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Review of PhD thesis of MSc Daniele Cecchetti
„The impact of electromagnetic fields (EMF) on the germination, morphology and physiological responses of seeds"
performed under the supervision of Prof. Adriana Szmidt-Jaworska and Dr. Agnieszka Pawełek as a co-supervisor in the Department of Plant Physiology and Biotechnology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Poland

The review is based on a letter from the Dean of the Faculty of Biological and Veterinary Sciences, dated October 28, 2024. This letter informs me of my appointment as a reviewer for the above-mentioned doctoral dissertation following the resolution of the Disciplinary Council of Biological Sciences at Nicolaus Copernicus University in Toruń, No. 77, dated October 25, 2024.

The application of Mr. Daniele Cecchetti for a doctoral degree is based on two original and thematically related scientific works.

1. Original scientific article
   Daniele Cecchetti, Agnieszka Pawełek, Joanna Wyszkowska, Marcel Antoszewski, and Adriana Szmidt-Jaworska. Treatment of winter wheat (*Triticum aestivum* L.) seeds with electromagnetic field influences germination and phytohormone balance depending on seed size. *Agronomy*, 2022, 12(6), 1423, https://doi.org/10.3390/agronomy12061423;
2. Manuscript of an original scientific article
   Daniele Cecchetti, Agnieszka Pawełek, Joanna Wyszkowska, and Adriana Szmidt-Jaworska. Promotion of germination, morphological and physiological response of aged *Triticum aestivum* seeds after treatment with electromagnetic field (EMF). The manuscript was submitted to the *Plant Growth Regulation*.

In the beginning, I must note and correct the information provided by Mr. Daniele Cecchetti in his doctoral dissertation. Namely, the PhD student indicates that the basis for applying for the academic degree of doctor is two publications. However, by now, only the first work is

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a publication, while the second work is not a publication but a manuscript of a publication that was submitted to a journal. To avoid ambiguities, I will use the names Publication and Manuscript in the next part of this review.

The information about the PhD student's contribution to the research is provided in the PhD thesis, which is just below the bibliographic data of the Publication and the Manuscript. The Publication and the Manuscript are multi-authored, but it is essential to highlight that the PhD student is the first author of both works. The PhD student's contribution to the Publication was significant, involving methodology, investigation, statistical analysis, visualization, and original draft preparation. The PhD student's involvement in the Manuscript was even more substantial, including conceptualization, methodology, investigation, statistical analysis, visualization, preliminary draft preparation, and review and editing. As the first author of the Publication and the Manuscript, it is clear that Mr. Daniele Cecchetti's contribution was significant at each stage of the study, from conceptualization through experiment implementation to dissemination of results.

The doctoral thesis is composed of the following parts:

- Acknowledgment,
- Table of contents,
- List of publications (including the Publication and the Manuscript being the basis of the dissertation, as well as additional publications representing scientific achievements of the PhD student),
- List of abbreviations,
- Abstract in English,
- Abstract in Polish,
- Introduction,
- Hypothesis and aim of the study,
- Discussion of the obtained results (divided into two sections dedicated to the Publication and the Manuscript),
- Summary and conclusions,
- Laboratory skills acquired during the PhD study,
- References,
- Copy of the Publication,
- Copy of the Manuscript.

The abstracts in both English and Polish are overly lengthy, as each spans three typewritten pages. Reading them gives the impression of a description of the research rather than a concise summary of the key objectives, results obtained, and conclusions.

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In the "Introduction", the PhD student briefly described the fundamental processes occurring during seed germination and the deterioration of seed condition due to aging. Attention was also drawn to the possibilities of improving seed quality. The procedure of seed priming is mentioned here, and it has been used for years in agriculture and horticulture to improve the condition of seeds. One innovative type of such priming can be treating seeds with an electromagnetic field (EMF). This part of the Introduction presents information on the types and physical parameters of the magnetic field, including EMF. In my view, this fragment could be more concise given that the research aimed to determine the effect of only one type and strength of the magnetic field (EMF, 50 Hz, 7 mT) on the germination of wheat seeds. The Introduction ends with a set of information on the effect of the magnetic field on plants, which I find to give proper background to the study.

The next part of the doctoral thesis is a chapter entitled "Hypothesis and aim of the study". In this chapter, the PhD student presents a brief hypothesis and a detailed list of research goals that were pursued in the study. Unfortunately, the hypothesis is formulated laconic, and a list of particular research goals dominates the section.

In my opinion, the chapter "Discussion of the obtained results" raises the most reservations. First of all, this chapter is not a typical scientific description of the research and obtained results, but it is a kind of story of a doctoral student about the research he conducted. In addition, this chapter contains mainly a description but not a discussion of the experimental results obtained. This description is also not balanced in relation to the Publication and the Manuscript. The PhD student devoted less than three pages to the description of the results contained in the Publication, while the description of the data presented in the Manuscript is much more extensive and detailed and covers eight pages. However, regardless of the length of the description, my assessment of this part of the doctoral dissertation cannot be high. There are many irregularities, ambiguities, and mistakes here, as well as inconsistencies between this chapter and the Publication and the Manuscript. In order not to be unfounded, I will only mention, for example, the lack of any experimental introduction. It is not known how seeds were treated with the EMF, whether they were dry or imbibed seeds, how long the aging period was, or in what external conditions the germination and growth of the seedling took place. Other examples - there is talk about six germination parameters that were tested, but these parameters are not mentioned in the text. The PhD student refers to figures included in the Publication, which illustrate changes in selected germination parameters over time (0-72 hours); however, some of the figures referred to only results from a one-time point (72 hours). The most confusing and hard-to-understand fragment of the Dissertation is a description of changes in the level of IAA and ABA. This fragment is on page 27 of the dissertation and already belongs to the description of results presented in the Manuscript. The PhD student describes the results of changes in the content of two phytohormones in aged seeds but relates them to changes in the content of hormones in young seeds that are presented in the Publication. Unfortunately, the PhD student does not clearly inform the reader about this

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connection, so it is not easy to understand the observations described. Furthermore, only figures from the Manuscript are cited here, which is insufficient. It would be more appropriate to reference figures from both the Manuscript and the Publication in this description.

The chapter "Summary and Conclusions" deserves a positive assessment because the research results are presented here point by point, along with their concise and substantive comments. This chapter highlights an innovative approach to seed improvement, including the improvement of aged seeds, by applying a non-invasive and ecologically friendly method that utilizes EMF. Additionally, the chapter provides a significant commentary on the value of the collected results and their potential applications in agriculture. Following this chapter, there is a list of skills acquired by the PhD student during the doctoral studies, as well as a list of 67 references.

The research of Mr. Daniele Cecchetti, well described in the Publication and the Manuscript, concerns the improvement of wheat seeds (*Triticum aestivum* L.) by EMF in terms of better performance in germination and seedling stages. In my opinion, the PhD student's research is new, innovative, valuable, and of great importance because the knowledge obtained is admittedly basic, but importantly, it can be translated into new agricultural applications aimed at improving crop yield. Currently, these are critical issues because the intensifying climate change and the constantly growing human population are leading to a gradual shrinkage of agricultural areas on a global scale. Obtaining sufficient amounts of food and feed is therefore becoming increasingly important and challenging, especially if this production were not to be predatory and destructive to the environment. Nevertheless, through biological progress in this area of human activity, it is possible to improve the parameters of seed materials or create new varieties of crops that make better use of environmental conditions, the yield of which will meet the increasingly high requirements placed. It should also be emphasized that wheat is one of the most important crop plants worldwide; thus, Mr. Daniele Cecchetti's research is additionally valuable.

To generalize, the most valuable outcome of Mr. Daniele Cecchetti's research is the identification of different responses of small and big seeds to EMF exposure. In more detail, the following achievements can be listed:

- ✓ identification that young small seeds of wheat germinated considerably faster and reflected better germination parameters compared to big seeds. However, the response of big seeds to EMS was more pronounced than the response of small seeds;
- ✓ evidencing that small seeds aged slower than the big seeds, which was reflected in better germination parameters, and the positive response of these seeds to EMF in terms of germination and root and coleoptile growth parameters was also more pronounced than in aged big seeds;
- ✓ discovering that the content of IAA was lower in young small seeds (and isolated embryos) than in young big seeds, while ABA content was even two-fold higher in big seeds than in

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small seeds during the experimental period. EMF lowered the level of IAA similarly in both small and big seeds. ABA content also decreased in both types of seeds, but the effect of EMF was more apparent in big seeds;

- ✓ showing that the content of IAA was decreasing during the experimental period in both types of young whole seeds, while the IAA content in the isolated embryos of the young big seeds was considerably increasing during the experimental period, reaching in controls even a four-fold increase in the last time point of the experiment (72 h). On the other hand, ABA content also successively decreased in both types of whole young seeds. Still, no pronounced time-dependent changes in the content of this phytohormone were observed in isolated embryos. The decrease in IAA and ABA content caused by EMF was both in small and big seeds and isolated embryos, but the most spectacular reduction during the experimental period was observed in IAA content in isolated embryos;
- ✓ demonstrating that aged seeds exposed to EMF showed improved membrane integrity and lower H_2O_2 content, indicating reduced accumulation of reactive oxygen species and oxidative damages. Additionally, EMF elevated α-amylase activity, enhancing the breakdown of storage starch, which altogether resulted in a faster growth rate;
- ✓ describing the changes caused by EMF in phytohormone content in aged small and big seeds as well as in isolated embryos, emphasizing the increase in IAA and GA and decrease in ABA and JA contents, which resulted in the positive effect of EMF on seed germination.

While reviewing the doctoral dissertation, I encountered several ambiguities, and I had a few questions. Therefore, I would like to request that the PhD student address and clarify the following issues.

- o What was the basis for choosing the parameters and the time of EMF operation? Why was EMF with a frequency of 50 Hz and a strength of 7 mT used? Why was the time of EMF operation 24 hours? Were any preliminary studies carried out to establish the experimental conditions for EMF operation? How can we be sure that the applied EMF was not, for example, too weak or too strong and that the observed results were not as pronounced as they could be under other EMF parameters?
- o Why were dry seeds subjected to EMF, not swollen ones? Seeds are the kind of spore form of plants, and naturally, dry seeds are much more resistant to external factors or aging than imbibed seeds. Could EMF in imbibed seeds have more spectacular effects, both positive and negative, for example, in terms of seed germination parameters and phytohormone content changes?
- o Wheat seeds germinate hypogeally and are non-photoblastic seeds, which raises the question of the reasons for conducting some of the experiments under continuous light conditions. Why were the light conditions applied in part of the experiments?
- o The experiments conducted on isolated embryos demonstrated that the changes in phytohormone content were different from those observed in the whole seeds. Thus, it was

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justified to distinguish the whole seeds from the isolated embryos in the research, but it can only be concluded after the fact. So, I would like to understand the reasons behind the decision also to conduct experiments on isolated embryos.

- The regulation of starch degradation during germination of cereal grains was well described by Thomas and Rodriguez already in the 1990s (Thomas and Rodriguez, Plant Physiology, 1994, 106: 1235-1239). In short, the key element in this regulation is the level of glucose, a degradation product of storage starch. Glucose, but also sucrose, regulates the breakdown of storage starch through changes in the expression of selected amylase and gibberellin genes. I will, therefore, ask the PhD student to answer the question of whether and how knowledge of this regulatory system can be used to interpret the results concerning the changes in amylase activity and gibberellins content caused by EMF presented in the Manuscript.
- In the presented studies, seeds were exposed to EMF immediately before they were sown on filter paper, i.e., at the beginning of the imbibition and germination, and the effects were observed within a maximum of 72 hours. In the case of aging seeds, the aging process first took place for 30 months, and only after this period was EMF applied. Could the application of EMF at the beginning of the aging period, or some time in advance before sowing, could also have positive effects? Could the action of EMF slow down the aging of seeds in such circumstances? The questions, therefore, come down to the issue of the durability of the effect of EMF on seeds and the advisability of storing previously EMF-treated seeds. Such an earlier improvement in the condition of seeds and their later storage takes place, for example, during hydro-, osmo-, halo-, or matriconditioning. In these cases, however, the aging processes are intensified, and the possible storage time of conditioned seeds is shortened compared to unconditioned seeds.
- In closing, I have three serious doubts regarding the statistical analysis of the results presented in both the Publication and the Manuscript. Therefore, I would like to ask the doctoral student for detailed explanations and a response to the following reservations.
  1) Why were two different tests (ANOVA and t-test) used to analyze the same type of data? I do not doubt that ANOVA was the right choice, but why and how was the t-test used? I want to point out that the t-test allows for the determination of the statistical significance of differences only between two sets of data, i.e., between two means. Therefore, it is impossible to use this test to analyze differences between several or a dozen means simultaneously. Nevertheless, such an analysis was done, as indicated by the small letters placed in tables and graphs. Such letter designations are used when visualizing the results of statistical analysis performed using ANOVA and not the t-test. Graphically, the results of the statistical analysis performed using the t-test are presented using asterisks and are only related to one pair of means compared with each other.
  2) In what constellations were the results presented in all tables, both in the Publication and in the Manuscript, analyzed? It isn't easy to know what data set was analyzed simultaneously. For example, in Table 1 from the Publication, how were these data

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analyzed? In rows? In columns? Separately for light conditions and separately for darkness? Or maybe all the data from the table were treated as one set and analyzed all together? How do you read these letter symbols, especially since the letter 'a' is next to almost every mean value? And finally, how was the t-test used here?

3) In the results presented in all tables of the Publication and the Manuscript, the letter 'a' dominates. This is incomprehensible because such a designation indicates an almost complete lack of differences between the data presented in the tables, which seems impossible looking at these data. I have similar reservations about the graphs. For example, in Figure 6A in the Publication – the difference in IAA content in coleoptiles of small seeds treated with EMF (black bar) and the IAA content in control coleoptiles of large seeds (red bar) is almost twofold. Still, there is a letter 'b' above both bars. So, is there no statistical significance in the differences between such clearly different IAA content? Many other examples of this type can be seen in the graphs in the Manuscript – Figures 5D, 5E, and 5G, almost all parts of Figure 6 (in Figure 6B, some differences are nearly three-fold, but the letter designations are the same; the letter 'a'), as well as in most parts of Figure 7. Suppose the letter designations for statistics in the Publication and the Manuscript are correct. In that case, the vast majority of differences are not significant, and this is hard to believe from looking at the tables and graphs.

In conclusion, I positively rate Mr. Daniel Cecchetti's doctoral dissertation. His work concerns previously unexplored aspects of plant growth and development and represents a significant contribution to research on the effects of electromagnetic fields (EMF) on plants. The comments and questions I raised in my review do not detract from the significance of the results presented. Instead, they are meant to contribute to the scientific discussion expected during the public defense of the dissertation. I hereby state that the doctoral thesis of Mr. Daniele Cecchetti meets the requirements outlined for postgraduate dissertations in Article 187 of the Act of 20 July 2018 - The Law on Higher Education and Science (consolidated text Journal of Laws of 2023, item 742). Therefore, I respectfully request the Disciplinary Council of Biological Sciences at Nicolaus Copernicus University in Toruń to allow Mr. Daniele Cecchetti to proceed to the following stages of the doctoral process.

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Poznań, December 16, 2024.

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