SZKOŁA GŁÓWNA
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Warsaw, December 12, 2024

The review of the PhD Thesis of Daniele Cecchetti under the title: „ The impact of electromagnetic fields (EMF) on the germination, morphology and physiological responses of *Triticum aestivum* seeds". The review was commissioned by the Scientific Council of the Discipline of Biological Sciences (Institute of Biology) of Nicolaus Copernicus University in Toruń. The doctoral thesis was performed at the Department of Plant Physiology and Biotechnology of Nicolaus Copernicus University in Toruń, under the supervision of Prof. Dr. habil. Adriana Szmidt-Jaworska, and co-supervision of Dr. Agnieszka Pawełek.

Formal assessment

Scientific and substantive value of the dissertation

Changing environmental conditions resulting from human activity significantly impact plants, especially cultivated species. Furthermore, the growing world population creates the need to find solutions related to food and energy production (preferably "green energy"). Improving the quality of plants, including seeds, is the research direction of particular importance to humanity. Nonionizing electromagnetic fields constitute a stimulus able to force a specific plant response. From literature data on this issue, it is known that plant responses to EMF involve various alterations at the level of biochemical reactions and modifications in the gene transcription. This factor impacts the content of reactive oxygen species (ROS). Free oxygen radicals are molecules linked to stress responses. Seeds are the basis of plant production. The high quality of seeds strongly impacts crop success, related to the economic aspect. Another issue is the effect of seed aging on their vigor (the reduction of life span) associated with crop loss.

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The research topic undertaken by the PhD student is linked to the scientific problem of the effect of physical stimulus (electromagnetic fields, EMF) on improving the germination rate of winter wheat (*Triticum aestivum* L.) seeds depending on the grain size and storage time (seed aging). The specific parameters of the EMF (frequency, intensity, and duration), the type of seeds being treated, and environmental conditions influence seeds (e.g. germination rate). Therefore, the results presented by the PhD student constitute an important contribution to this issue and provide new information for modern knowledge.

This dissertation is a compact study with two publications, which have been attached. The first one has already been published (MDPI), and the second one has been submitted to Plant Growth Regulation (Springer):

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).
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 (submitted, IF 3.5).

If the second paper is accepted for publication, the total IF will be: 7.2.

The publications included in the doctoral dissertation are thematically coherent and constitute an original solution to a scientific problem. These publications are multi-authored. The PhD student is the first author of both publications but is not the corresponding author. There are no separate declarations by co-authors about their participation in individual publications. However, the PhD Student's statement (included in the doctoral dissertation) is consistent with the statements of all authors of the attached publications. Therefore, I conclude that his participation was important to verify the hypotheses and achieve the purpose of the doctoral thesis.

Daniele Cecchetti's contribution to 1st paper: methodology, investigation, statistical analysis, original draft preparation, and visualization. Contribution to 2nd paper: conceptualization, methodology, investigation, statistical analysis, preliminary draft preparation, review and

editing, visualization. Moreover, the statement regarding PhD student's laboratory skills acquired during the research (p. 34) indicates the author's significant contribution to obtaining the results. Thus, the doctoral dissertation meets all formal conditions specified in currently applicable legal acts.

This dissertation, written in English, has a total of 97 pages. It has a typical layout for this type of research work, divided into an introduction (7 pages), hypothesis and aim of the study (1 page), discussion of the obtained results (12 pages), summary and conclusions (3 pages), laboratory skills acquired during the PhD (1 page), references (the list contains 67 cited literature items). The doctoral dissertation is preceded by a summary in Polish and English, and provided with a table of contents, and a list of abbreviations. Copies of the publication are attached. From the Introduction part related to the review of available literature, presented by the PhD student, it can be concluded that Daniele Cecchetti is well-versed in his chosen research topics. The hypotheses and the aim of the work were formulated clearly. The methodology is adequate for the research undertaken. Scientific literature selected correctly. I suggest that it would be worth adding citations: Bailly et al. 2008(the „oxidative window" in seed biology), Ali et al. 2024 (EMF impact on maize seeds), Fabrissin et al. 2021 (seed priming and seed longevity, with the description of seed priming treatment).

Assessment of editorial correctness and detailed comments

The work is written in generally correct scientific language. However, the dissertation contains some editorial, stylistic, and grammatical errors (e.g. pp. 16, 20, 26, 28, 29). Minor editorial errors also in the introduction in Polish (pp. 7, 8, 9). These errors do not affect the substantive quality of the work.

Despite the attachment of the publications, the Introduction lacks the characteristics of the experimental material and the description of experimental parameters regarding seed germination, other than EMF (light and temperature). A partial description of the type of wheat seeds can be found in the second publication (p. 65) but is missing precisely in the Introduction part. There are no photographs of the experimental material. Comparisons of large and small seeds, scheme of the general structure of the wheat kernel/seedling, including the most important elements. Fig. 3 from publication is of good quality but the Fig. 1 is not. There are also no photographs of the germination of old wheat grains (along with controls) in

various combinations. These photographs included in the Introduction part would improve its quality. Also, Fig. 4 attached by Daniele Cecchetti is unreadable (p. 94). A larger diagram, perhaps with other colors than in the publication, would make the review easier. In this work, I would also like to see a diagram describing the mode of action of selected hormones on seed dormancy (also the definition of seed dormancy in this dissertation is missing) and seed germination/aging. Such a scheme would improve the doctoral dissertation. One of the elements of the PhD student's research was to analyse the hydrogen peroxide (H_2O_2) content in terms of seed aging. The importance of ROS in seed biology is described by the "oxidation window" model (Bailly et al. 2008), a concept that could have been included in this work by the PhD student. In addition, one or two sentences about the effect of EMF on, for example, membrane potential or the interaction of metabolites would also improve the Introduction part. The PhD student did not provide the full word of the unit ((Tesla), which is less important), even in the list of abbreviations. As for the abbreviations, they are not consistently used when referring to EMF and gibberellins (GA) (pp. 22, 28, 29, 30).

I included further detailed comments related to this dissertation and the attached publications in the questions addressed to the PhD student.

Questions to the author:

* P. 11: How will the PhD student respond to the statement: "Generally, bigger seeds are more successful in producing seedlings and may germinate earlier than smaller seeds...", with: "...small wheat seeds exhibited a notably faster germination rate and achieved superior morphophysiological parameters compared to their big counterparts." (p. 31)?
* P. 12 (bottom of the page): Do the mentioned effects of seed aging apply to dormant (not imbibed) or germinating seeds?
* P. 12: Can the PhD student specify: "Seeds are generally classified based on their storage requirements...".
* P. 13: The PhD student's experiments are based on the pre-treatment of wheat grains (immediately before germination). In the work, the author discusses the issue of priming. Have wheat seeds ever been subjected to EMF priming and germination in the PhD student's laboratory (or elsewhere)? In the author's

opinion, is the pre-treatment of non-aged seeds better, or would priming of non-aged seeds be better using EMF? And what method of improving the quality of seeds would be better for aged seeds?

- P. 13: What did the author mean: "...seeds remain biologically inactive..."?
- P. 18: Were the EMF parameters (used in experiments) tested by the PhD student or researchers from the PhD student's team, or taken from the literature? If from the literature, for the seeds of what plant species?
- The PhD student's experiments are related to free forms of hormones. Does the author know how the levels of bound forms of selected hormones and ABA catabolites change? Could a PhD student suggest what the concentration of these forms of hormones would be?
- The author also mentions the important function of the IAA. Is there a known effect of EMF on IAA metabolism or polar auxin transport in seeds/seedlings?
- P. 22: Why did the PhD student decide to determine the level of H_2O_2 and not some form of free radicals?
- The quality of seeds is related to their viability. Were any other seed viability tests performed, e.g. TTC staining?
- P. 25: Did the PhD student measure the content of storage compounds, e.g. starch?
- P. 25: What did the author mean: "...EMF treatment has a different impact on seed size..."?
- My question is related to the ABA content in aged seeds. But isn't it better if aged seeds have a higher ABA content, which allows them to maintain reduced metabolic activity during storage? Is it common that improving the quality of aged seeds is associated with lowering the content of ABA?
- Has the PhD student ever extended the culture of wheat seedlings to observe the growth and development of plants (long-term effect of EMF) developing from small and large grains and aged ones?

Conclusion

I positively evaluate the doctoral dissertation submitted for review by M.A. Daniele Cecchetti and I declare that it meets the conditions specified in art. 190 section 2 of the Act of July 20,

2018, Law on Higher Education and Science (Journal of Laws of 2023, item 742, as amended). Therefore, I am appealing to the Disciplinary Council of Biological Sciences of the Nicolaus Copernicus University in Toruń for admission of Daniele Cecchetti, M.A. to the following stages of the PhD procedure.

Warsaw, December 12, 2024

Urszula Krasuska

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