds.

3.4 Number of roots of germinated seedlings growing from aged small and big seeds 72 h AS

The root system architecture is a vital part of the plant and the number of roots in seedlings represents the changes in the physiological processes that take place during the germination stages. The caryopsis express root development that includes primary root and seminal roots (**Table 4**). The analysis has shown that under the control conditions, no differences between the number of roots in the aged small and big seeds were noticed. EMF pre-sowing treatment had no significant impact on the number of roots in the small seeds, compared to untreated control. However, as seen in **Table 4** the EMF-treated big seeds, compared to the control were stimulated as follows: 264% increase in the 5-root class and 63% decrease in the 0-2 root class.

3.5 Monitoring of water absorption and membrane integrity of aged wheat seeds

Further, the effects of seed size and EMF treatment on membrane permeability of aged seeds of wheat were analysed (**Fig 3**). The result obtained for water uptake assay (**Fig 3B**) in control conditions have shown statistically significant difference at 4 h, where the small seeds show lower (12%) water content compared to the big seeds, while small seeds show higher (5%) water content at 12 h AS compared to the big seeds. EMF treatment affected the water uptake only in the small aged seeds, compared to the untreated controls. Aged treated small seeds show a decrement (3%) of the water content at 8 h when compared to control.

In membrane integrity assay (**Fig 3A**) revealed that there is no difference in the control groups in the electrolyte leakage between small and big seeds. EMF treatment significantly increased the electrolyte leakage in big aged seeds by 21% at 2 h, compared to the control. However, values of electrolyte leakage decrease after the treatment of big seeds by 12% at 16 h.

3.6 Amylase activity and H_2O_2 content in germinating seeds

The aim was to determine how the ageing process and EMF treatment influence α-amylases activity and the change in H_2O_2 levels in germinating seeds (**Fig 4**). In control conditions there was no difference in α-amylase activity between small and big seeds. However, a stimulatory effect of EMF exposure on amylase activity was revealed in small seeds at 8, 48 and 72 h AS.

To test the H_2O_2 content in aged wheat seeds in control conditions groups of small and big seeds were analysed. It was observed that the H_2O_2 level in aged small seeds is higher compared to big ones, with significant statistical differences at the five time points: 8 h AS (7%), 12 h AS (24%), 20 h AS (6%), and at 48 h AS (36%). EMF treatment caused significant increases in H_2O_2 levels in small seeds at 4 h AS (28%), 48 h AS (3%) and 72 h AS (15%), compared to untreated controls. However, in EMF-treated big seeds significant rises in H_2O_2 levels were noted at 8 h AS (4%), 12 h AS (13%) and 20 h AS (8%), compared to controls.

3.7 Phytohormonal content of gibberellins, IAA, ABA, SA, and JA in germinating aged seeds and in seedlings, in control conditions and after EMF treatment

The quantitative determination of phytohormone concentration was conducted in aged small and big seeds, in controls and EMF-treated samples. Whole seeds (0-72 h AS; **Fig 5**), isolated embryos (8-48 h AS; **Fig 6**), as well as roots and coleoptiles at 72 h AS (**Fig 7**), were used for this analysis.

Gibberellins are key regulators promoting plant growth and cell division (Lymperopoulos et al. 2018). Our study revealed that the concentration of particular gibberellin varied from 6 to 25 ng per g fresh weight and depended on plant organs, time after seeding, and exposure to EMF.

In control conditions, significant changes in GA_1 level were detected only in the whole seeds at 48 h AS, where small seeds contained more (100%) GA_1 compared to the big seeds. EMF treatment affected GA_1 level almost exclusively in the whole big seeds. From 8 to 24 h AS, the EMF-treated big seeds had approximately 5-12 ng per g fresh weightof GA_1 compared to the untreated controls with undetectable levels of GA_1. At 48 h AS, the GA_1 level in EMF-treated big seeds was significantly higher (177%) compared to the untreated control.

In control conditions from 0 h AS to 48 h AS, the whole small seeds contained more GA_3 compared to the whole big seeds. After EMF treatment, GA_3 level significantly increased in whole small and big seeds at 16 h AS, compared to untreated controls. Additionally, in whole small seeds at 8 h AS, the GA_3 content rose from an undetectable level in untreated control to ~2.5 ng in EMF-treated seeds. Furthermore, an increase in GA_3 level was noted in the isolated embryos from small EMF-treated seeds at 8 h AS (46%) compared to the control. Moreover, in 3-day-old roots growing from the big seeds, EMF exposure resulted in a significant increase (87%) in GA_3 content compared to untreated control.

There was no significant difference in GA_4 content between small and big seeds in control conditions. After EMF treatment, specific changes in GA_4 levels were noted in whole seeds, as well as in embryos. With the whole seeds, a significant increase in GA_4 level was observed in EMF-treated small seeds (152%) and big seeds (106%) at 8 h AS, as well as in EMF-treated small seeds (322%) at 24 h AS, compared to untreated controls. However, at 72 h AS, a reverse seeds response to EMF treatment was observed, where a significant drop (80%) in GA_4 level in whole small seeds was noted, compared to untreated control. In isolated embryos, EMF treatment increased GA_4 level from undetectable to approximately 1.8 – 4.4 ng in the embryos of small seeds in the early hours of germination (8 h AS and 16 h AS). There were no changes in GA_4 levels in 3-day-old organs under control conditions or after EMF treatment.

In all analysed tissues, significant changes in GA_7 level were observed only after EMF treatment. In whole seeds, a rise in GA_7 content was noted in EMF-treated small (93%) and big (450%) seeds, but only at 8 h AS. In the embryos, only a slight decrease (24%) in GA_7 level occurred in EMF-treated big seeds compared to untreated control. However, in roots growing from EMF-treated big seeds, a significant rise (39%) in GA_7 content was noted compared to control.

In the control conditions, there were significant differences in IAA levels between small and big seeds during the germination process. With the whole seeds, the amount of IAA in the small seeds was 149% and 74% higher than in the big seeds at 24 h AS and 72 h AS, respectively. With the embryos, a significant increase in IAA amount (63%) was detected in small seeds compared to big seeds.

EMF treatment positively affected IAA level in both small and big seeds. In whole small seeds, EMF exposure caused the increase in IAA level at 8 h AS (62%) and at 16 h AS (89%) compared to untreated controls. However, in whole big seeds after EMF treatment, the IAA level was higher at 8 h AS (126%), 24 h AS (95%), and 72 h AS (72 %) compared to controls.

In the embryos of small and big seeds, EMF exposure affected IAA level differently at 8 h and 16 h AS. For EMF-treated embryos of small seeds, the IAA level first dropped by 19% (8 AS) and then rose by 94 % (6 h AS) compared to untreated controls. In the embryos of big seeds, EMF exposure caused an increase in IAA level by 60% (8 h AS) and a 42% decrease at 16 h AS compared to controls. In 3-day-old organs, IAA level was not affected by seed size or EMF treatment.

In control conditions, the whole small seeds contained more ABA than the big seeds at 24 h AS (35%) and at 72 h AS (81%). In the embryos of control samples of big seeds, two large increases in ABA levels were detected at 8 h AS (207%) and 16 h AS (538%) compared to the embryos of small seeds.

EMF treatment affected ABA level mainly in big seeds. After EMF exposure at 8 h AS and 24 h AS, the whole big seeds contained more ABA than untreated controls. However, in EMF-treated embryos of big seeds, the ABA level significantly decreased at 8 h AS (57%) and 16 h AS (81%), compared to controls. EMF effect on small seeds was detected only in whole seeds, causing an 18% increase at 24 h AS and a 34% reduction at 72 h AS, compared to untreated controls. Similarly, in roots and coleoptiles growing from EMF-treated big seeds, a significant decrease in ABA level (35%) compared to untreated controls was revealed.

SA was present in high concentration in all analysed samples. In controls, the whole small seeds at 8 h AS contained 53% more SA than the whole big seeds.

With the whole seeds, EMF treatment caused a decrease in SA level at 16 h AS in big seeds (by 38%), and at 24 h AS in big seeds (by 47%) as well as in small seeds (by 38%), compared to untreated controls. In contrast, EMF-treated embryos of big seeds contained higher SA level at 8 h AS (72%) and 24 h AS (41%) than their controls. At 24 h AS, EMF-treated embryos of small seeds also expressed higher SA level (31%) compared to untreated control.

Concerning 3-day-old organs, EMF treatment significantly affected SA level only in coleoptiles growing from big seeds, causing an increment of 60% compared to untreated control.

Examination of JA levels revealed different JA content and detectability depending on plant organ and treatment conditions. During the 72-h germination period, JA was not detected in the whole seeds, regardless of seed size or EMF exposure. JA level was affected by EMF treatment only in the embryos of big seeds where at 8 h AS and 16 h AS, JA amount increased by 122% and 31% respectively, compared to untreated control.

After EMF exposure, 3-day-old roots growing from small seeds expressed a 30% decrease in the level of JA, compared to the control. Also, in the case of coleoptiles growing from big seeds, EMF treatment caused a significant reduction by 45% in JA amount, compared to untreated control.

4 Discussion

In this study, new findings on the effect of electromagnetic fields on the imbibition and germination of wheat grains that have been stored and lost their natural vigour are presented. The study also examined changes in seed's amylolytic enzyme activity, membrane permeability and a profile of hormone changes that serve as key indicators of seed viability. We showed that higher temperature and humidity conditions during storage caused a significant loss of the ability of ageing seeds to germinate and emerge from the soil (**Table 1**). For orthodox seeds like wheat seeds, storage conditions of low moisture and low temperature are important factors to maintain their viability (van Treuren et al. 2018).

The coleorhiza emergence speed and germination parameters of the control sample of aged small seeds without EMF treatment were significantly lower than the control sample of aged big seeds (**Table 2, Fig 2**). In a previous study in control conditions, fresh small seeds of wheat expressed faster coleorhiza and radicle emergence compared to fresh big seeds (Cecchetti et al. 2022). This shows that suboptimal storage conditions used in the current study influence seed germination depending on seed size. Results obtained from artificially

407 aged seeds of lentil (*Lens culinaris* Medik.) showed that aged seeds express lower emergence percentage
408 compared to fresh seeds, but independent on seed size. However, lentil plants growing from aged small seeds
409 produced lesser grain yield per unit area than plants growing from aged big seeds (Ghassemi-Golezani et al.
410 2014). Also, for artificially aged seeds of rice, significantly lower germination rates compared to seeds without
411 ageing treatment were observed, and the radicle was more sensitive to ageing, compared to the coleoptile
412 (Zheng et al. 2024). A study of eight woody plant species showed that small seeded species are the fastest to
413 germinate (Duncan et al. 2019). However, other reports prove that traits of large-sized seeds allow growing
414 plants to cope better in stressful conditions (Lebrija-Trejos et al. 2016; Ghassemi-Golezani et al. 2014).
415 EMF exposure stimulated coleorhiza and radicle emergence in both small and big aged seeds of wheat,
416 while the highest values of stimulation were observed for aged big seeds (**Table 2**). This is in line with Maffei
417 (2018) observation that EMF improves seed germination. Bhardwaj et al. (2016) noted an enhancement of
418 germination and seedling vigour in pea (*Pisum sativum* L.) seeds subjected to natural ageing and then treated
419 with pulsed magnetic field (PMF).
420 Analysis of physiological parameters in control conditions showed higher seedling fresh mass and
421 coleoptile length of aged small seeds when compared to aged big seeds (**Table 3**). These results are similar to
422 those obtained from fresh and aged seeds of sorghum (*Sorghum bicolor* L.) where seedlings developed from
423 small seeds have longer shoots compared to big seeds (Yousif, 2010). Also, for fresh seeds of wheat small
424 seeds produced seedlings with longer coleoptiles than big seeds in control conditions (Cecchetti et al. 2022).
425 However, the difference in shoot length between fresh small and fresh big seeds was more pronounced than
426 for shoots growing from the aged seeds.
427 In our study, the majority of seedlings had three roots (**Table 4**). In wheat seedlings the number of root
428 is generally five but this value varies depending on germination conditions, time after seeding, and plant variety
429 (Golan et al. 2018). Low frequency of seedlings with bigger number of roots indicates that few new seminal
430 roots developed from their primordial state, which can be an index of a promoted seedling recovery from stress
431 such as the ageing process. The count of the relative frequency of roots in wheat seedlings developed from
432 aged big seeds treated with EMF showed an increase in the number of seedlings with more than 3 roots and a
433 decrease in the number of seedlings with less than 3 roots, compared to untreated controls (**Table 4**). Golan
434 and colleagues (2018) proposed that in water stress conditions, rapid recovery of roots is essential for survival
435 of wheat seedlings with high root count, therefore without a substantial root system.
436 Aged small seeds of wheat at 4 h AS exhibited significantly lower water absorption compared to aged
437 big seeds in control conditions. This corresponds to significantly slower coleorhiza emergence revealed in aged
438 small seeds, compared to aged big seeds in control conditions. While long-term preservation of the seeds in
439 conditions of high RH (above 40%) causes progressive seed's absorption of the water (Hay et al. 2022), this
440 can be responsible for observed slower water absorption during the first hours of germination of aged small
441 seeds of wheat. In following hours of germination in control conditions, at 12 h AS water absorption of aged
442 small seeds of wheat was higher compared to aged big seeds. Similarly, small seeds of *Acacia mangium* showed
443 higher water absorption compared to the big seeds until 120 hours of imbibition (Oliveira et al. 2016). EMF

444 treatment affected electrolyte leakage of only aged big seeds, where an increment of ion leakage was noted at
445 2 h AS, followed by a reduction at 6 h AS when compared to untreated controls (**Fig 3A**). The reduction of ion
446 leakage at later hours of germination in aged big seeds treated with EMF may suggest the activation of repair
447 processes as confirmed in other experiments which tested the priming effect in aged seeds (Hasanuzzaman and
448 Fotopoulos, 2019). Monitoring of changes in membrane integrity can be a relevant method to investigate the
449 acquisition of plant tolerance to stressful conditions (Bajji et al. 2002). Similarly, other results obtained from
450 wheat suggest the promotional effects of EMF (10 kHz, 30 mT) treatment on the seed's membrane integrity
451 (Paycz et al. 2013).
452 Higher level of H_2O_2 observed in germinating aged small seeds compared to aged big seeds in control
453 conditions may indicate the presence of oxidative stress in aged small seeds of wheat (**Fig 4A**). Excessive
454 levels of ROS present in the plant cell start a cascade of events causing oxidative stress (Bienert et al. 2006).
455 At 48 h and 72 h AS, in EMF-treated aged small seeds, an increment of H_2O_2 level and α-amylase activity was
456 observed, compared to untreated controls (**Fig 4**). Increased H_2O_2 production in mitochondria due to enhanced
457 respiration rate may be a result of the activated mechanism of cell recovery from stress (Smirnoff and Arnaud,
458 2019). Increased α-amylase activity in plant cells makes maltose more accessible through the hydrolysis of the
459 starch, and subsequently promotes the growth of important organs, such as roots and stems (Zhang et al. 2021).
460 In control conditions, at particular hours AS, aged small seeds of wheat contained significantly higher
461 levels of IAA, GA_1, and SA compared to aged big seeds. This can be connected to the proven important role
462 of IAA and GA in the regulation of seed longevity (Pellizzaro et al. 2020). The accumulation of SA in the aged
463 small seeds can be related to a reduction in the activity of ROS scavenging enzymes, such as catalases, as was
464 shown in oxidatively-stressed plants of rice (Shim et al. 2003). EMF exposure caused different changes in SA
465 level in whole-aged seeds and embryos of wheat. After EMF treatment 24 h AS, SA level dropped in whole
466 aged small and big seeds, and rose in isolated embryos of aged small and big seeds, compared to untreated
467 controls (**Fig 5G, 6G**). Exogenous application of SA can induce tolerance to multiple environmental stresses
468 in plants (Wang et al. 2023). Moreover, it was shown that endogenous SA is required in response to chilling
469 stress in cucumber seedlings (Dong et al. 2014). Therefore, the accumulation of SA observed in wheat embryos
470 can be a part of the specific cell invigoration process after EMF exposure.
471 During 30 months of the natural ageing process, ABA content in small and big whole wheat seeds
472 greatly decreased compared to fresh seeds (5-fold drop in small seeds and 10-fold drop in big seeds) (Cecchetti
473 et al. 2022). Storing the seeds at 60% RH may cause a partial but consecutive imbibition of the seeds leading
474 to a gradual reduction in the seed's ABA content (Hay et al. 2022; Sano and Marion-Poll, 2021). In control
475 conditions, at 8 h AS and 16 h AS, ABA content in embryos of aged big seeds was notably higher than in
476 embryos of aged small seeds (**Fig 6F**). Mosher et al. (2010) reported that in higher RH conditions, there can
477 be a connection between increased amounts of ABA and SA accumulation in plant cells. EMF exposure caused
478 a vast decline in ABA content in wheat embryos of aged big seeds (at 8 and 16 h AS), compared to untreated
479 controls (**Fig 6F**). Similarly, lower ABA levels were observed in seeds of red clover and sunflower primed

with EMF of 5.28 MHz and 0.74 mT, compared to unprimed controls (Mildažienė et al. 2019; Ivankov et al. 2021).

Changes in SA level were the only significant changes in phytohormone content noted in 72-h-old organs of wheat in control conditions, where coleoptiles growing from aged small seeds contained more SA compared to coleoptiles of aged big seeds (**Fig 7G**). This corresponds to the observed longer coleoptiles growing from aged small seeds compared to coleoptiles from aged big seeds in control conditions (**Table 3**). It is reported that the role of SA in plant growth and development depends on its concentration, plant growth conditions and developmental stages, and the application of optimal concentrations of SA can stimulate plant growth in normal and stressful conditions (Koo et al. 2020). EMF treatment caused an increase in particular gibberellin content in 72-h-old wheat organs growing from aged big seeds (**Fig 7**). In roots and coleoptiles growing from EMF-treated aged big seeds, the content of GA_3 and GA_7, respectively, was higher compared to the untreated controls (**Fig 7 B, D**). The increment of GA_3 level was also noted for roots and aboveground parts of 6-day-old pea seedling growing from seeds treated with SMF (30 mT and 85 mT) (Podleśny et al. 2021). The decrease in ABA level in roots and coleoptiles growing from EMF-treated aged big seeds of wheat compared to untreated controls is associated with the observed increment of GA (GA_7) level in these organs (**Fig 7**). In Arabidopsis, the promotion of GA biosynthesis was mediated by H_2O_2 which in turn up-regulates ABA catabolism, probably through a nitric oxide (NO) signal (Liu et al. 2010). EMF treatment had a stimulatory effect on studied gibberellin (GA_1, GA_3, GA_4, GA_7) content more often in whole seeds than in the isolated embryos of aged small and aged big seeds of wheat (**Fig 5, 6**). Meanwhile, in wheat embryos of aged small and big seeds, GA_4 was the most stimulated gibberellin after EMF exposure. An increase in particular gibberellin contents after EMF treatment has also been noted for seeds of pea and tomato (GA_3), as well as of red clover (GA_7) (Podleśny et al. 2021; Anand et al. 2019, Mildažiene et al. 2019). A broad range of developmental and physiological responses of plants is controlled jointly by light receptors phytochromes and plant hormones, including gibberellins and auxin (Liu et al. 2021). The involvement of phytochrome and cryptochrome in plant response to magnetic field exposure has already been reported (Agliassa et al. 2018; Pooam et al. 2019; Maffei, 2014). Phytochromes and cryptochromes play important roles in the control of germination and growth processes in plants, acting as promoters that interact with different transcription factors. Phytochrome interacting factors (PIFs) are among the main transcription factors regulating phytohormone-mediated plant environmental adaptations (Li et al. 2024). It was shown that *PIF3* gene expression level in wild-type Arabidopsis seedlings and photoreceptor-deficient mutants was significantly affected in the presence of geomagnetic field (GMF) or near null magnetic field (NNMF) (Agliassa et al. 2018).

Conclusions

Seed ageing is a natural process that leads to a progressive deterioration of seed quality. For some time now, static and alternating magnetic fields have been used as a promising tool to improve germination and seedling growth. Existing literature shows that the sensitivity of plants to electromagnetic fields varies depending on

i.e. plant species, developmental stage of the plant, and physical characteristics of magnetic field exposure. Our research confirms this factor-dependent plant reactivity to EMF (50 Hz, 7 mT) treatment and also sheds new light on the physiological and cellular processes underlying the mechanism of EMF action in wheat plants. It was revealed that storage conditions affect wheat seed vigour and small seeds are more subjected to the negative effects of ageing processes compared to big seeds. Interestingly, pre-sowing exposure of ageing wheat seeds to EMF resulted in better germination kinetics and seedling growth dynamics especially in small seeds, compared to their untreated control group. The association between germination features, H_2O_2, and the membrane integrity changes indicates the purposeful influence of EMF on the improvement of these parameters depending on seed size. Moreover, the change in the profile of phytohormones present in EMF-treated germinating seeds and isolated embryos, i.e. specific increases in the level of IAA and the decreases in the amount of SA, JA, and ABA also confirms the positive effect of EMF in strengthening the studied growth traits and cellular processes. Thus, the obtained results indicate the relationship between the germination of winter wheat and the seed ageing process, seed size, phytohormones, α-amylase, and H_2O_2 levels in control conditions and after pre-sowing seed treatment with EMFs (50 Hz, 7 mT). Our results open a new perspective on understanding the reactions initiated by the electromagnetic field and indicate the possibility of using the electromagnetic field as a technique supporting the recovery of wheat seeds subjected to natural ageing.

References

Abdollahi F, Niknam V, Ghanati F, Masroor F, Noorbakhsh SN (2012) Biological effects of weak electromagnetic field on healthy and infected lime (*Citrus aurantifolia*) trees with phytoplasma. *The Scientific World Journal*, 716929, doi: 10.1100/2012/716929

Agliassa C, Narayana R, Christie JM, Maffei ME (2018) Geomagnetic field impacts on cryptochrome and phytochrome signaling. *Journal of Photochemistry and Photobiology B: Biology,* 185: 32–40, https://doi.org/10.1016/j.jphotobiol.2018.05.027

Ali MF, Ahmad MSA, Gaafar ARZ, Shakoor A (2024) Seed pre-treatment with electromagnetic field (EMF) differentially enhances germination kinetics and seedling growth of maize (*Zea mays* L.). *Journal of King Saud University-Science*, 36 (5), 103184, doi.org/10.1016/j.jksus.2024.103184

Anand A, Kumar, A, Thakur M, Koul A (2019) Hydrogen peroxide signaling integrates with phytohormones during the germination of magnetoprimed tomato seeds. *Scientific Reports*, 9: 8814

551 Baghel L, Kataria S, Jain M (2019) Mitigation of adverse effects of salt stress on germination, growth,
552 photosynthetic efficiency and yield in maize (*Zea mays* L.) through magnetopriming. *Acta*
553 *Agrobotanica*, 72 (1), doi:10.5586/aa.1757
554
555 Bajji M, Kinet J-M, Lutts S (2002) The use of the electrolyte leakage method for assessing cell
556 membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation*, 36, 61–
557 70, doi:10.1023/A:1014732714549
558
559 Bernard GC, Lockett A, Asundi S, Mitchell IL, Egnin M, Ritte I, Idehen O, Okoma PM (2024)
560 Magnetic fields in plant development: unravelling the complex interplay from phenotypic responses
561 to molecular dynamics. *American Journal of Biomedical Science and Research*, 21, 376–378,
562 doi:10.34297/AJBSR.2024.21.002854
563
564 Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development,
565 germination and dormancy, 3rd Edition. Springer New York, New York, NY
566
567 Bhardwaj J, Anand A, Pandita VK, Nagarajan S (2016) Pulsed magnetic field improves seed quality
568 of aged green pea seeds by homeostasis of free radical content. *Journal of Food Science and*
569 *Technology*, 53: 3969–3977, doi: 10.1007/s13197-016-2392-8
570
571 Bienert GP, Schjoerring JK, Jahn TP (2006) Membrane transport of hydrogen peroxide. *Biochimica*
572 *et Biophysica Acta (BBA)-Biomembranes*, 1758, 994–1003, doi: 10.1016/j.bbamem.2006.02.015.
573
574 Bieńkowski P, Wyszkowska J (2015) Technical aspects of exposure to magnetic fields of extremely
575 low frequencies (ELF) in biomedical research. *Occupational Medicine*, 66, 185–197,
576 https://doi.org/10.13075/mp.5893.00164
577
578 Biswas S, Seal P, Majumder B, Biswas AK (2023) Efficacy of seed priming strategies for enhancing
579 salinity tolerance in plants: An overview of the progress and achievements. *Plant Stress*, 9, 100186,
580 https://doi.org/10.1016/j.stress.2023.100186
581
582 Cecchetti D, Pawełek A, Wyszkowska J, Antoszewski M, Szmidt-Jaworska A (2022) Treatment of
583 Winter Wheat (*Triticum aestivum* L.) Seeds with electromagnetic field influences germination and

584 phytohormone balance depending on seed size. *Agronomy*, 12 (6), 1423,
585 https://doi.org/10.3390/agronomy12061423
586
587 Chen Y, Chen D, Liu Q (2017) Exposure to a magnetic field or laser radiation ameliorates effects of
588 Pb and Cd on physiology and growth of young wheat seedlings. *Journal of Photochemistry and*
589 *Photobiology B: Biology*, 169, 171–177, https://doi.org/10.1016/j.jphotobiol.2017.03.012
590
591 Chen Y, Li R, He J-M (2011) Magnetic field can alleviate toxicological effect induced by cadmium
592 in mungbean seedlings. *Ecotoxicology*, 20, 760–769, doi: 10.1007/s10646-011-0620-6
593
594 Corbineau F (2012) Markers of seed quality: from present to future. *Seed science research*, 22, S61–
595 S68, doi:10.1017/S0960258511000419
596
597 Dong C-J, Li L, Shang Q-M, Liu X-Y, Zhang Z-G (2014) Endogenous salicylic acid accumulation is
598 required for chilling tolerance in cucumber (*Cucumis sativus* L.) seedlings. *Planta*, 240, 687–700,
599 doi: 10.1007/s00425-014-2115-1
600
601 Duncan C, Schultz N, Lewandrowski W, Good MK, Cook S (2019) Lower dormancy with rapid
602 germination is an important strategy for seeds in an arid zone with unpredictable rainfall. *PLoS One*,
603 14, e0218421, https://doi.org/10.1371/journal.pone.0218421
604
605 Dziwulska-Hunek A, Niemczynowicz A, Kycia RA, Matwijczuk A, Kornarzyński K, Stadnik J,
606 Szymanek M (2023) Stimulation of soy seeds using environmentally friendly magnetic and electric
607 fields. *Scientific Reports*, 13, 18085, https://doi.org/10.1038/s41598-023-45134-y
608
609 Ellis RH (2022) Seed ageing, survival, and the improved seed viability equation; forty years on. *Seed*
610 *Science and Technology*, 50 (2), 1–20, doi:10.15258/sst.2022.50.1.s.01
611
612 Farooq M, Usman M, Nadeem F, ur Rehman H, Wahid A, Basra SM, Siddique KH (2019) Seed
613 priming in field crops: Potential benefits, adoption and challenges. *Crop and Pasture Science*, 70,
614 731–771, doi:10.1071/CP18604
615

616 Farzpourmachiani S, Majd A, Arbabian S, Dorranian D, Hashemi M (2015) Effect of extremely low
617 frequency electromagnetic fields on antioxidant enzymes in valerian (*Valeriana officinalis* L.).
618 *Iranian Journal of Plant Physiology*, 5(4), 1465–1472
619
620 Galland P, Pazur A (2005) Magnetoreception in plants. *Journal of Plant Research*, 118, 371–389,
621 doi:10.1007/s10265-005-0246-y
622
623 Gebeyehu B (2020) Review on: Effect of seed storage period and storage environment on seed
624 quality. *International Journal of Applied Agricultural Sciences*, 6, 185–190,
625 doi:10.11648/j.ijaas.20200606.14
626
627 Ghaemi M, Majd A, Iranbakhsh A, Dorranian D (2020) Seed priming with electromagnetic field
628 improved growth, nutrition, and metabolism of *Salvia nemorosa* L. *Iranian Journal of Plant*
629 *Physiology*, 10, 3315–3324
630
631 Ghassemi-Golezani K, Chadordooz-Jeddi A, Zehtab-Salmasi S (2014) Effects of seed size and ageing
632 on field performance of lentil (*Lens culinaris* Medik.) under different irrigation treatments. *Acta*
633 *agriculturae Slovenica*, 103, 158–166, doi:10.14720/aas.2014.103.2.1
634
635 Golan G, Hendel E, Méndez Espitia GE, Schwartz N, Peleg Z (2018) Activation of seminal root
636 primordia during wheat domestication reveals underlying mechanisms of plant resilience. *Plant Cell*
637 *and Environment,* 41, 755–766, doi:10.1111/pce.13138
638
639 Hammer Q, Harper DA (2001) Past: paleontological statistics software package for education and
640 data analysis. *Palaeontologia Electronica*, 4 (1), 1-9
641
642 Hasanuzzaman M, Fotopoulos V (eds) (2019) Priming and pretreatment of seeds and seedlings:
643 Implication in plant stress tolerance and enhancing productivity in crop plants. Springer Nature,
644 doi:10.1007/978-981-13-8625-1
645
646 Hay FR, Rezaei S, Buitink J (2022) Seed moisture isotherms, sorption models, and longevity.
647 Frontiers in Plant Science, 13, 891913, doi:10.3389/fpls.2022.891913
648

649 Hendry GA (1993) Oxygen, free radical processes and seed longevity. *Seed Science Research*, 3,
650 141–153, https://doi.org/10.1017/s0960258500001720
651
652 Huang B-R, Taylor HM, McMichael BL (1991) Growth and development of seminal and crown roots
653 of wheat seedlings as affected by temperature. *Environmental and Experimental Botany*, 31, 471–
654 477, https://doi.org/10.1016/0098-8472(91)90046-Q
655
656 Ivankov A, Zukiene R, Nauciene Z, Degutyte-Fomins L, Filatova I, Lyushkevich V, Mildažiene V
657 (2021) The Effects of red clover seed treatment with cold plasma and electromagnetic field on
658 germination and seedling growth are dependent on seed colour. *Applied Sciences*, 11, 4676,
659 doi:10.3390/app11104676
660
661 Kaya MD, Ergin N, Harmanci P, Kulan EG (2024) Seed priming as a method of preservation and
662 restoration of sunflower seeds. *Oilseeds and Fat, Crops and Lipids*, 31, 4, doi:10.1051/ocl/2024003
663
664 Kibinza S, Vinel D, Côme D, Bailly C, Corbineau F (2006) Sunflower seed deterioration as related
665 to moisture content during ageing, energy metabolism and active oxygen species scavenging.
666 *Physiologia Plantarum*, 128, 496–506, doi10.1111/j.1399-3054.2006.00771.x
667
668 Koo YM, Heo AY, Choi HW (2020) Salicylic acid as a safe plant protector and growth regulator.
669 *The Plant Pathology Journal*, 36, 1-10, doi:10.5423/PPJ.RW.12.2019.0295
670
671 Lebrija-Trejos E, Reich PB, Hernández A, Wright SJ (2016) Species with greater seed mass are more
672 tolerant of conspecific neighbours: a key driver of early survival and future abundances in a tropical
673 forest. *Ecology Letters*, 19, 1071–1080, https://doi.org/10.1111/ele.12643
674
675 Li G, Kazmi A, Feng M, Hou H (2024) Phytochrome-interacting factors (PIFs) regulate
676 phytohormone-mediated plant environmental adaptation. *Environmental and Experimental Botany*,
677 105610, https://doi.org/10.1016/j.envexpbot.2023.105610
678
679 Liu Y, Jafari F, Wang H (2021) Integration of light and hormone signaling pathways in the regulation
680 of plant shade avoidance syndrome. *aBIOTECH*, 2, 1- 15, doi:10.1007/s42994-021-00038-1
681

682 Liu Y, Ye N, Liu R, Chen M, Zhang J (2010) H_2O_2 mediates the regulation of ABA catabolism and
683 GA biosynthesis in Arabidopsis seed dormancy and germination. Journal of Experimental Botany,
684 61, 2979–2990, doi: 10.1093/jxb/erq125
685
686 Lutts S, Benincasa P, Wojtyla L, Kubala S, Pace R, Lechowska K, Quinet M, Garnczarska M (2016)
687 Seed priming: new comprehensive approaches for an old empirical technique. *New Challenges in*
688 *Seed Biology-Basic and Translational Research Driving Seed Technology,* 1-30, doi: 10.5772/64420
689
690 Lymperopoulos P, Msanne J, Rabara R (2018) Phytochrome and phytohormones: working in tandem
691 for plant growth and development. Frontiers in Plant Science, 9, 1037, doi: 10.3389/fpls.2018.01037
692
693 Maffei ME (2014) Magnetic field effects on plant growth, development, and evolution. *Frontiers in*
694 *Plant Science*, 5, 445, https://doi.org/10.3389/fpls.2014.00445
695
696 Maffei ME (2018) Plant responses to electromagnetic fields. *Biological and Medical Aspects of*
697 *Electromagnetic Fields*, Fourth Edition. CRC Press, pp. 89–110
698
699 McDonald MB (1999) Seed Deterioration: Physiology Repair and Assessment, Seed Science and
700 Technology, Vol. 27, No. 1, 1999, pp. 177-237.
701
702 Mildažienė V, Aleknavičiūtė V, Žūkienė R, Paužaitė G, Naučienė Z, Filatova I, Lyushkevich V,
703 Haimi P, Tamošiūnė I, Baniulis D (2019) Treatment of common sunflower (*Helianthus annus* L.)
704 seeds with radio-frequency electromagnetic field and cold plasma induces changes in seed
705 phytohormone balance, seedling development and leaf protein expression. *Scientific Reports*, 9, 6437,
706 doi:10.1038/s41598-019-42893-5
707
708 Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Annals of*
709 *Chemistry,* 31, 426–428, https://doi.org/10.1021/ac60147a030
710
711 Mosher S, Moeder W, Nishimura N, Jikumaru Y, Joo S-H, Urquhart W, Klessig DF, Kim S-K,
712 Nambara E, Yoshioka K (2010) The lesion-mimic mutant cpr22 shows alterations in abscisic acid
713 signaling and abscisic acid insensitivity in a salicylic acid-dependent manner. *Plant Physiology*, 152,
714 1901–1913, doi: 10.1104/pp.109.152603
715

716 Nakamoto T, Oyanagi A (1994) The direction of growth of seminal roots of *Triticum aestivum* L. and
717 experimental modification *Annals of Botany*. 73, 363–367, https://doi.org/10.1006/anbo.1994.1045
718
719 Oliveira DL, de Smiderle OJ, Schuertz Paulino PP, Graças Souza A (2016) Water absorption and
720 method improvement concerning electrical conductivity testing of *Acacia mangium* (Fabaceae) seeds.
721 *Revista de Biología Tropical*, 64, 1651–1660
722
723 Pawelek A, Wyszkowska J, Cecchetti D, Dinka MD, Przybylski K, Szmidt-Jaworska A (2022) The
724 physiological and biochemical response of field bean (*Vicia faba* L.(partim)) to electromagnetic field
725 exposure is influenced by seed age, light conditions, and growth media. *Agronomy*, 12, 2161,
726 https://doi.org/10.3390/agronomy12092161
727
728 Payez A, Ghanati F, Behmanesh M, Abdolmaleki P, Hajnorouzi A, Rajabbeigi E (2013) Increase of
729 seed germination, growth and membrane integrity of wheat seedlings by exposure to static and a 10-
730 KHz electromagnetic field. *Electromagnetic Biology and Medicine*, 32, 417–429,
731 doi:10.3109/15368378.2012.735625
732
733 Pellizzaro A, Neveu M, Lalanne D, Ly Vu B, Kanno Y, Seo M, Leprince O, Buitink J (2020) A role
734 for auxin signaling in the acquisition of longevity during seed maturation. *New Phytologist*, 225, 284–
735 296, doi: 10.1111/nph.16150
736
737 Pietruszewski S, Kania K (2010) Effect of magnetic field on germination and yield of wheat.
738 *Internatinal Agrophysics*, 24, 297–302
739
740 Podleśny J, Podleśna A, Gładyszewska B, Bojarszczuk J (2021) Effect of pre-sowing magnetic field
741 treatment on enzymes and phytohormones in pea (*Pisum sativum* L.) seeds and seedlings. *Agronomy*,
742 11, 494, https://doi.org/10.3390/agronomy11030494
743
744 Pooam M, Arthaut L-D, Burdick D, Link J, Martino CF, Ahmad M (2019) Magnetic sensitivity
745 mediated by the *Arabidopsis* blue-light receptor cryptochrome occurs during flavin reoxidation in the
746 dark. *Planta*, 249, 319–332, doi: 10.1007/s00425-018-3002-y
747
748 Pritchard HW (2020) Diversity in seed longevity amongst biodiverse seeds. *Seed Science Research*,
749 30 (2), 1-6, doi:10.1017/S0960258520000306

750
751 Pu C-H, Lin S-K, Chuang W-C, Shyu T-H (2018) Modified QuEChERS method for 24 plant growth
752 regulators in grapes using LC-MS/MS. *Journal of Food and Drug Analysis*, 26, 637–648,
753 doi:10.1016/j.jfda.2017.08.001
754
755 Rifna EJ, Ramanan KR, Mahendran R (2019) Emerging technology applications for improving seed
756 germination. *Trends in Food Science and Technology*, 86, 95–108, doi:10.1016/j.tifs.2019.02.029
757
758 Roberts EH (1973) Predicting the Storage Life of Seeds. *Seed Science and Technology*, 1, 499-514
759
760 Sano N, Marion-Poll A (2021) ABA metabolism and homeostasis in seed dormancy and germination.
761 International Journal of Molecular Sciences, 22, 5069, https://doi.org/10.3390/ijms22105069
762
763 Sarraf M, Kataria S, Taimourya H, Santos LO, Menegatti RD, Jain M, Ihtisham M, Liu S (2020)
764 Magnetic field (MF) applications in plants: An overview. *Plants*, 9, 1139,
765 doi: 10.3390/plants9091139
766
767 Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis.
768 *Nature methods*, 9, 671–675, doi:10.1038/nmeth.2089
769
770 Shabrangy A, Ghatak A, Zhang S, Priller A, Chaturvedi P, Weckwerth W (2021) Magnetic field
771 induced changes in the shoot and root proteome of barley (*Hordeum vulgare* L.). *Frontiers in Plant*
772 *Science*, 12, 622795, https://doi.org/10.3389/fpls.2021.622795
773
774 Shim I-S, Momose Y, Yamamoto A, Kim D-W, Usui K (2003) Inhibition of catalase activity by
775 oxidative stress and its relationship to salicylic acid accumulation in plants. *Plant Growth Regulation*,
776 39, 285–292, doi:10.1023/A:1022861312375
777
778 Smirnoff N, Arnaud D (2019) Hydrogen peroxide metabolism and functions in plants. *New*
779 *Phytologist*, 221, 1197–1214 , doi:10.1111/nph.15488
780
781 Trebbi G, Borghini F, Lazzarato L, Torrigiani P, Calzoni GL, Betti L (2007) Extremely low frequency
782 weak magnetic fields enhance resistance of NN tobacco plants to tobacco mosaic virus and elicit
783 stress-related biochemical activities. *Bioelectromagnetics*, 28, 214–223, doi:10.1002/bem.20296

van Treuren R, Bas N, Kodde J, Groot SP, Kik C (2018) Rapid loss of seed viability in ex situ conserved wheat and barley at 4°C as compared to-20°C storage. *Conservation physiology*, 6, coy033, doi:10.1093/conphys/coy033

Wang X, Miao J, Kang W, Shi S (2023) Exogenous application of salicylic acid improves freezing stress tolerance in alfalfa. *Frontiers in Plant Science*, 14, 1091077, https://doi.org/10.3389/fpls.2023.1091077

Yousif AA (2010) Effect of seed age, size and moisture content on seed quality of sorghum (*Sorghum bicolor* L. Moench). *Research Journal of Agriculture and Biological Sciences*, 6, 522–529

Zhang Q, Pritchard J, Mieog J, Byrne K, Colgrave ML, Wang J, Ral JF (2021) Overexpression of a wheat α-amylase type 2 impact on starch metabolism and abscisic acid sensitivity during grain germination. *The Plant Journal*, 108, 378–393, doi: 10.1111/tpj.15444

Zheng Q, Teng Z, Zhang J, Ye N (2024) ABA inhibits rice seed ageing by reducing H_2O_2 accumulation in the radicle of reeds. *Plants,* 13, 809, doi:10.3390/plants13060809

Ziegler V, Paraginski RT, Ferreira CD (2021) Grain storage systems and effects of moisture, temperature and time on grain quality-A review. *Journal of Stored Products Research*, 91, 101770, doi:10.1016/j.jspr.2021.101770

Legend for Tables

Table 1 Ageing monitoring of winter wheat (*Triticum aestivum*) seeds stored at 10 ± 1 °C and 24.5 ± 0.5 °C, with relative humidity (RH) of 40% and 60 % respectively. Data are shown as mean values of 50 seeds (n = 3) with standard error (± SE). Letters indicate significant differences ($p < 0.05$, one-way ANOVA, Tukey's test) between different storage conditions and following months of seed storage for final seed germination and final seedling emergence

Table 2 Germination kinetics of aged winter wheat seeds (*Triticum aestivum*) in control condition and after EMF treatment. The coleorhiza emergence and radicle emergence were evaluated in small and big seeds. The showed data represent the mean values of 50 seeds (n = 3), while the bars represent standard error (± SE). Letters indicate significant differences (between particular seed group ($p < 0.05$; two-tailed t-test;)

820 **Table 3** Determination of physiological parameters of 72-hours-old seedlings growing from aged
821 small and big seeds of winter wheat (*Triticum aestivum*), in controls and after EMF treatment. Data
822 are shown as mean values of 50 seeds (n = 3) with standard error (± SE). Different letters indicate
823 significant differences at $p < 0.05$ (one-way ANOVA, Tukey's test)

824 **Table 4** Relative frequency (%) of roots in aged winter wheat (*Triticum aestivum*) seedlings 72 h
825 AS, growing for small and big seeds, in controls and after EMF treatment. Data are shown as mean
826 values of 50 seeds ($n = 3$), and bars represent standard error (± SE). Different letters indicate
827 significant differences at $p < 0.05$ (two-tailed t-test)

828

829

830 **Legend for Figures**

831 **Fig 1** Selection of intact and not germinated aged winter wheat (*Triticum aestivum*) seeds stored at
832 24.5 ± 0.5°C and 60% RH, based on seed size expressed as length, width and thickness: 1) small
833 seeds; 2) big seeds. Comparison of dead germinated with broken coleorhiza (Co) seeds observed
834 during the selection process: 3) seed with partially detached scutellum (Sc) and dry emerged root
835 (Ro); 4) seed with developed coleoptile (Cp) and dry emerged root (Ro). **B)** Morphological
836 characterisation of roots in aged winter wheat (*Triticum aestivum*) seeds kept in continuous darkness
837 for 72 h after seeding (AS) on moistened filter paper: 1) three emerged roots observed, with one
838 primary and two seminal roots (considered as germinated and normal); 2) and 3) four or five emerged
839 roots observed, with one primary and three or four seminal roots, respectively (considered as
840 germinated and normal); 4) no emerged roots present, but an emerged coleoptile observed (considered
841 as non-germinated and abnormal); 5 and 6) one or two emerged roots observed, but only seminal
842 roots (considered as germinated and abnormal). The distinction between primary roots (a) and
843 seminal roots (b) is indicated

844 **Fig 2** Evaluation of parameters of germination kinetic for coleorhiza emergence (A, B, C, D, E and
845 F) and radicle emergence (G, H, I, J, K and L) of small (S) and big (B) seeds of winter wheat (*Triticum*
846 *aestivum*) in control conditions (C) and after EMF treatment (T). MGT - Mean Germination Time,
847 CVt - Coefficient of Variability of germination time, CVG - Coefficient of velocity of germination,
848 GI - Germination index, t50 - Median germination time, G - Germinability. Data are shown as mean
849 values of 50 seeds ($n = 3$). Significant differences are marked as dark grey boxes ($p < 0.05$; two-tailed
850 t-test)

851 **Fig 3** Electroconductivity (A) and water absorption (B) changes during the first 24 h AS, in controls
852 and EMF-treated big and small aged wheat seeds. Data are the means of 30 big and 35 small seeds (n
853 = 3) and bars represent standard error (± SE). The symbols indicate significant differences (two-tailed
854 t-test): (*) indicates significant differences between EMF-treated and control groups of aged small
855 seeds ($p < 0.05$); (@) indicates significant differences between EMF-treated and control groups of
856 aged big seeds ($p < 0.05$); (&) indicates significant differences between control groups ($p < 0.05$); (#)
857 indicates significant differences between EMF-treated group ($p < 0.05$)

858 **Fig 4** H_2O_2 content (A) and α-amylase activity (B) in small and big seeds germinating for 72 h, in
859 controls and after EMF treatment. Data are the means (n = 3) and bars represent standard error (±
860 SE). Different symbols indicate significant differences (one-way ANOVA, Tukey's test): (*) indicates
861 significant differences between EMF-treated and control groups of aged small seeds ($p < 0.05$); (@)
862 indicates significant differences between EMF-treated and control groups of aged big seeds ($p <$

0.05); (&) indicates significant differences between control groups ($p < 0.05$); (#) indicates significant differences between EMF-treated group ($p < 0.05$)

Fig 5 Determination of endogenous levels of GA_1 (A), GA_3 (B), GA_4 (C), GA_7 (D), IAA (E), ABA (F), and SA (G) phytohormones in small and big seeds of winter wheat (*Triticum aestivum*), in controls and after EMF treatment. GA_1, GA_3, GA_4, GA_7 - gibberellins, IAA - indol-3-acetic acid, ABA - abscisic acid, SA - salicylic acid, JA - jasmonic acid. Data are shown as mean values ($n = 3$) and the bars represent standard error (± SE). ND indicates "no detected". Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test)

Fig 6 Determination of endogenous levels of GA_1 (A), GA_3 (B), GA_4 (C), GA_7 (D), IAA (E), ABA (F), SA (G), and JA (H) phytohormones in embryos of small and big seeds of winter wheat (*Triticum aestivum*), in controls and after EMF treatment. GA_1, GA_3, GA_4, GA_7 - gibberellins, IAA - indol-3-acetic acid, ABA - abscisic acid, SA - salicylic acid, JA - jasmonic acid. Data are shown as mean values ($n = 3$) and the bars represent standard error (± SE). ND indicates "no detected". Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test)

Fig 7 Determination of endogenous levels of GA_1 (A), GA_3 (B), GA_4 (C), GA_7 (D), IAA (E), ABA (F), SA (G), and JA (H) phytohormones in 72-h-old roots and shoots (coleoptiles) growing from small and big seeds of winter wheat (*Triticum aestivum*), in controls and after EMF treatment. GA_1, GA_3, GA_4, GA_7 - gibberellins, IAA - indol-3-acetic acid, ABA - abscisic acid, SA - salicylic acid, JA - jasmonic acid. Data are shown as mean values ($n = 3$) and the bars represent standard error (± SE). Different letters indicate significant differences at $p < 0.05$ (two-tailed t-test)

Competing Interests

All authors declare they have no financial interests.

Author Contributions

Preliminary conceptualization by Adriana Szmidt-Jaworska and Agnieszka Pawełek; conceptualization by Adriana Szmidt-Jaworska, Daniele Cecchetti and Agnieszka Pawełek; methodology by Daniele Cecchetti, Agnieszka Pawełek and Joanna Wyszkowska; investigation by Daniele Cecchetti adnd Agnieszka Pawełek; statistical analysis by Daniele Cecchetti; preliminary draft preparation by Daniele Cecchetti; original draft preparation by Adriana Szmidt-Jaworska and Agnieszka Pawełek; review and editing by Adriana Szmidt-Jaworska, Agnieszka Pawełek, Daniele Cecchetti and Joanna Wyszkowska; visualization by Daniele Cecchetti, Agnieszka Pawełek and Adriana Szmidt-Jaworska; supervision by Adriana Szmidt-Jaworska; co-supervision Agnieszka Pawełek; funding by Adriana Szmidt-Jaworska.

901 Table 1

902

	Control time	Storage conditions	
		10 ± 1 °C and 40% RH	24.5 ± 0.5 °C and 60% RH
Final Seed Germination (%)	October 2019	98 ± 0.13 a	96 ± 0.17 a
	April 2020	96.66 ± 0.13 a	94.66 ± 0.26 ab
	October 2020	94.66 ± 0.13 ab	90.66 ± 0.13 ab
	April 2021	95.34 ± 0.13 ab	86.66 ± 0.22 abc
	October 2021	93.34 ± 0.20 ab	83.34 ± 0.31 bc
	April 2022	94 ± 0.17 ab	78.66 ± 0.23 c
Final Seedling Emergence (%)	October 2019	92 ± 0.13 ab	88 ± 0.30 ab
	April 2020	92.66 ± 0.13 ab	90.66 ± 0.23 abc
	October 2020	91.34 ± 0.18 ab	87.34 ± 0.13 abc
	April 2021	93.34 ± 0.21 a	84.66 ± 0.22 abc
	October 2021	88.66 ± 0.22 abc	79.34 ± 0.23 bc
	April 2022	88 ± 0.23 abc	76 ± 0.09 c

903

904 Table 2

905

	Time after seeding (h)	Small seeds		Big seeds	
		Control	Treated	Control	Treated
Number of emerged coleorhiza (%)	0	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a
	4	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a
	8	0 ± 0.00 c	0 ± 0.00 c	12 ± 0.2 b	23 ± 0.36 a
	12	16 ± 0.35 d	23 ± 0.26 c	32 ± 0.25 b	45 ± 0.07 a
	16	45 ± 0.19 c	56 ± 0.19 b	58 ± 0.19 b	73 ± 0.15 a
	20	71 ± 0.50 a	80 ± 0.16 a	80 ± 0.27 a	91 ± 0.13 a
	24	87 ± 0.10 c	90 ± 0.09 b	84 ± 0.32 abc	97 ± 0.05 a
Number of emerged radicles (%)	0	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a
	4	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a
	8	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a
	12	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a	0 ± 0.00 a
	16	0 ± 0.00 a	0 ± 0.00 a	1 ± 0.58 a	2 ± 1.00 a
	20	11 ± 0.76 c	17 ± 0.60 ab	9 ± 0.31 bc	17 ± 0.31 a
	24	39 ± 0.50 c	58 ± 0.37 a	42 ± 0.70 bc	53 ± 0.23 ab
	48	80 ± 0.24 ab	80 ± 0.27 b	85 ± 0.22 a	91 ± 0.20 ab
	72	84 ± 0.18 a	81 ± 0.19 a	89 ± 0.35 a	92 ± 0.17 a

906

907 Table 3

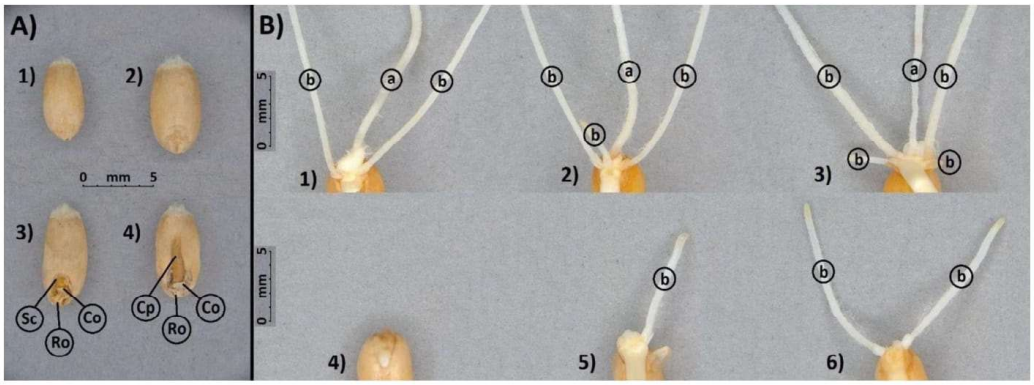
Physiological parameters			Control	Treated
Dry mass (mg)	Root Weight	Small seeds	1.90 ± 0.11 b	2.43 ± 0.15 a
		Big seeds	1.05 ± 0.14 c	1.65 ± 0.23 b
	Coleoptile Weight	Small seeds	1.96 ± 0.06 a	2.07 ± 0.20 a
		Big seeds	1.02 ± 0.12 b	0.99 ± 0.16 b
	Seedling Weight	Small seeds	26.39 ± 0.23 b	26.52 ± 0.23 b
		Big seeds	36.57 ± 0.61 ab	38.1 ± 0.35 a
Fresh mass (mg)	Root Weight	Small seeds	14.80 ± 0.58 a	15.29 ± 0.97 a
		Big seeds	16.67 ± 0.88 a	17.79 ± 1.07 a
	Coleoptile Weight	Small seeds	13.97 ± 0.62 b	15.40 ± 0.76 a
		Big seeds	13.27 ± 0.66 b	13.62 ± 0.96 ab
	Seedling Weight	Small seeds	80.15 ± 1.52 b	80.83 ± 1.48 b
		Big seeds	93.92 ± 1.33 a	101.67 ± 1.51 a
Organ Length (cm)	Root Length	Small seeds	34.63 ± 1.18 b	40.36 ± 1.20 a
		Big seeds	36.62 ± 0.95 ab	37.22 ± 1.00 a
	Coleoptile Length	Small seeds	18.77 ± 0.71 b	20.89 ± 0.68 a
		Big seeds	17.61 ± 0.57 c	18.01 ± 0.58 b

908

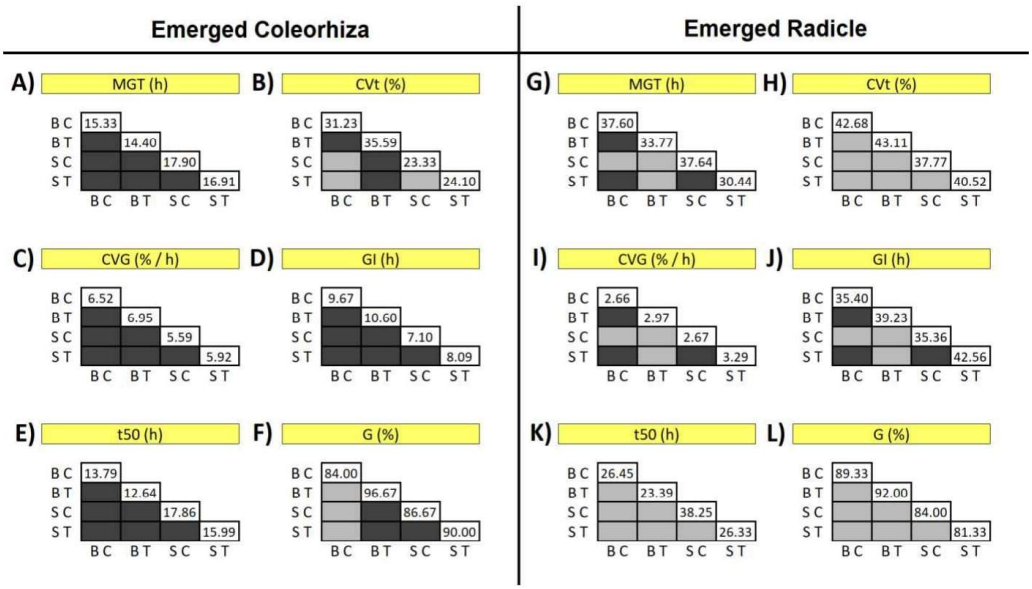
909 Table 4

Relative frequency of number of roots (%)	Small seeds		Big seeds	
	Control	Treated	Control	Treated
0 (abnormal)	2 ± 0.06 a	1.5 ± 0.13 a	1 ± 0.09 a	1 ± 0.09 a
1 (1 seminal; abnormal)	3 ± 0.09 a	1 ± 0.09 a	2.5 ± 0.11 a	1 ± 0.09 a
2 (2 seminals; abnormal)	14.5 ± 0.08 a	9 ± 0.09 a	10.5 ± 0.07 a	3.5 ± 0.04 a
3 (2 seminals and 1 primary; normal)	76.5 ± 0.06 ab	82 ± 0.03 a	80 ± 0.02 ab	80 ± 0.03 b
4 (3 seminals and 1 primary; normal)	3 ± 0.12 b	5.5 ± 0.03 ab	4.5 ± 0.07 ab	9.5 ± 0.10 a
5 (4 seminals and 1 primary; normal)	1 ± 0.09 b	1 ± 0.09 ab	1.5 ± 0.06 b	5 ± 0.03 a
Sum of seeds with abnormal roots (0 – 2)	19.5 ± 0.10 ab	11.5 ± 0.06 ab	14 ± 0.04 a	5.5 ± 0.09 b
Sum of seeds with more than 3 roots (4 – 5)	4 ± 0.11 b	6.5 ± 0.06 b	6 ± 0.04 ab	14.5 ± 0.07 a

Fig 1



921 **Fig 2**


Emerged Coleorhiza
Emerged Radicle
A) MGT (h)
B) CVt (%)
C) CVG (% / h)
D) GI (h)
E) t50 (h)
F) G (%)
G) MGT (h)
H) CVt (%)
I) CVG (% / h)
J) GI (h)
K) t50 (h)
L) G (%)
BC BT SC ST
15.33 14.40 17.90 16.91
31.23 35.59 23.33 24.10
6.52 6.95 5.59 5.92
9.67 10.60 7.10 8.09
13.79 12.64 17.86 15.99
84.00 96.67 86.67 90.00
37.60 33.77 37.64 30.44
42.68 43.11 37.77 40.52
2.66 2.97 2.67 3.29
35.40 39.23 35.36 42.56
26.45 23.39 38.25 26.33
89.33 92.00 84.00 81.33

922

Fig 3

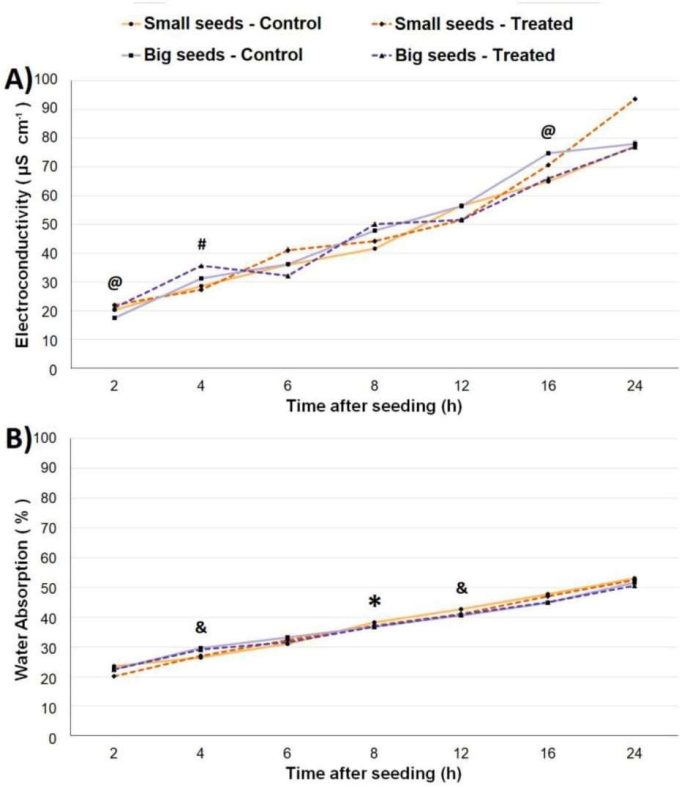
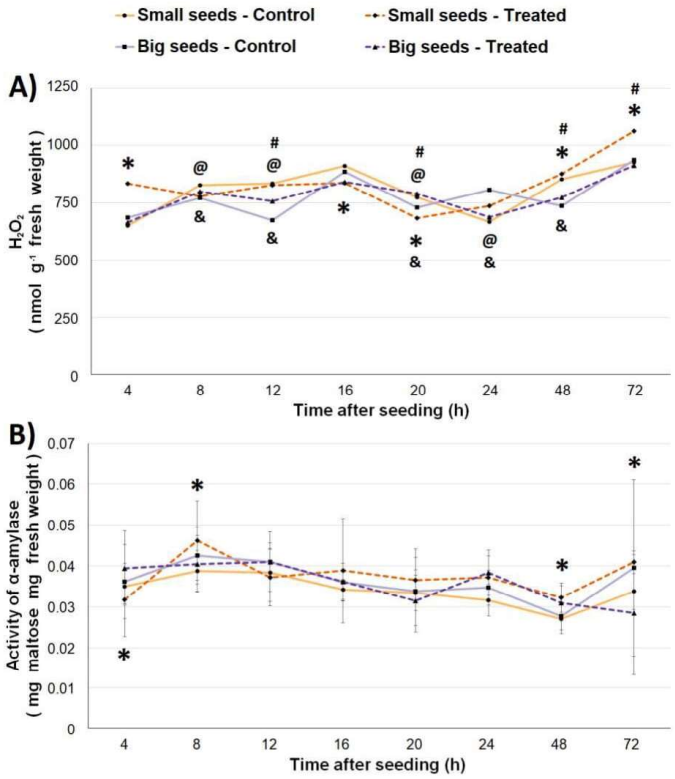
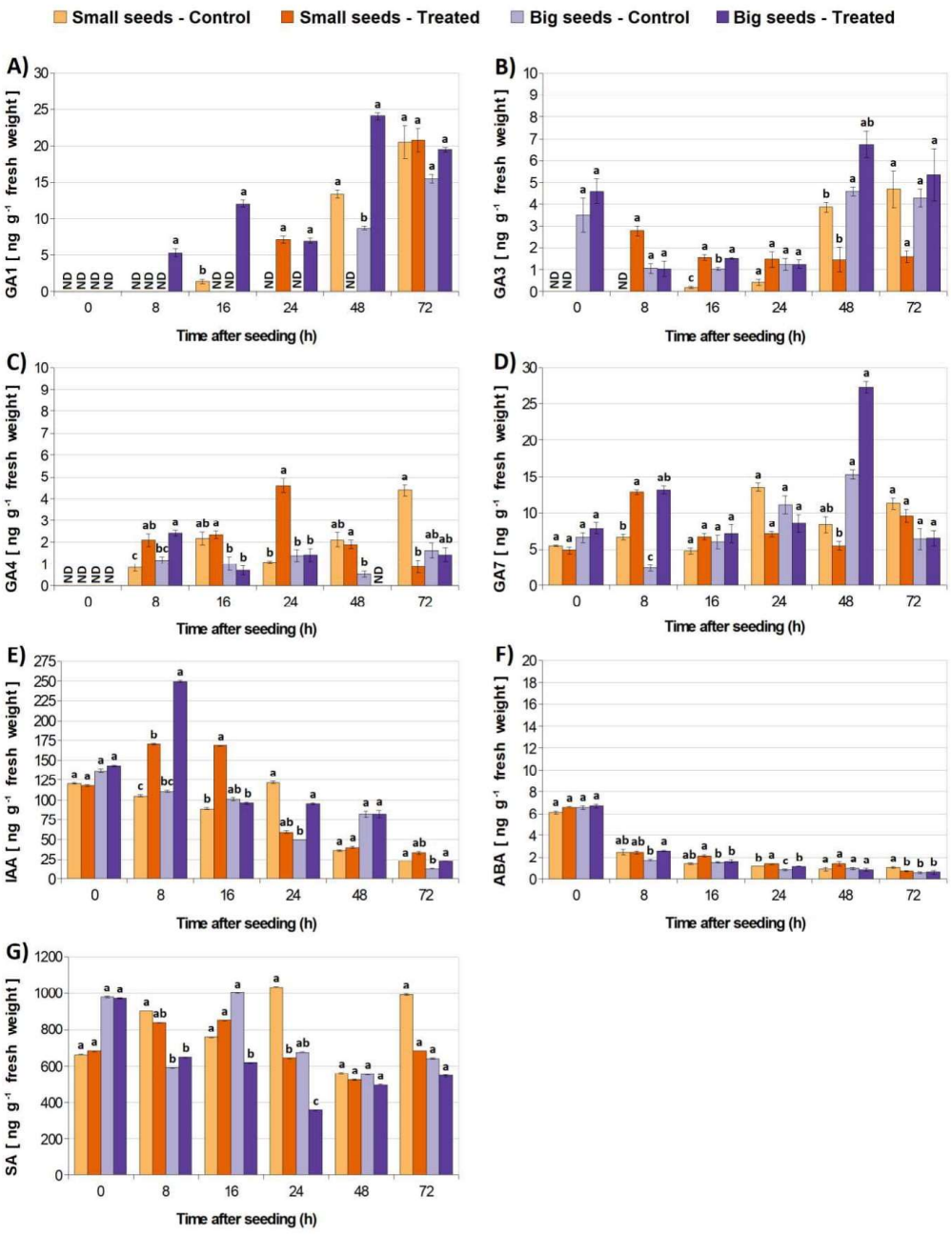

Small seeds - Control
Small seeds - Treated
Big seeds - Control
Big seeds - Treated
A)
Electroconductivity (µS cm⁻¹)
Time after seeding (h)
B)
Water Absorption (%)
Time after seeding (h)

Fig 4

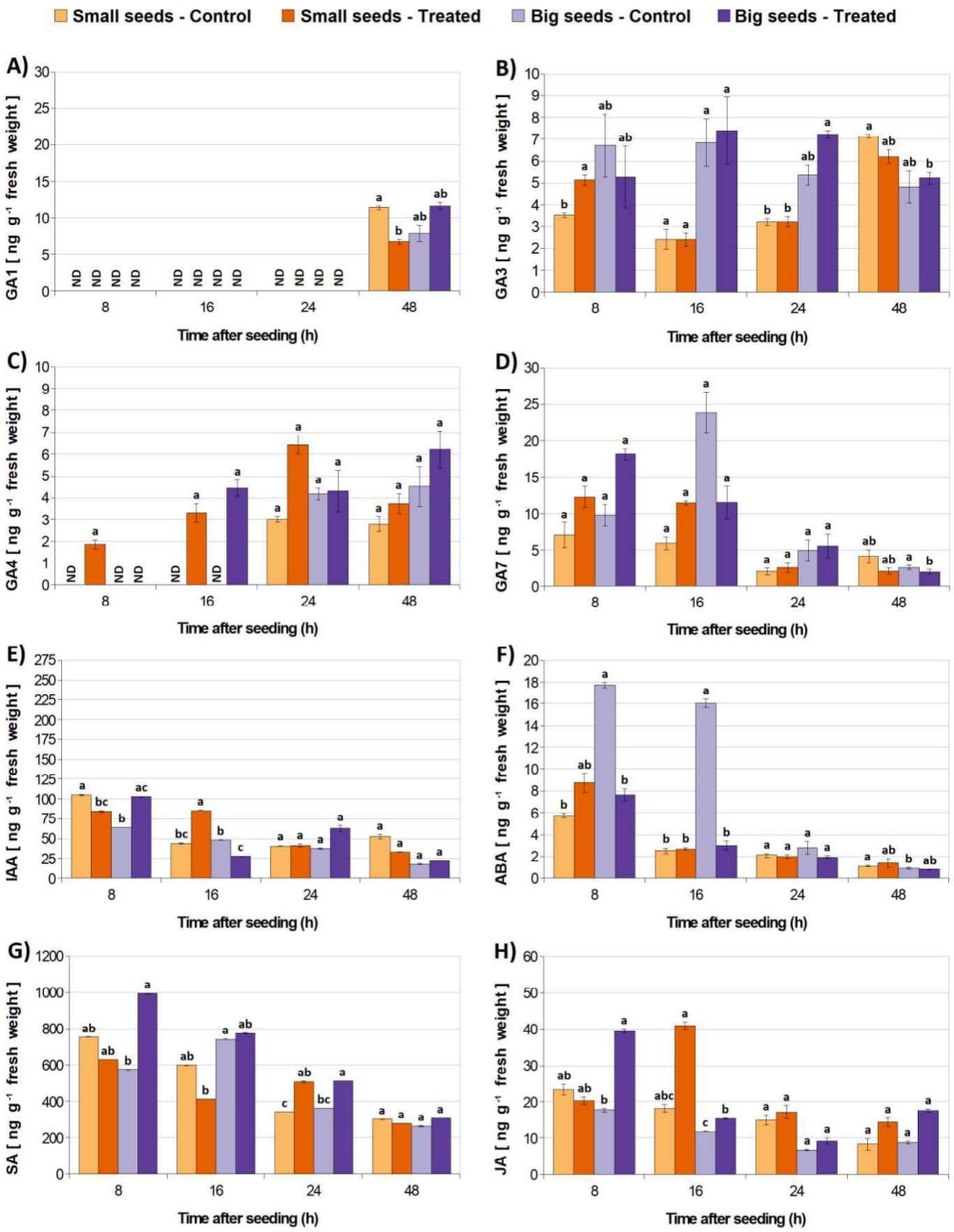

Small seeds - Control
Small seeds - Treated
Big seeds - Control
Big seeds - Treated
A)
H_2O_2 (nmol g^{-1} fresh weight)
Time after seeding (h)
B)
Activity of α-amylase (mg maltose mg fresh weight)
Time after seeding (h)

962 **Fig 5**


Small seeds - Control
Small seeds - Treated
Big seeds - Control
Big seeds - Treated
A)
GA1 [ng g⁻¹ fresh weight]
B)
GA3 [ng g⁻¹ fresh weight]
C)
GA4 [ng g⁻¹ fresh weight]
D)
GA7 [ng g⁻¹ fresh weight]
E)
IAA [ng g⁻¹ fresh weight]
F)
ABA [ng g⁻¹ fresh weight]
G)
SA [ng g⁻¹ fresh weight]
Time after seeding (h)

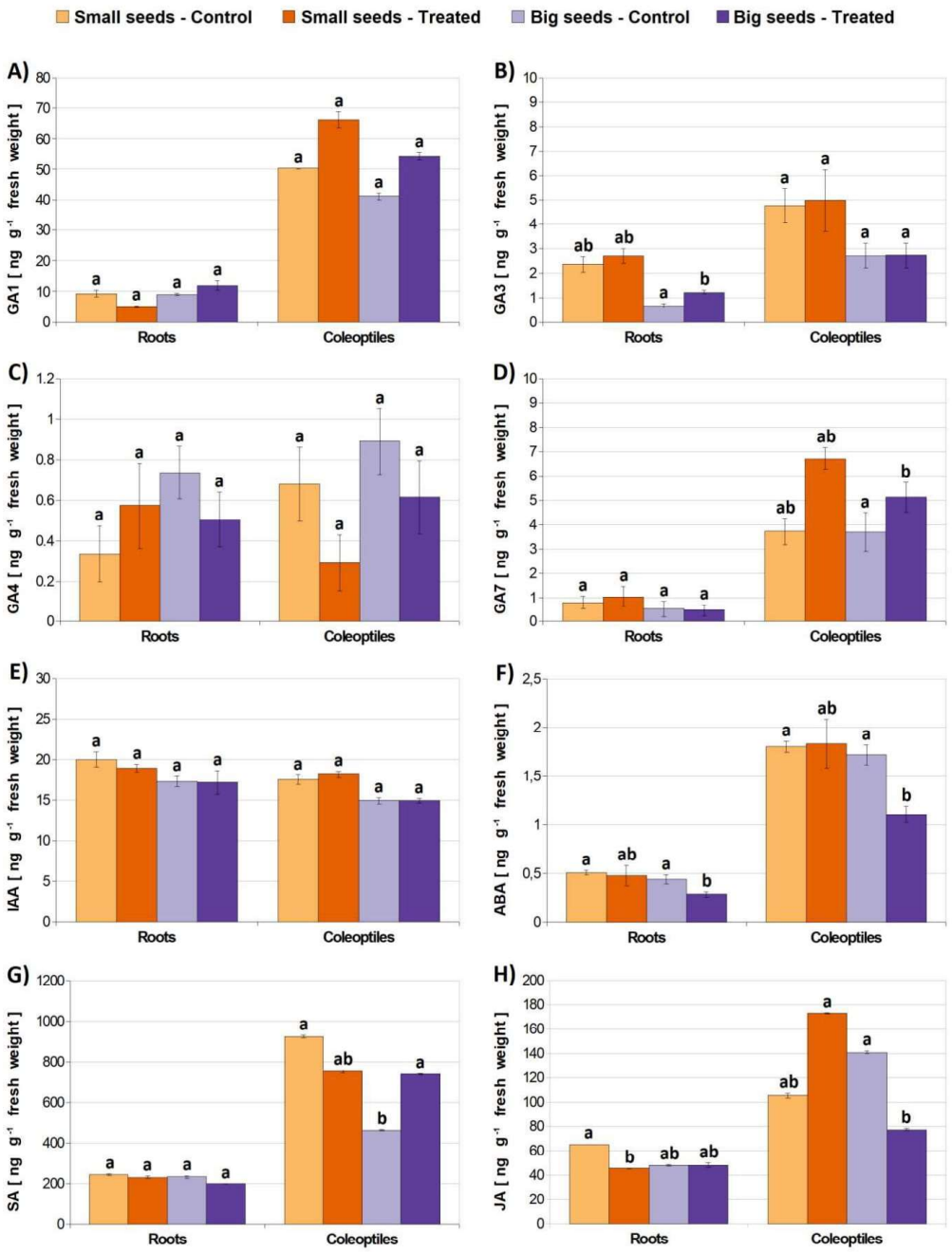
963

964 **Fig 6**


Small seeds - Control
Small seeds - Treated
Big seeds - Control
Big seeds - Treated
A)
GA1 [ng g-1 fresh weight]
B)
GA3 [ng g-1 fresh weight]
C)
GA4 [ng g-1 fresh weight]
D)
GA7 [ng g-1 fresh weight]
E)
IAA [ng g-1 fresh weight]
F)
ABA [ng g-1 fresh weight]
G)
SA [ng g-1 fresh weight]
H)
JA [ng g-1 fresh weight]
Time after seeding (h)
8
16
24
48
ND

965

966 **Fig 7**


Small seeds - Control
Small seeds - Treated
Big seeds - Control
Big seeds - Treated
A)
GA1 [ng g-1 fresh weight]
B)
GA3 [ng g-1 fresh weight]
C)
GA4 [ng g-1 fresh weight]
D)
GA7 [ng g-1 fresh weight]
E)
IAA [ng g-1 fresh weight]
F)
ABA [ng g-1 fresh weight]
G)
SA [ng g-1 fresh weight]
H)
JA [ng g-1 fresh weight]
Roots
Coleoptiles

967

968

35