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# The impact of electromagnetic fields (EMF) on the germination, morphology and physiological responses of *Triticum aestivum* seeds.

**(Dissertation for a doctoral degree)**

# Wpływ pola elektromagnetycznego (PEM) na kiełkowanie, morfologię i reakcje fizjologiczne nasion *Triticum aestivum*.

**(Rozprawa doktorska)**

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## Publications forming the basis of the doctoral dissertation

1. **Cecchetti Daniele**, Pawełek Agnieszka, Wyszkowska Joanna, Antoszewski Marcel, Szmidt-Jaworska Adriana (2022). Treatment of winter wheat (*Triticum aestivum* L.) seeds with electromagnetic field influences germination and phytohormone balance depending in seed size. *Agronomy*, 12 (6), 1423; https://doi.org/10.3390/agronomy12061423 (IF 3,7).

**Contribution to 1st paper:** methodology, investigation, statistical analysis, original draft preparation, visualization

2. **Cecchetti Daniele**, Pawełek Agnieszka, Wyszkowska Joanna, Szmidt-Jaworska Adriana (2024) Promotion of germination, morphological and physiological response of aged *Triticum aestivum* seeds after treatment with electromagnetic field (EMF). *Plant Growth Regulation* (IF 3,5).

**Contribution to 2nd paper:** conceptualization, methodology, investigation, statistical analysis, preliminary draft preparation, review and editing, visualization

## The additional publications representing scientific achievements

1. Pawelek Agnieszka, Wyszkowska Joanna, **Cecchetti Daniele**, Dinka Mergi Daba, Przybylski Krzysztof, Szmidt-Jaworska Adriana (2022) The physiological and biochemical response of field bean (*Vicia faba* L. (partim)) to electromagnetic field exposure is influenced by seed age, light conditions, and growth media. *Agronomy*, 2022, 12 (9), 2161; https://doi.org/10.3390/agronomy12061423 (IF 3,7)
2. Pawelek Agnieszka, Owusu Samuel Acheaw, **Cecchetti Daniele**, Zielińska Adrianna, Wyszkowska Joanna (2022) What evidence exists of crop plants response to exposure to static magnetic and electromagnetic fields? A systematic map protocol. *Environmental Evidence*, 11, 37; https://doi.org/10.1186/s13750-022-00292-w (IF 2,9)

## List of abbreviations

ABA = Abscisic Acid

AMF = Alternating Magnetic Field

AS = After Sowing

EF = Electric Field

EH-MF = Extremely-High Magnetic Field

EL-MF = Extremely-Low Magnetic Field

ELF = Extremely Low Frequency

EMF = Electromagnetic Field

GA(n) = Gibberellin, where (n) is the gibberellin identification number 1, 3, 4 or 7

GMF = Geomagnetic Field

$H_2O_2$ = Hydrogen Peroxide

H-MF = High Magnetic Field

IAA = Indole-3-Acetic Acid

JA = Jasmonic Acid

LC-MS/MS - Liquid Chromatography Tandem Mass Spectrometry

LF = Low Frequency

L-MF = Low Magnetic Field

MF = Magnetic Field

NIR = Non-Ionizing Radiation

PMF = Pulsating Magnetic Field

RF = Radio Frequency

RH = Relative Humidity

ROS = Reactive Oxygen Species

SA = Salicylic Acid

SMF = Static Magnetic Field

## Abstract in English

The application of electromagnetic fields (EMFs) in agriculture has garnered increasing attention in recent years, primarily due to its potential to enhance plant growth parameters and improve the physiological responses of plants to a wide array of environmental stress factors. The use of EMFs represents an innovative approach to mitigating these stresses by promoting plant resilience and overall growth efficiency. Despite this growing interest, the precise mechanisms through which EMFs influence plant processes remain widely unexplored, particularly in relation to how they interact with seed size and quality.

Most existing research has focused on the broad benefits of EMF exposure on plant growth, but the finer details - such as how seed characteristics like size and aging influence the outcomes of EMF treatments - have not been studied. Addressing this gap is crucial, as seeds are the foundation of crop productivity, and their ability to germinate and grow effectively can make or break agricultural success. My research aimed to delve deeper into these interactions by exploring how EMF exposure affects winter wheat (*Triticum aestivum* L.) seeds. In this study, the seeds were divided into two size groups - small and big - and two quality categories - freshly harvested (referred to as "young") and aged seeds. This dual categorization allowed for a comprehensive analysis of how seed size and age impact the effectiveness of EMF treatments, offering a more nuanced understanding of its potential applications.

The first phase of the study focused on young, freshly harvested wheat seeds. These seeds were exposed to EMFs at a frequency of 50 Hz and an intensity of 7 mT, while a control group of seeds was grown under identical conditions without EMF exposure. The experimental design aimed to evaluate a range of growth and physiological parameters, including germination kinetics, early-stage growth, and the concentration of key phytohormones, specifically, indole-3-acetic acid (IAA) and abscisic acid (ABA), within different parts of the seeds, such as roots and coleoptiles.

The results were significant. EMF exposure was found to accelerate both germination and early growth processes, particularly in big seeds. Big seeds exposed to EMFs exhibited faster germination rates and more vigorous early growth compared to their non-EMF-exposed counterparts, especially when germinated in darkness. This suggests that EMFs may enhance the metabolic processes involved in early plant development, possibly by influencing the uptake and utilization of stored nutrients within the seed. In contrast,

despite the registration of increments for several factors, smaller seeds treated with EMFs performed really similar to those kept under control conditions, showing almost the same faster germination kinetics and seedling growth parameters. These differential responses between seed sizes highlight the complexity of the interaction between seed characteristics and EMF exposure, suggesting that the optimal use of EMF treatment in agriculture may need to be tailored based on seed size.

The study also examined the changes in phytohormone concentrations in response to EMF exposure. Notably, IAA levels were raised up in EMF-treated seeds, particularly in the root and coleoptile tissues. Meanwhile, ABA, a hormone that inhibits growth and promotes dormancy under stressful conditions, showed decreased levels in EMF-exposed seeds. This hormonal modulation suggests that EMF exposure may shift the internal hormonal balance for foster the growth-promoting signals, thereby accelerating early-stage development.

In the second phase of the research, the focus shifted to aged seeds that had been stored for 30 months under controlled conditions. Seed aging is a natural process that reduces the vitality and vigor of seeds, leading to slower germination rates, reduced seedling growth, and lower overall crop yields. Aging is often accompanied by biochemical changes, such as the accumulation of reactive oxygen species (ROS), loss of membrane integrity, and reduced enzymatic activity, all of which can negatively impact the ability of seeds to germinate and grow. The aim of this phase was to assess whether EMF exposure could mitigate the detrimental effects of aging on seed performance.

At first, the aged seeds were exposed to EMFs at a frequency of 50 Hz and intensity of 7 mT, with comparisons made to a control group of aged seeds that were not exposed to EMFs. Various physiological and biochemical parameters were evaluated, including germination rates, early-stage growth, membrane integrity, hydrogen peroxide ($H_2O_2$) levels, α-amylase activity, and changes in the concentrations of phytohormones such as gibberellins (GAs), IAA, ABA, jasmonic acid (JA), and salicylic acid (SA). The discovered results indicated that seed size continued to play a significant role in how seeds responded to EMF exposure, even in aged samples. Storage had a more pronounced negative effect on the smaller seeds, which exhibited greater reductions in germination rates and slower early growth parameters when compared to the big seeds. However, pre-sowing EMF treatment significantly improved the performance of aged seeds, particularly the smaller ones. EMF exposure enhanced germination kinetics and promoted stronger early-stage growth in these seeds, suggesting that the treatment could counteract some of the negative effects of aging by stimulating metabolic activity and improving seedling vigor.

Biochemical analyses revealed that EMF treatment positively influenced key physiological markers of seed conditions. For example, aged seeds exposed to EMFs demonstrated improved membrane integrity and reduced $H_2O_2$ levels, indicating a lower accumulation of ROS and less oxidative damage. Additionally, α-amylase activity, critical for breaking down starch reserves during germination, was higher in EMF-treated seeds, providing the energy necessary for more rapid growth. Changes in phytohormone levels also reflected the positive impact of EMFs, with increases in growth-promoting hormones like gibberellins and IAA, and decreases in stress-related hormones such as ABA and JA. The ability of EMFs to enhance germination rates, promote early-stage growth, and modulate hormonal pathways in both young and aged seeds suggests that EMF exposure could be a promising tool for boosting crop yields, particularly in challenging growing conditions where seed quality may be compromised.

One of the most significant findings of this research is the differential response of small and big seeds to EMF exposure. While big seeds appeared to benefit more from EMF treatment, particularly in terms of early growth under dark conditions in both young and aged seeds, smaller young seeds show to have a natural advantage and perform better under control conditions and then their ability is compromised after being subjected to aging conditions. This suggests that the effectiveness of EMF treatment may depend on seed size and potentially other factors such as the specific crop species or environmental conditions.

The findings suggest that EMF treatment holds significant potential as a tool for enhancing seed viability, particularly in the face of environmental stressors and aging-related decline in seed quality.

## Abstract in Polish

Zastosowanie pól elektromagnetycznych (ang. EMF – Electromagnetic Field) w rolnictwie skupia w ostatnich latach coraz większą uwagę, głównie ze względu na potencjał czynników fizycznych w poprawie parametrów wzrostu roślin oraz wzmocnieniu odpowiedzi na czynniki stresowe. Zastosowanie EMF stanowi innowacyjne podejście do łagodzenia stresów, poprzez zwiększanie odporności roślin oraz poprawę efektywności ich wzrostu. Pomimo rosnącego zainteresowania, dokładne mechanizmy, za pomocą których EMF wpływa na procesy roślinne i wywoływane efekty, pozostają w dużej mierze nieodkryte.

Większość prowadzonych badań dotyczyła ogólnych korzyści wpływu EMF na wzrost roślin, jednak bardziej szczegółowe aspekty, takie jak jakość materiału siewnego i wielkość nasion nie były do tej pory przedmiotem badań. Temat który realizowałem miał na celu głębsze zrozumienie tych interakcji poprzez zbadanie, jak ekspozycja na EMF wpływa na nasiona i proces kiełkowania pszenicy ozimej (*Triticum aestivum* L.). Badane nasiona podzielono na dwie grupy pod względem wielkości, małe i duże oraz na dwie kategorie jakości,. świeżo zebrane (nazywane „młodymi") oraz nasiona starzejące się. Taki podział umożliwił kompleksową analizę wpływu wielkości i wieku nasion na procesy fizjologiczne zachodzące podczas kiełkowania, skuteczność traktowania nasion EMF, jednocześnie oferując bardziej złożone zrozumienie potencjalnych zastosowań tego czynnika.

Pierwsza faza badań dotyczyła młodych, świeżo zebranych nasionach pszenicy. Nasiona te były wystawione na działanie EMF o częstotliwości 50 Hz i natężeniu 7 mT, podczas gdy grupa kontrolna nasion wzrastała w identycznych warunkach bez ekspozycji na EMF. Projekt eksperymentu miał na celu ocenę szeregu parametrów wzrostu, jak kinetyka kiełkowania, wzrost we wczesnej fazie oraz koncentracji kluczowych fitohormonów, zwłaszcza kwasu indolilo-3-octowego (IAA) i kwasu abscysynowego (ABA) zarówno w nasionach jak i w korzeniach i koleoptylach.

Osiągnięto ciekawe i znaczące wyniki. Wykazano, że ekspozycja nasion na EMF przyspiesza zarówno kiełkowanie, jak i procesy wczesnego ich wzrostu, szczególnie w przypadku dużych nasion. Wykazane szybsze tempo kiełkowania i bardziej dynamiczny wzrost we wczesnej fazie w porównaniu do nasion z warunków kontrolnych, był bardziej widoczny gdy nasiona kiełkowały w ciemności. Wyciągnięto więc wniosek, że EMF

może wspierać procesy metaboliczne uruchamiane we wczesnych etapach wzrostu roślin, prawdopodobnie wpływając na pobieranie i wykorzystanie zgromadzonych w nasionach składników odżywczych. Nasiona mniejsze z kolei lepiej radziły sobie w warunkach kontrolnych, wykazując szybsze kiełkowanie i wzrost siewek,. Te różne reakcje nasion różnej wielkości podkreślają złożoność interakcji między cechami nasion, a wpływem EMF, sugerując, że optymalne wykorzystanie EMF w rolnictwie może wymagać dostosowania primingu do wielkości nasion.

W trakcie badań przeanalizowano również zmiany poziomu fitohormonów w odpowiedzi na ekspozycję na EMF. Zauważalny wzrost poziomu IAA został odnotowany w nasionach poddanych EMF oraz w tkankach korzeni i koleoptyla siewek. Z kolei poziom ABA, hormonu hamującego wzrost i promującego stan uśpienia w warunkach stresowych, był obniżony po traktowaniu nasion EMF. Ta modulacja hormonalna sugeruje, że ekspozycja na EMF może przesuwać wewnętrzną równowagę hormonalną na korzyść sygnałów promujących wzrost, przyspieszając tym samym rozwój wczesnych faz wzrostu.

W drugiej fazie badań skupiono się na starszych nasionach , które były przechowywane przez 30 miesięcy w kontrolowanych warunkach. Starzenie się nasion jest naturalnym procesem, który zmniejsza ich żywotność i wigor, prowadząc do wolniejszego kiełkowania, słabszego wzrostu siewek i niższych plonów ogólnych. Procesowi starzenia towarzyszą liczne zmiany biochemiczne, takie jak gromadzenie reaktywnych form tlenu (ROS), utrata integralności błon i zmniejszona aktywność enzymatyczna, co negatywnie wpływa na zdolność nasion do kiełkowania i wzrostu. Celem analiz było więc zbadanie czy ekspozycja takich nasion na EMF może złagodzić negatywne skutki wywołane procesem starzenia.

Zastosowano analogiczne warunki doświadczalne, EMF o częstotliwości 50 Hz i natężeniu 7 mT, a porównania dokonano z grupą kontrolną nie poddaną działaniu EMF. Ocenie poddano różne parametry fizjologiczne i biochemiczne, w tym wskaźniki kiełkowania, wczesny wzrost, integralność błon, poziomy nadtlenku wodoru ($H_2O_2$), aktywność α-amylazy oraz zmiany w stężeniach fitohormonów, takich jak gibereliny (GA), IAA, ABA, kwas jasmonowy (JA) i kwas salicylowy (SA).

Wyniki pokazały, że wielkość nasion nadal odgrywała znaczącą rolę w odpowiedzi nasion na ekspozycję w EMF. Przechowywanie miało bardziej negatywny wpływ na mniejsze nasiona, które cechowały znacznie obniżone wskaźniki kiełkowania i wolniejszy wzrost w porównaniu do nasion większych. Jednakże, przedsiewne traktowanie nasion EMF znacznie poprawiło kinetykę kiełkowania starszych nasion, szczególnie tych mniejszych. EMF znacząco wspierało wzrost na wczesnych etapach kiełkowania u tych nasion, sugerując, że ekspozycja na EMF może przeciwdziałać niektórym negatywnym skutkom starzenia się, poprzez stymulację aktywności metabolicznej i poprawę wigor.

Analizy biochemiczne wykazały, że traktowanie EMF pozytywnie wpłynęła na kluczowe fizjologiczne wskaźniki kondycji nasion. Starsze nasiona poddane działaniu EMF wykazywały poprawioną integralność błon oraz niższe stężenia $H_2O_2$, co wskazuje na mniejsze gromadzenie ROS i uszkodzenia oksydacyjne. Ponadto aktywność α-amylazy, która jest kluczowa dla mobilizacji skrobi podczas kiełkowania, była wyższa w nasionach traktowanych EMF, co przekładało się na szybsze tempo wzrostu. Zmiany w poziomach fitohormonów, wzrost IAA i GA, a spadek ABA i JA, również odzwierciedlały pozytywny wpływ EMF na kiełkujące nasiona.

Podsumowując, jednym z najważniejszych odkryć tej pracy jest odmienna reakcja małych i dużych nasion na działanie pola elektromagnetycznego. Podczas gdy duże nasiona pozytywnie reagowały na EMF, co miało swoje przełożenie na zmiany na wczesnych etapach kiełkowania, mniejsze nasiona lepiej sprawdzały się w warunkach kontrolnych. Sugeruje to, że efekt ekspozycji na EMF może zależeć od wielkości nasion, a także od innych czynników, takich jak gatunek rośliny czy warunki środowiskowe.

Tak więc, poprzez zbadanie zależności między ekspozycją na EMF, wielkością nasion i ich wiekiem, przeprowadzone badania pozwoliły zgłębić fizjologiczne i biochemiczne mechanizmy leżące u podstaw kiełkowania nasion i wzrostu siewek. Wyniki wskazują, że ekspozycja nasion na EMF ma znaczący potencjał jako narzędzie służące do poprawy ich żywotności, , szczególnie w obliczu stresorów środowiskowych oraz starzenia się.

## 1. Introduction

The seed is a crucial stage in the life cycle of higher plants, essential for the survival of the species. It serves as the plant's dispersal unit, capable of enduring the interval between seed maturation and the establishment of the next generation as a seedling after germination. The primary function of seeds is to protect the embryo and sense environmental cues, aligning germination with the seasons. Successful seed germination and the development of a normal seedling are vital for the propagation of plant species. Seed germination is a complex process that relies on the precise balance of internal factors (such as phytohormones and endosperm decay) and external factors (such as light and temperature). The transition from dormancy to germination starts when the dry seed encounters water and concludes when the radicle has emerged through the seed coats. This interaction triggers internal processes at various organizational levels of the plant, from molecular and genetic to biochemical, cellular, and physiological (Carrera-Castaño et al., 2020).

The quantity and quality of seeds, including seed size, are crucial traits from an evolutionary perspective for the survival and propagation of plant species. Seed size is genetically regulated and results from the growth programs of the diploid embryo, the triploid endosperm, and the diploid maternal ovule (Guo et al., 2022; Sundaresan, 2005). Additionally, epigenetic and environmental factors, particularly temperature, water, and light, also influence seed development (Li & Li, 2016; Huang et al., 2023). While plant species exhibit a range of seed sizes, the average can vary among individuals within a single population (Sakai & Sakai, 1996). Generally, bigger seeds are more successful in producing seedlings and may germinate earlier than smaller seeds, allowing them to capture environmental resources more effectively and grow larger and faster than seedlings from later-germinating smaller seeds. There is an evolutionary trade-off between producing numerous small seeds, each with a lower chance of survival, and fewer big seeds, most of which successfully produce seedlings. Bigger seeds, with their greater storage reserves, are typically better able to emerge from depth compared to smaller seeds of the same species (Naylor, 2017). Consequently, it can be inferred that big seeds may also endure long periods of dormancy without showing signs of aging (Naylor, 2017).

The decline in seed viability and quality, encompassing attributes such as germinability and vigor, is significantly influenced by the seeds' tolerance to dehydration. Three critical factors regulate this process: the moisture content of the seeds in equilibrium with the relative humidity of their storage environment, the temperature at which the seeds are stored, and the composition of the surrounding gaseous environment (De Vitis et al., 2020; Solberg et al., 2020; Nadarajan et al., 2023).

Seed storage is a commone practice due to the seasonal nature of agricultural crops, the demands of breeding programs, and the need to mitigate risks associated with crop failures. The ability to store seeds effectively ensures that they remain viable and capable of germinating when needed. However, despite the implementation of optimal storage conditions, seeds inevitably undergo deterioration over time. This deterioration is a natural process that involves a gradual loss of seed viability and vigor (Bewley et al., 2013; McDonald, 1999).

Seed deterioration, often named as aging, encompasses a range of changes that lead to diminished seed quality. This decline in quality can be attributed to both intrinsic natural processes and extrinsic environmental factors. Key factors influencing seed deterioration include the initial condition of the seed at the time of storage, its history prior to storage, moisture content or ambient relative humidity, the temperature at which the seeds are stored, the duration of storage, and exposure to various biotic agents such as fungi, bacteria, and insects (Ziegler et al., 2021).

Seeds are generally classified based on their storage requirements into two main categories: orthodox and recalcitrant. Orthodox seeds can endure desiccation and maintain their viability for extended periods when kept in a dry state. This resilience allows them to be stored for long durations without significantly losing quality (Roberts, 1973). On the other hand, recalcitrant seeds, also known as non-orthodox seeds, are highly sensitive to drying and do not survive long-term storage in dry conditions. These seeds require specific conditions to remain viable and are typically unsuited for long-term storage in conventional methods. Even under ideal storage conditions, orthodox seeds will eventually lose their viability. This inevitable decline occurs as seeds age and can manifest in several ways, including delayed field establishment, abnormalities in seedling development, and in some cases, a complete failure of seedling emergence (Bewley et al., 2013; Ziegler et al., 2021). Although the precise mechanisms underlying seed aging are still being studied, several common molecular-level effects have been identified. These include a) disruption of cellular membranes, b) decreased enzyme activity, c) reduced respiration rates, d) diminished efficiency of antioxidant systems, e) lipid peroxidation,

f) impairment of protein synthesis systems, g) depletion of food reserves, and h) damage to genetic integrity (Bewley et al., 2013; Gebeyehu, 2020; Hendry, 1993; Kibinza et al., 2006; McDonald, 1999).

Understanding these physiological and biochemical changes is essential for ensuring successful seedling establishment and crop production. Detailed exploration of these cellular parameters could significantly enhance our ability to assess and predict seed quality. Such insights could lead to the development of improved strategies for maintaining seed viability throughout storage periods, ultimately advancing our capacity to manage and preserve seeds more effectively (Corbineau, 2012; Pritchard, 2020).

Damage due to the seed aging process can be significantly mitigated or even stopped by employing a range of treatments throughout various stages of agronomic management. These treatments are crucial for maintaining seed viability and quality, and they can be effectively applied from the beginning of the crop management cycle, from pre-sowing by harvesting to the storage processes.

In agriculture, advancing techniques to enhance plant growth and yield has always been a central focus. The primary objectives are to boost food production and minimize waste (Savin & Slafer, 2019). This focus has driven the development of innovative methods and fostered a multidisciplinary approach that integrates various research fields. A significant category of techniques in this field is referred to as priming (Paparella et al., 2015; Paul et al., 2022). Priming methods are designed to prepare plants to better withstand environmental stress and improve their resistance.

Seed priming is a widely employed pre-sowing technique favored by seed producers, farmers, and gardeners for improving different components of seed performances, such as germination speed, final germination rate, seedling vigour, uniformity, and resilience to both biotic and abiotic stresses (Lutts et al., 2016; Rifna et al., 2019; Biswas et al., 2023; Kaya et al., 2024). This treatment is typically applied close to the sowing time, either before (pre-sowing) or after (post-sowing) the seeds are planted. Of these, pre-sowing priming is the most thoroughly researched and widely used method in agriculture. This approach involves treating seeds prior to the onset of germination (Lutts et al., 2016).

Pre-sowing priming presents several key benefits: 1) it prevents damage to seedlings since the seeds are treated while in their dormant state, making them less prone to mechanical stress during the sowing process; 2) it maintains the priming effects until sowing time, as the seeds remain biologically inactive and unaffected by environmental conditions during this period; and 3) it is relatively low-cost, which makes it a practical and accessible solution for enhancing seed performance.

Various priming treatments have been developed, yet none has been universally proven effective or beneficial for all crop types (Farooq et al., 2019). The success of these treatments depends on a range of factors, such as the plant species or genotypes involved, the priming agents' water potential, the treatment's length, and the surrounding environmental conditions.

Today, priming techniques are generally divided into two main categories: conventional and modern. Conventional methods include hydro-priming, osmo-priming, bio-priming, and chemo-priming. In contrast, modern techniques encompass nutri-priming, nano-priming, and physical-priming. Each category presents distinct advantages and is suited to specific crop types and environmental conditions. Nevertheless, the variable effectiveness of these techniques underscores the necessity for tailored approaches that address the particular needs of individual agricultural scenarios and crop species (Amir et al., 2024).

Among physical-priming techniques, investigating the effects of magnetic fields (MFs) on seeds has garnered considerable attention (Bera et al., 2022; Sarraf et al., 2020). These techniques are esteemed for their eco-friendliness, relative affordability, and non-invasive nature and have demonstrated beneficial impacts on plant production (Bera et al., 2022; Ramesh et al., 2020; Shabrangy et al., 2021; Waskow et al., 2021). The application of magnetic fields to plants can be categorized into two principal types based on the nature of the magnetic fields and the sources used to generate them (Maffei, 2022). The first category encompasses the different types of magnetic fields, including:

- Static Magnetic Fields (SMFs): Also referred to as permanent magnetic fields, these are produced by permanent magnets, commonly known as lodestones. SMFs are prevalent in nature and are characterized by their constant, unchanging magnetic force.
- Alternating Magnetic Fields (AMFs): These fields are generated by alternating current sources and vary periodically in strength and direction.
- Pulsating Magnetic Fields (PMFs): Produced by pulsed current sources, these fields exhibit a periodic fluctuation in strength, rather than a continuous alteration.

Magnetic fields, in general, are vector fields capable of exerting forces on moving electric charges and magnetic materials. When an electric current flows, SMFs, AMFs, and PMFs can be produced through direct, alternating, or pulsed currents, respectively. A moving charge subjected to a magnetic field experiences a force perpendicular to its velocity and the direction of the magnetic field. This interaction can influence various physiological processes within seeds, potentially enhancing germination and growth outcomes.

Since electric current generates an electric field (EF), it can induce a MF when it flows near a magnetizable material. Given that individual components of magnetic fields and electric fields cannot be isolated from one another, the term "electromagnetic fields" (EMFs) is more appropriate when referring to SMFs, AMFs, and PMFs generated by electric currents.

The second category of magnetic field application is based on the strength or intensity of the MFs. This classification facilitates a comparison between geomagnetic field (GMF) and artificially generated MFs (Lanza & Meloni, 2006; Maffei, 2022). The GMF has an intensity that typically ranges from 30 to 70 µT. This reference value is used to categorize generated magnetic fields into several types:

- Superweak, Conditionally Zero, or Extremely-Low Magnetic Fields (EL-MFs): fields with values below 100 nT.
- Weak or Low Magnetic Fields (L-MFs): fields with intensities ranging from 100 nT to 0.5 µT.
- High Magnetic Fields (H-MFs): fields with intensities from 0.5 µT to 100 µT.
- Very High Magnetic Fields (VH-MFs): fields with intensities exceeding 100 µT up to 15 T.

Beyond intensity, the frequency (Hz) of the magnetic fields also plays a crucial role in physics and biology (Maffei, 2022). This allows for the subdivision of magnetic fields into:

- Extremely Low Frequency (ELF): ranging between 0 Hz ≤ 30 Hz.
- Low Frequency (LF): from 30 Hz to 300 Hz.
- Intermediate Frequencies (IF): between 300 Hz and 10 MHz.
- Radio Frequencies (RF): from 10 MHz to 300 GHz.

MFs that are falling into these categories are considered non-ionizing radiation (NIR).

The impact of MFs on biological systems is influenced by the frequency of the fields, which dictates the expression of various biophysical and biochemical properties (Maffei, 2022). A key threshold for examining these effects is 1 MHz. Frequencies below 1 MHz generally activate tissues' biochemical and biophysical processes, whereas frequencies above this threshold primarily produce thermal effects. Although the precise mechanisms by which MFs exert their effects are not yet fully understood, their importance is well-documented in several contexts, including bird and insect migration (Mouritsen, 2022; Wan et al., 2020), bacterial magnetotaxis (Mandal, 2021), and human health (Maffei, 2022; Daish et al., 2018; Van Huizen et al., 2019). Additionally, the effects of EMFs on plant systems represent a growing area of research (Sarraf et al., 2020; Maffei, 2018).

Extensive research has been conducted to investigate the impact of SMFs and EMFs on crucial plant functions. Findings from numerous studies indicate that varying magnetic field frequencies can positively influence the growth and development of a range of plant species, enhancing overall yield and productivity (Vashisth & Joshi, 2017; Efthimiadou et al., 2014; De Souza et al., 2014; Luo et al., 2022; Radhakrishnan & Kumari, 2012). Several investigations, in particular, have demonstrated that magnetic fields can significantly improve seed germination rates (Podleśny et al., 2004; Florez et al., 2014; Konefał-Janocha et al., 2018; Pauzaite et al., 2018). In addition to the type of magnetic field used, affect the duration of exposure and the intensity are critical factors affecting the outcomes of SMF and EMF applications. Furthermore, the impact of magnetic fields on plants is influenced by specific factors such as plant species, genotype, physiological state, and environmental conditions (Aguilar et al., 2009; Cakmak et al., 2010; Peñuelas et al., 2004). Despite these advancements, the precise mechanisms by which magnetic fields affect plant cells remain poorly understood. It has been suggested that the biological effects of magnetic fields may be mediated through alterations in reactive oxygen species (ROS) levels and cytosolic calcium concentrations, which subsequently lead to various cellular responses, including changes in gene expression and enzymatic activities, ultimately affecting cell metabolism (Kaur et al., 2021). Additionally, the involvement of cryptochromes (blue-light receptors) and the role of auxin signaling in magnetic field-regulated plant growth have also been documented (Jin et al., 2019).

Wheat, a staple cereal crop from the Poaceae family, is integral to global food and feed production (Shewry & Hey, 2015). Over recent years, the yield of winter wheat (*Triticum aestivum* L.) has seen significant growth, driven by advances in cultivar genetics and more intensive agricultural practices (Wójcik-Gront et al., 2021). Nonetheless, with the anticipated global food demand projected to rise by approximately 35–56% from 2010 to 2050 (van Dijk et al., 2021), there is an urgent need to enhance the yield of major crops like wheat. In addition, the environmental impact of intensive farming practices has spurred interest in more sustainable approaches to boost crop production.

Wheat has been at the forefront of research into biomagnetism, with early studies exploring its response to magnetic fields. A notable early investigation in 1963 by Pittman exposed wheat to a magnetic field of 0.065 T (Pittman, 1963). Subsequent research revealed that exposing wheat to a weak magnetic field at 16 (2/3) Hz and 20 μT significantly enhanced germination rates and increased root fresh and dry weights (Fischer et al., 2004). Additionally, exposure to static magnetic fields with energies of 6217 and 24,868 J/m$^3$ promoted early growth in wheat (Martinez et al., 2002). These findings suggest EMFs with LF applied during pre-sowing treatments can positively impact seed development. Consequently, utilizing EMF stimulation may be an effective strategy for improving wheat growth and yield, offering a promising tool for advancing agricultural productivity more sustainably.

## 2. Hypothesis and aim of the study

The main hypothesis of my doctoral thesis posits that EMFs act as a stimulus for the germination process and enhance the quality of seed material. This research was designed to elucidate the mechanisms through which EMFs affect wheat (*Triticum aestivum* L.). The specific objectives of the experimental procedures are as follows:

- identifying an effective method for seed classification based on seed size
- investigating the impact of light conditions on germination parameters of freshly harvested seeds treated with EMFs (50 Hz, 7 mT)
- comparing phytohormone profile alterations induced by EMF exposure
- examining changes in germination parameters of aging seeds
- assessing the potential of EMFs to enhance seed condition and their role in the priming procedure.

The findings from these investigations are detailed in two original articles (Cecchetti et al., 2022; Cecchetti et al., 2024, submitted to a scientific journal), which are included as appendices.

## 3. Discussion of the obtained results

Electromagnetic field (EMF), as a permanent element of the environment, affects the development of life on Earth, and its changes influence physiological, biochemical, genetic, and biophysical processes. In the literature, information indicates the positive impact of electromagnetic fields, primarily 50Hz, on processes occurring during plant ontogenesis. However, the explanation of observed phenomena is unknown. My research aimed to elucidate the mechanisms by which EMFs influence wheat germination. Therefore, my study concentrated on two principal areas of investigation. The first area explored the impact of electromagnetic fields on the germination processes of seeds immediately after harvest, assessing how these fields influence early seedling development and initial growth phases (**as detailed in paper 1**). The second area examined the effects of electromagnetic fields on seeds that have been stored and have begun to undergo the aging process, investigating how such fields might affect seed viability, germination rates, and overall quality during long-term storage (**as presented in paper 2**). This dual focus allowed for a comprehensive understanding of EMF's effects on newly harvested and aging seeds.

### Paper 1.

This study aimed to delve into the mechanisms through which electromagnetic fields (EMF) impact crop plants, with a particular emphasis on wheat (*Triticum aestivum* L.), a key cereal crop of global agricultural importance. The main objective of the research was to investigate whether variations in seed size can influence the plant's response to EMF treatment, thereby affecting germination rates, early growth dynamics, and overall seedling development. When I followed the analysis of zing both small and big wheat seeds, the study sought to uncover size-dependent responses that could provide not only new knowledge but also new insights into optimizing EMF applications in agricultural practices.

A comprehensive evaluation of germination kinetics was conducted, that included parameters such as the rate of coleorhiza and radicle emergence, as well as early seedling growth under both continuous light and dark conditions. Control and EMF-treated seeds were assessed to understand how exposure to electromagnetic fields alters these processes. The dual environment setup allowed me to understand how light conditions interact with EMF treatment to influence plant development at critical early stages, as well allowed me to focus my investigations on other parameters. In addition to germination behaviour, the study also investigated the biochemical changes induced by EMF exposure, particularly focusing on the levels of two key phytohormones: indole-3-acetic acid (IAA), a growth-promoting auxin, and abscisic acid (ABA), a hormone involved in stress responses and seed dormancy. The hormonal profiling permitted me to determine if the EMF treatment led to shifts in IAA and ABA concentrations, which could explain the observed variations in germination speed and seedling vigor.

In wheat seeds, two distinct events characterize the germination process, with specific organs leading these phases. The coleorhiza, a protective layer of the emerging root, is the first visible tissue to appear during germination (0–24 h after sowing, AS). This is followed by the breaking of the coleorhiza as the primary root emerges, marking the completion of germination (0–72 h AS). In my study of germination in young wheat seeds under continuous light (**Figures 4A** and **4B**) and darkness (**Figures 4C** and **4D**), I found that seed size significantly influenced the germination process in both control and EMF-treated seeds. The biggest seeds exhibited the most pronounced changes in continuous darkness compared to other conditions and treatments (**Tables 1** and **2**). Smaller seeds germinated faster than bigger ones in control conditions under both light settings. However, when analysing seeds treated with EMF, I observed substantially increasement in coleorhiza emergence in treated seeds compared to controls, with the greatest increases seen under dark conditions. A similar pattern was observed for radicle emergence, with EMF-treated seeds showing similar increments at the same timepoints as those observed for coleorhiza emergence.

Following my initial observations, I selected additional parameters to assess germination speed better. In both studies described in my thesis, six germination parameters were analysed to measure coleorhiza and radicle emergence in small and big seeds under continuous light and darkness, with or without EMF priming (**Tables 3** and **4**). The results showed that bigger seeds exhibited the most significant changes in continuous darkness across several parameters. In contrast, young small seeds displayed the highest number of significant changes in germination parameters in both light and darkness compared to bigger seeds. However, when analysing the effects of EMF on young seeds, the most notable differences were observed in bigger seeds grown in darkness. In this case, the results indicated that EMF treatment had the most significant impact on the germination of bigger seeds when compared to the control. The observed increments in germination kinetics parameters for big seeds under light conditions may be attributed to enhanced water movement, driven by the heat generated from continuous light exposure. This effect parallels the results of big seeds exposed to EMF under darkness. These findings suggest that , bigger seeds might face a physical disadvantage related to the storage material in the embryo, potentially impacting water absorption, which is a critical factor for the rapid emergence of coleorhiza and the radicle. The results from this study underscore the need to consider both light conditions and seed size in the analysis of early germination stages.

Measurements of morphological parameters, such as root and coleoptile length, as well as fresh and dry weight in wheat seedlings (72 h after sowing), serve as valuable indicators for identifying differences based on selected traits like seed size and for assessing the effects of EMF treatment (**Table 5**). When comparing growth parameters among control samples of young seeds, seedlings from smaller seeds exhibited longer roots and coleoptiles than those from bigger seeds. However, upon comparing EMF-treated seeds with their respective controls, I observed that treated big seeds displayed the most significant increase in both root and coleoptile length. These results suggest that EMF treatment primarily enhances germination and physiological parameters in bigger seeds. Consequently, bigger seeds appear more responsive to EMF treatment during the vigorous growth phase of germination. This highlights the importance of seed size in guiding future research on physical priming techniques like EMF, particularly in optimizing growth outcomes.

Analyzing phytohormone levels is an effective way to understand the responses occurring within plant tissues. Detecting changes in their concentrations provides insights into how plants grow or respond to stimuli like EMF. In my study, the phytohormones indole-3-acetic acid (IAA) and abscisic acid (ABA) were measured during the selected germination period (**Figures 5** and **6**). Despite variations in phytohormone levels across time points and seed sizes under dark conditions, several key observations can be summarized: 1) in control whole seeds and embryos, IAA level was initially higher in small seeds during the early hours of germination, while in big seeds, the increase occurred later (**Figures 5A, 5B,** and **6A**); 2) EMF exposure significantly reduced IAA concentration in whole small seed tissues during the early hours of germination compared to controls (**Figure 5A**), whereas in big seeds, EMF treatment led to an increase in IAA content in both whole seeds and embryos (**Figures 5A** and **5B**); 3) regarding ABA, control samples of whole seeds, embryos and roots revealed that big seeds contained higher ABA levels than small seeds when dry and at the onset of germination (**Figures 5C, 5D,** and **6B**); 4) EMF exposure, on the other hand, resulted in a significant reduction of ABA content in big seeds compared to control, at several time points (**Figures 5C, 5D,** and **6B**). The observed lower IAA level in small seeds may indicate an earlier dormancy breaking compared to big seeds. This aligns with the known role of IAA in regulating seed dormancy in cereals, where the coleorhiza plays a crucial role in breaking dormancy and is associated with reduced IAA levels. My findings confirm that EMF treatment affects seeds differently based on their size. Overall, these results underscore the important role phytohormones play in driving the faster germination and growth of morphological parameters following EMF treatment.

### Paper 2.

The objective of the subsequent study was to explore the priming effects of electromagnetic field (EMF) treatment (50 Hz, 7 mT) on the germination and early growth dynamics of aging wheat (*Triticum aestivum* L) seeds, with a specific emphasis on how this physical stimulus influences various physiological and biochemical processes. Aging seeds often exhibit a decline in vigor and viability, making it crucial to develop effective methods to improve their performance. By applying EMF treatment, my research attempted to assess its potential to enhance the recovery of these aging seeds and support their germination and seedling development.

The study involved a comprehensive examination of various biochemical changes induced by EMF treatment, both in the seeds and the resulting seedlings. These changes included alterations in membrane integrity, a key indicator of cell stability and function, and shifts in hydrogen peroxide ($H_2O_2$) levels, which are closely associated with oxidative stress and signaling during germination. Another critical factor evaluated was α-amylase activity, an enzyme crucial for the breakdown of starch reserves in seeds, which provides the necessary energy for attempting germination and early seedling growth. In addition to these biochemical markers, the study also investigated changes in the levels of important phytohormones, which play a vital role in regulating plant growth and development. Specifically, my research focused on the concentrations of gibberellins (GAs), which are key regulators of germination; indole-3-acetic acid (IAA), an important auxin involved in cell elongation and growth; abscisic acid (ABA), which is known to inhibit germination and promote dormancy; salicylic acid (SA), a signaling molecule involved in defense responses; and jasmonic acid (JA), another hormone implicated in both stress responses and developmental processes.

The insights gained from this study aim to shed light on the underlying mechanisms by which EMF treatment influences seed germination, focusing on how it might reverse or mitigate the negative effects of aging. My research not only provides a deeper understanding of the physiological and hormonal responses to EMF but also highlights the potential of this method as a practical and efficient approach to improving seed quality, especially for deteriorating or less viable seed stocks. By enhancing germination rates and early growth parameters, EMF priming could offer a valuable tool for agricultural practices, particularly in addressing challenges related to seed aging and crop productivity.

Several factors contribute to the aging process in seeds, but those that play critical roles in accelerating or mitigating seed deterioration are temperature and relative humidity (RH). For my study, I carefully selected a controlled environment to induce artificial aging, setting the RH at 60% and maintaining a temperature of 24.5 ± 0.5°C. These specific environmental conditions are known to enhance the oxidative stress experienced by seeds, leading to biochemical changes that gradually compromise seed vigor, viability, and germination potential. Over a storage period of 30 months under these conditions, I observed a marked decline in the germination rate of the aged seeds. This reduction in viability was particularly evident when comparing the data from seeds stored under aging conditions with those stored under more favourable control conditions - at a cooler temperature of 10 ± 1°C with an RH of 40%. Seeds stored under these control conditions maintained a higher germination rate, indicating that cooler temperatures and lower humidity significantly slow down the aging process and preserve seed vitality for a longer duration. This comparison (**Table 1**) underscores the detrimental effects of prolonged exposure to high temperature and humidity on seed conditions. In addition to comparing aged seeds with those in controlled conditions, I also analysed young freshly harvested seeds to serve as a baseline for evaluating the impact of extended storage on seed performance. The results indicated that small seeds exhibited slower germination in aged wheat seeds compared to bigger seeds, specifically in terms of coleorhiza and radicle emergence under control conditions. However, after exposure to EMF, a significant acceleration in both coleorhiza and radicle emergence was observed in small seeds compared to their respective controls.

In the analysis of aged seeds, the same germination parameters were evaluated for both small and big seeds, focusing on six germination velocity metrics, as well as observations of coleorhiza and radicle emergence (**Figure 2** and **Table 2**). Under control conditions, a noticeable reduction in germination speed was observed in aged small seeds compared to aged big seeds (**Figure 2A** and **2C-E)**. However, in aged seeds exposed to EMF treatment, a significant enhancement of both germination parameters and velocity was observed in both small and big seeds (**Figure 2A-G** and **2I-J)**. Interestingly, the EMF treatment had a more pronounced effect on promoting the germination of aged small seeds compared to both the control and big seeds (**Figure 2A, 2C-G** and **2I-J; Table 2)**.

In the analysis of aged seeds, I observed notable differences in morpho-physiological parameters between small and big seeds, with small seeds consistently displaying the highest values in both EMF-treated and control groups (**Table 3**). Seedlings derived from aged small seeds under control conditions exhibited longer roots and coleoptiles, as well as higher dry weights for both organs, when compared to those from aged big seeds. However, these parameters were significantly lower than those of young seeds of the same size, with the most pronounced reductions found in aged small seeds (the reduction was between 45 and 52% for small seeds, while it was between 33 and 40% for big ones). EMF treatment had a differential impact depending on seed size, with the most significant improvements in growth characteristics occurring in treated aged small seeds. Analysis of physiological parameters revealed that aged small seeds had a greater fresh mass and longer coleoptile length than aged big seeds when both were kept under control conditions.

The presence of organs with abnormal architecture often signals the influence of detrimental factors affecting plants, seedlings, or seeds (Copeland & McDonald, 2001). An abnormal number of roots, deviating from the expected average, indicates physiological responses to environmental and physiological changes. Although root numbers vary during germination, the presence of specific roots (primary and seminal) is crucial for promoting plant growth (**Figure 1B** and **Table 4**). In 72-hour-old seedlings, a moderate frequency (10-20%) of abnormal roots was observed in both seed sizes, but the highest value was recorded in small seeds, indicating that the aging process particularly affects this seed size. EMF treatment increase the number of seedlings with five roots in big seeds, and reduce the number of seedlings with zero to two roots. These observations suggest that EMF treatment plays a role in mitigating aging effects but primarily impacts specific seed tissues within the 72-hour post-sowing period. Aged big seeds showed less reduction in germination factors and morpho-physiological parameters compared to aged small seeds. Consequently, seeds with greater nutrient reserves and less damage from aging exhibited a more pronounced response to EMF treatment.

Changes in water absorption play a crucial role in determining how quickly seeds imbibe water, a key trigger for the physiological processes that lead to germination. This rapid water intake activates enzymes, softens seed tissues, and initiates metabolic activity necessary for growth. On the other hand, maintaining membrane integrity is equally important as it serves as a protective barrier, preventing excessive ion leakage from seed cells. Loss of membrane integrity, particularly in aged seeds, can lead to increased permeability, compromising seed viability and delaying or inhibiting germination (**Figure 3**). By assessing membrane stability, researchers can evaluate whether a specific treatment, such as exposure to electromagnetic fields (EMF), aids in preserving or restoring membrane function, thereby supporting the recovery of damaged or aged tissues (Bajji et al., 2002).

In present study, the analysis of membrane integrity in aged seeds showed no significant differences between the control groups (**Figure 3A**), indicating that aging itself may not have drastically affected membrane permeability under these specific conditions. However, big seeds treated with EMF displayed a notable reduction in ion leakage during the initial hours after sowing (AS), suggesting that the treatment helped reinforce membrane stability and limit the outflow of ions, which is a positive indicator of reduced cellular damage (**Figure 3A**). On the other hand, the water uptake analysis revealed more significant changes in small seeds than big ones (**Figure 3B**). Both control and EMF-treated small seeds exhibited more dynamic water absorption patterns, indicating that these seeds may respond more sensitively to changes in environmental conditions and treatments (**Figure 3B**). This heightened water absorption could be a double-edged sword; while it may accelerate the germination process, it could also expose small seeds to greater risks of membrane damage if they cannot maintain ion balance. The data suggest that EMF treatment has a different impact on seed size, with bigger seeds benefiting more from enhanced membrane stability and small seeds showing a greater change in water uptake dynamics. This variation in response highlights the complex interaction between seed size, water absorption, and the protective effects of EMF on seed physiology.

Detecting changes in α-amylase activity and $H_2O_2$ content effectively assesses the physiological alterations occurring in aged seeds and plant tissues (**Figure 4**). An increase in α-amylase activity enhances the breakdown of starch, making it more available as an energy source, which in turn supports the growth of key plant organs (Zhang et al., 2021). Elevated $H_2O_2$ levels, often associated with plant responses to adverse environmental conditions or tissue damage, initiate a cascade of oxidative stress events. This, in turn, triggers the production of molecules that mitigate stress and enhance plant survivability (Bienert et al., 2006).

In the analysis of α-amylase activity, only aged seeds exposed to EMF exhibited a stimulatory effect, particularly in small seeds compared to the control group (**Figure 4B**). On the other hand, $H_2O_2$ levels showed variability between control seeds, with higher concentrations in small seeds than big ones (**Figure 4A**). EMF treatment caused significant alterations in $H_2O_2$ levels in both small and big aged seeds (**Figure 4A**). The most pronounced changes were observed in treated small seeds, where $H_2O_2$ content initially increased, then decreased, and subsequently surged again at the final timepoint of the observation period (**Figure 4A**). The persistently high levels of $H_2O_2$ in aged small seeds across multiple time points suggest that these seeds were under considerable stress. The contrasting patterns between $H_2O_2$ and α-amylase levels indicate the initiation of repair processes, enhancing metabolic activity in the treated small seeds compared to the controls (Hasanuzzaman & Fotopoulos, 2019). This likely reflects an increase in mitochondrial respiration, the primary site of $H_2O_2$ production, signaling the recovery of the seeds from stress (Smirnoff & Arnaud, 2019). The data I collected highlight the oxidative stress induced by the aging process in small seeds and demonstrate the stimulatory effects of EMF treatment in aiding the recovery of these seeds from aging-related damage.

In my research, I conducted an in-depth analysis of selected phytohormones, focusing on aged seeds grown in darkness and categorized by size. This analysis was significantly enhanced by advancements in the phytohormone extraction procedure, allowing for a more comprehensive evaluation of hormone profiles (**Figures 5, 6,** and **7**). The goal was to detect any changes in hormonal levels between 1) the common phytohormones (IAA, and ABA) in both aged and young seeds to understand better how the aging process impacts germination and plant development; 2) other phytohormones determined only in aged seeds (GAs, JA, and SA) to understand which other physiological response is triggered in the plant to tolerate the natural aging stress.

When I analyzed the IAA and ABA contents, the main difference between young and aged seeds was an interesting discovery (Figures 5E-F, 6E-F, and 7E-F)ly., While both IAA and ABA were analyzed, only ABA exhibited significant differences in content between the aged and young seeds (**Figure 5F**). Despite the aging process, the levels of IAA did not show notable variations between aged and young seeds during the early stages of germination (**Figure 5E**). However, in the later stages, small aged seeds demonstrated higher IAA level than their bigger counterparts. This suggests that IAA may play a more prominent role in the growth and development of smaller seeds as they age. Additionally, when seeds of both sizes were treated with EMF, their IAA levels shifted noticeably compared to the controls. The most pronounced changes were observed in treated small aged seeds, where IAA levels initially dropped 8 hours after sowing (AS) before rising again at 16 hours AS, indicating a potential regulatory role for IAA in response to EMF treatment (**Figure 5E**). In terms of ABA, the results were clearer when compared to those obtained from IAA. The analysis revealed a significant, tenfold decrease in ABA content in whole-aged seeds compared to young seeds, highlighting the impact of aging on this important stress-related hormone (**Figure 5F**). ABA is known to inhibit germination and promote dormancy, so its reduction in aged seeds suggests a loss of these protective mechanisms as seeds deteriorate. This fact can also explain the observation of seed germinated inside the contained during the sorting of seeds to be used in this experiment (**Figure 1A**). Furthermore, when examining ABA levels in the whole aged seeds sorted by size, it was found that small seeds contained higher ABA levels than big seeds, possibly reflecting a greater stress response in smaller seeds (**Figure 5F**). The EMF treatment also influenced ABA content, with a significant reduction observed in the roots and coleoptiles of treated big seeds compared to their untreated counterparts (**Figure 7F**). This suggests that EMF may help mitigate the negative effects of aging by lowering ABA levels, particularly in embryos of big seeds, which could lead to improved growth and germination rates (**Figures 6F** and **7F**). Overall, these findings provide valuable insights into how IAA and ABA level changes base on both seed size and EMF treatment in the context of seed aging. The differential effects of EMF on small and big seeds, as reflected in their phytohormonal profiles, suggest that EMF treatment could be a promising tool for improving the germination and growth of aged seeds, particularly for those that are more prone to stress-related deterioration.

Analyzing the gibberellins (GA1, GA3, GA4, and GA7) content was a critical part of this research due to the pivotal roles these hormones play as regulators of plant growth, development, and cell division (**Figures 5A-D, 6A-D** and **7A-D**). Gibberellins are essential for several developmental stages (e.g. promoting germination, and stem elongation), making those molecules crucial to examine how aging and external treatments like EMF influence their levels. Measuring gibberellin content in aged seeds provided insights into which specific gibberellins are most affected by the aging process and how EMF treatment may mitigate or enhance these effects.

In control conditions, GA1 levels were almost undetectable in whole seeds and embryos across most time points (**Figures 5A** and **6A**). GA1 levels were firstly detected in aged small seeds, 16 hours after sowing (AS), and later on their levels were higher in small seeds when was observed the first time the GA1 level in to control big seeds, 48 hours AS (**Figure 5A**). This suggests that GA1 might play a more prominent role in small seeds as they age. Interestingly, EMF treatment had a marked influence on GA1 levels, leading to a significant increase in GA1 content. This effect was particularly noticeable in treated big seeds, where GA1 levels became detectable and were higher than in untreated control seeds, indicating that EMF might stimulate gibberellin production or its activation in bigger seeds during aging (**Figure 5A**). When examining GA3 levels in whole seeds, aged big seeds exhibited higher GA3 content under control conditions, while GA3 was undetectable in aged small seeds (**Figure 5B**). This suggests that GA3 plays a more significant role in big seeds under natural aging conditions. However, after EMF treatment GA3 levels significantly increased in aged small seeds at 8 and 16 hours AS mostly, in both embryos and whole seeds, indicating that EMF may have a stronger stimulating effect on GA3 production or mobilization in smaller seeds (**Figures 5B** and **6B**). The detectable GA3 levels in small seeds appeared early after sowing, highlighting a potential role for GA3 in the early stages of recovery from aging stress in these seeds, while in aged big seeds the changes in GA3 content is detected during the 72-h-stage of germination (**Figures 5B** and **7B**). Regarding GA4, changes in its content were observed during the later stages of the germination process (**Figures 5C** and **6C**). Among the controls, small seeds exhibited the most substantial increases in GA4 content, suggesting that GA4 may become more prominent as the germination process in small seeds (**Figures 5C** and **6C**). EMF treatment further elevated GA4 levels in aged small seeds compared to controls, reinforcing the idea that EMF enhances the hormonal regulation of growth and recovery, particularly in smaller seeds. In contrast, significant changes in GA7 content were only noted in seeds treated with EMF, with the most pronounced increases occurring in treated big seeds, indicating that GA7 might respond specifically to external treatments like EMF in bigger seeds (**Figure 5D**).

The final two phytohormones analyzed in aged wheat seeds were salicylic acid (SA) and jasmonic acid (JA) (**Figures 5G, 6G-H** and **7G-H**). SA is widely known for its role in plant recovery from biotic and abiotic stress, and it shares molecular pathways and physiological responses with other phytohormones discussed in this thesis. It functions as a key signaling molecule involved in defense responses and stress tolerance.

In control conditions, the most notable observation was the high SA content in aged small seeds during the early time points of the experiment, suggesting that small seeds might rely more heavily on SA-mediated stress responses (**Figure 5G**). After EMF treatment, a reduction in SA levels was observed in both whole seeds of treated big and small seeds, (**Figure 5G**). In other hands, in tissues from embryos, treated big seeds maintained higher SA content at more time points compared to their untreated counterparts (**Figures 6G** and **7G**). In contrast to SA, jasmonic acid (JA) was not detected in whole seeds during the 72-hour germination period. This non-detection was mostly caused by the higher background during the analysis conducted at the LC-MS/MS, implying that there are numerous molecules within a whole seed that possess the same m/z ratio as JA. However, during the analysis of the JA level in tissues from embryos, it was possible to detect clearly the content of JA. In embryos EMF treatment led to an increase in JA levels in big seeds, particularly during the early time points (**Figure 6H**). This suggests that JA may be involved in responding to specific stress signals induced by EMF. A reduction in JA content was observed in the coleoptile tissues of treated seeds, indicating that JA's role in these specific tissues might differ depending on external treatments or stress factors (**Figure 7H**). Those observations imply that JA plays a more specialized role in stress responses and its production can be limited to only some tissues in the seeds during the germination process.

Summarizing, these analysis of gibberellins, SA, and JA highlight the complex hormonal responses in aged seeds, emphasizing the importance of these phytohormones in modulating growth, stress recovery, and the effects of EMF treatment during the germination process. The data suggest that while big and small seeds respond differently to aging and EMF, specific phytohormones like GA3, GA4, and SA play critical roles in mediating these responses across different seed sizes.

Overall, my research underscores the significant benefits of using electromagnetic field (EMF) treatment to boost seeds subjected to aging stress. The findings confirm that EMF treatment can effectively mitigate some negative effects associated with seed aging, thereby enhancing seed resilience and overall performance. This treatment not only addresses the damage caused by prolonged exposure to stressful conditions but also supports the seeds' ability to recover and thrive.

Furthermore, the study highlights the versatility of EMF treatment in improving the characteristics of individual seeds, regardless of their size. Seed size often influences various aspects of seed viability, growth, and adaptability. By applying EMF treatment, we can address the unique needs of seeds based on their size, leading to improvements in their growth potential and stress tolerance. This is particularly relevant for optimizing seed performance in diverse agricultural and environmental conditions.

In essence, my research show that EMF treatment can be a valuable tool for enhancing seed quality and survival. It offers a practical solution for addressing the challenges posed by aging and stress, thereby promoting better seed condition and development. This approach can potentially improve seed outcomes on a broader scale, ensuring that seeds, whether small or big, can achieve their maximum potential and contribute effectively to agricultural productivity and sustainability.

## 4. Summary and Conclusions

My doctoral dissertation provided an in-depth investigation into the physiological changes experienced by wheat seeds of varying sizes, specifically small and big, over the initial 72 hours after sowing. The study concentrated on young and naturally aged seeds and assessed the impact of electromagnetic field (EMF) treatment. This approach was innovative and unprecedented in crop research, as the influence of EMF on seed physiology had not been explored before in such detail.

The research led to several key conclusions:

1) **Germination efficiency and morphological development:**

- **Findings**: The study revealed that young small wheat seeds exhibited a notably faster germination rate and achieved superior morphophysiological parameters compared to their big counterparts. Specifically, small seeds not only germinated more quickly but also developed more robust early-stage growth characteristics.
- **Implications**: This implicates that smaller seeds possess an intrinsic advantage during the early stages of growth, potentially due to their faster metabolic processes or more efficient utilization of available resources. This advantage becomes apparent under optimal conditions, where smaller seeds may demonstrate a more rapid and efficient growth trajectory.

2) **Aging tolerance:**

- **Findings**: In naturally aged wheat seeds, bigger seeds displayed a greater capacity to withstand the negative effects of aging compared to smaller seeds. This observation underscores the tolerance of bigger seeds to face aging-related stress.
- **Implications**: Seed size appears to be a crucial factor in determining a seed's ability to endure the adverse effects of aging. The bigger seeds might benefit from better nutrient reserves or a sturdier structural integrity, which contributes to their enhanced tolerance and ability to maintain viability over time.

3) **Impact of EMF treatment:**

- **Findings**: The application of EMF treatment had varied effects depending on seed size. EMF treatment proved to be most effective for big seeds among the young seeds, showing pronounced positive impacts. Additionally, EMF treatment was found to be even more advantageous when applied to small seeds from the naturally aged pool.

- **Implications**: These findings suggest that the efficacy of EMF treatment is influenced by both the initial condition and size of the seeds. This highlights the need for customized priming strategies based on seed characteristics to maximize the benefits of EMF treatment. Selecting the proper treatment according to seed size and age could lead to improved seed performance and tolerance against stress.

4) **Analysis of germination kinetics and phytohormonal content:**

- **Findings**: My dissertation conducted a detailed analysis of germination kinetics and phytohormonal content, focusing particularly on the critical time frame between 8 and 20 hours after sowing. This period was identified as crucial for observing the transient effects of EMF treatment and understanding the physiological changes during the early stages of seed germination.
- **Implications**: The combined analysis of germination kinetics and phytohormonal content provided valuable insights into how EMF treatment affects seed growth and development. By examining hormone levels and germination dynamics, my study shown the complex interactions between EMF treatment and seed physiology, offering a deeper understanding of how seeds respond to various stimuli.

5) **Implications for Future Research and Applications:**

**5.1) Seed priming techniques**:

- **Impact**: My research highlights the significant role of seed size in determining the outcomes of EMF treatment, which could determine to lead significantly implications for the development of seed priming techniques. Understanding how different seed sizes respond to EMF treatment can guide future research and practical applications, leading to more effective and targeted strategies for seed enhancement**.**

**5.2) Comprehensive methodology**:

- **Impact**: Using both germination kinetics and phytohormonal analysis, alongside other investigative methods such as $H_2O_2$ content, α-amylase activity, and root architecture studies, provides an efficient framework for studying seed physiology. This holistic approach is essential for gaining a thorough understanding of seed responses to stress factors and treatments, ultimately contributing to improvements in seed viability and tolerance to stresses.

Overall, the dissertation underscores the importance of integrating innovative techniques like EMF treatment with a comprehensive analysis of seed physiology to advance agricultural practices. The findings offer valuable insights that can enhance seed performance and contribute to sustainable agricultural productivity.

## 5. Laboratory skills acquired during the PhD:

- Implementation of various plant cultivation techniques, including in vitro methods and pot-based growing systems, specifically analyzing germination rates and conducting comprehensive morpho-physiological assessments to evaluate seed performance and growth dynamics.
- Selection and meticulous optimization of seed storage conditions, accompanied by continuous monitoring of aged wheat seeds to track changes over time and assess the impact of different storage environments on seed viability and quality.
- Detailed morphological observation employing a high-resolution imaging microscopy, combined with the use of the ImageJ software program to analyze and quantify structural features and physiological responses of plant tissues.
- Conducting spectrophotometric analyses to accurately quantify hydrogen peroxide ($H_2O_2$) content and measure α-amylase activity, essential for understanding oxidative stress levels and enzymatic activity in seeds.
- Utilization of QuEChERS-based methods for efficient and effective extraction of phytohormones from seed samples, ensuring high recovery rates and accurate representation of hormone concentrations.
- Application of chromatographic techniques, such as solid-phase extraction (SPE), for the purification and separation of phytohormones, facilitating detailed analysis and characterization of individual hormone compounds.
- Advanced phytohormone analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to achieve precise and sensitive quantification of hormone levels, enabling a thorough investigation of hormonal changes and their effects on seed physiology.
- Comprehensive statistical data analysis employing a range of software tools, including PAST for statistical analysis, OpenOffice for data management, and Microsoft Excel for data organization and visualization, to ensure robust interpretation and presentation of research findings.

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