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Review

of a doctoral dissertation entitled "The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development" written by Sena Turkan, MSc at the Faculty of Biological and Veterinary Sciences, NICOLAUS COPERNICUS UNIVERSITY (NCU) IN TORUŃ, under the supervision of Grażyna Dąbrowska, Dr. Habil., Professor at NCU (Supervisor) and Agnieszka Mierek-Adamska, PhD (Co-supervisor)

Research background

RelA/SpoT homologue (RSH) proteins, identified for the first time in bacteria as effectors of the stringent response to stressors, are able to synthesize and/or hydrolyze atypical nucleotides referred to as (p)ppGpp or alarmones. In plants, RSH genes regulate nuclear and chloroplast transcription, chloroplast translation, plant development, and responses to biotic and abiotic stresses. To date, RSH genes have been identified and described in a few mono-and dicotyledonous plant species such as *Oryza sativa*, *Arabidopsis thaliana*, *Nicotiana tabacum*, *Capsicum annnum,* and *Pharbitis nil*. A better understanding of the regulatory functions of alarmones can contribute to increasing tolerance in plants exposed to fungal pathogens and climate change-related environmental stressors, which is an important consideration in modern agriculture. The fact that the Author of the doctoral dissertation decided to analyze rapeseed (*Brassica napus* L) deserves special recognition because this oilseed crop is economically important, and Poland is one of the leading rapeseed producers in Europe.

A practical implication of the presented study is the development of seed biocoating composed of chitin, methylcellulose and *Trichoderma viride* spores, which provided a basis for submitting a patent application (Patent No: P.442362). The seed coating can be used as a biological control agent, thus delivering tangible benefits in terms of crop disease prevention.

Therefore, in my opinion, **the dissertation addresses a topical issue and has important theoretical and practical implications.** The doctoral research was conducted by Sena Turkan,

MSc under an interdisciplinary project, in collaboration with the Bydgoszcz University of Science and Technology and the University of Warmia and Mazury in Olsztyn.

Factual information about the doctoral dissertation

The doctoral dissertation submitted for review, entitled **"The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development"**, is based on a collection of three thematically related articles that have been published in scientific journals.

The dissertation consists of the following three articles:

P1: Dąbrowska G.B., **Turkan S.**, Tylman-Mojżeszek W., Mierek-Adamska A. *In silico* study of RSH (RelA/SpoT homologs) gene family and the expression analysis in response to PGPR bacteria and salinity in *Brassica napus*. International Journal of Molecular Sciences, 2021, 22(19): 10666. doi: 10.3390/ijms221910666.

P2: Turkan S., Mierek-Adamska A., Głowacka K., Szydłowska-Czerniak A., Rewers M., Jędrzejczyk I., Dąbrowska G.B. Localization, and expression of *CRSH* transcript, level of calcium ions, and cell cycle activity during *Brassica napus* L. seed development. Industrial Crops & Products, 2023, 195: 116439. doi: 10.1016/j.indcrop.2023.116439.

P3: Turkan S., Mierek-Adamska A., Kulasek M., Konieczna W.B., Dąbrowska G.B. New seed coating containing *Trichoderma viride* with anti-pathogenic properties. PeerJ, 2023, 11: e15392. doi: 10.7717/peerj.15392.

All of the above articles are original research papers published in scientific journals with impact factors, indexed in the Journal Citation Reports (JCR) database. The cumulative impact factor (IF) for the journals where articles P1 - P3 were published is 16.718 (in the year of publication), and the total number of points assigned by the Ministry of Education and Science is 440 (in the year of publication).

Articles P1 - P3 were written by multiple authors. The Candidate is the first author in two articles (P2, P3), the second author in one article (P1), and the corresponding author in one article (P3).

In addition to the above publications, the dissertation includes a synthetic description of an original solution to the research problem (in English), abstracts in English and Polish, and the contribution statements of the Candidate and the remaining co-authors. According to the statements detailing their percentage contributions to each article, Sena Turkan made substantial contributions to all works (P1-35%, P2-70%, and P3-65%); she participated in the most important stages of the research process, i.e. the development of the study concept,

conducting the study, data curation, interpretation and discussion of results, and preparation of the manuscript, which indicates that the Candidate is able to conduct independent research. The dissertation has a standard layout. The research problem, research hypotheses, aims and objectives of the study were described in sufficient detail, and the issues addressed by the study were presented concisely and succinctly.

In view of the above, the doctoral dissertation submitted for review meets the formal requirements specified in art. 187, section 3 and section 4 of the Act of 20 July 2018 - Law on Higher Education and Science (Journal of Laws 2018, item 1668, as amended).

Substantive assessment of the doctoral dissertation

The main aim of the study was to determine the structure and function of RSH proteins in rapeseed plants, homologous to bacterial stringent response proteins, and the role of the stringent response in the growth and development of plants and their adaptation to environmental stress. Moreover, as part of her research work, the Candidate developed an innovative seed coating with antimicrobial and growth-promoting properties for rapeseed plants.

Having carefully read the entire dissertation, I can conclude that the research articles that provided a basis for the dissertation form a cohesive body of work and address related issues, and therefore comply with the provisions of the Act of 20 July 2018 - Law on Higher Education and Science.

The specific objectives of the study were as follows:

1. to analyze nucleotide and amino acid sequences in three groups of RSH proteins in different species of the genus *Brassica* (in silico);
2. to analyze the expression of the C*RSH* gene (qPCR) and the localization of the *BnCRSH* transcript (fluorescence *in-situ* hybridization) in order to determine whether seed development in *B. napus* is regulated by the calcium-dependent stringent response;
3. to analyze the expression of the *BnRSH* gene in rapeseed seedlings in response to salinity stress and the presence of plant growth-promoting rhizobacteria (PGPR): *S. liquefaciens*, *Serratia plymuthica*, and *Massilia timonae*, and plant growth-promoting fungi (PGPF): *Trichoderma viride* (strains TvI and TvII) in order to elucidate the role of the stringent response in the plant response to abiotic and biotic factors;
4. to develop seed biocoating composed of chitin, methylcellulose and *Trichoderma viride* spores, and evaluate its influence on seed germination, seedling growth, and plant metabolism expressed in terms of the activity of superoxide dismutase (SOD) and the expression of RSH

genes in *B. napus*.

Based on the research findings, the Candidate formulated four general conclusions:

(P1) a total of 14 *RSH* genes were identified in the genome of the polyploid plant *B. napus*, and an analysis of amino acid sequences revealed that they could be divided into three subgroups similar to those found in other plant species, i.e. RSH1, RSH2/3, and CRSH;

(P1) the promoter regions of the identified genes harbor regulatory elements that respond to various environmental signals such as light, hormones, and factors evoking abiotic and biotic stresses; these findings suggest that RSH proteins play an important role in plant adaptation to stress conditions;

(P2) increasing levels of Ca^{2+} ions in developing *B. napus* seeds stimulate CRSH activity, which leads to the accumulation of alarmones whose increased concentrations inhibit the expression of nuclear and plastid genes, inducing seed dormancy in late stages of plant development;

(P1 and P3) biotic and abiotic factors exert varied effects on the expression of *BnRSH* genes, which indicates that the stringent response may be one of the pathways *via* which plant-growth promoting bacteria (PGPB) promote plant growth and development;

(P3) *T. viride* strains inhibit, under *in vitro* conditions, the growth of important plant pathogens such as *B. cinerea*, *F. culmorum* and *Colletotrichum* spp.;

(P3) the developed innovative seed coating exerted a beneficial effect on seedling growth by increasing the length and biomass of shoots and roots.

The merits of the reviewed dissertation include complexity, a clear structure, and a synthetic and clear presentation of scientific evidence. The writing style is appropriate, the dissertation is characterized by high levels of correctness, clarity and conciseness, and it has been properly edited. The dissertation provides valuable practical information that enabled the Candidate to submit a patent application. In my opinion, the development of innovative seed coating is an important scientific achievement. The seed coating can be widely used as part of a biological control strategy that, in an era of reduced fungicide application, can become an important component of crop protection programs. The submitted dissertation testifies to the research methodology skills of Sena Turkan, confirms her excellent knowledge of the addressed research problems, and demonstrates her ability to both conduct independent research and work collaboratively as a member of research teams.

Having analyzed the submitted dissertation, I would like to make some comments and ask

the Candidate the following questions:

1. What was the rationale behind choosing the species of pathogenic fungi analyzed in research article P3 since only one of them is a common rapeseed pathogen?
2. Will the efficacy of the developed seed coating be tested on artificially inoculated seedlings, in pot and field experiments?

The above questions detract nothing from my high opinion about the achievements described in the dissertation, and they are merely an expression of my intention to participate in a scientific debate and discussion. I believe that the presented research has considerable theoretical and practical potential, and should be continued in the future.

The doctoral dissertation submitted for review, entitled "**The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development**", consists of a collection of three thematically related articles. I have no doubt that this pioneering work provides new insights and original contributions to the existing body of knowledge. The presented findings are valuable from a scientific perspective, and have important practical implications. The three thematically related articles have been published in prestigious scientific journals: International Journal of Molecular Sciences (IF=6.208), Industrial Crops & Products (IF=6.449), and PeerJ, (IF=2.929). All of them address unique research topics and introduce new ideas, and research results have practical significance, as manifested by the submitted patent application. **I request that the dissertation be recognized with an award.**

Final evaluation statement

The doctoral dissertation by Sena Turkan, MSc, entitled "**The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development**", complies with the provisions of the Act of 20 July 2018 - Law on Higher Education and Science (**Journal of Laws 2018, item 1668, as amended**) regarding doctoral dissertations. **Therefore, I request that Sena Turkan, MSc be admitted to the subsequent stages of the procedure for the conferment of a doctoral degree by the Discipline Council for Biological Sciences at the Nicolaus Copernicus University in Toruń.**

10.08.2023 r. Adam Okorski