

PhD Thesis Acceptance Report  
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**Candidate's name and surname:** Sena Turkan

**PhD Thesis Title:** The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development

**Thesis Supervisor:** dr hab. Grażyna Dąbrowska, prof. NCU

**Assistant Supervisor / Second Supervisor/ Co-supervisor (if applicable):** dr Agnieszka Mierek-Adamska

**Reviewer:** dr hab. inż. Tomasz Warzecha, prof. URK

## THESIS EVALUATION

### 1. Formal evaluation

PhD thesis being under evaluation was completed in the Department of Genetics, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Toruń, Lwowska 1, 87-100 Torun, Poland. The supervisor of the thesis was dr hab. Grażyna Dąbrowska, prof. NCU, and co-supervisor dr Agnieszka Mierek-Adamska. The review was prepared based on the Resolution of Scientific Board of Discipline of Biological Sciences Nicolaus Copernicus University in Toruń no 44 from 29-th of June 2023, including information about designating me as reviewer of the PhD thesis of Sena Turkan entitled: “ The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development”. Presented for the review PhD thesis consists of three scientific articles with the description of each one in the separate part with the introduction, discussion and the summary of all achievements. The articles were published in recognized in the field of biology highly rated journals included in the JCR list, each one possessed high impact factor. The series of three articles are consistent and concern important biological phenomenon of gene expression regulation under various favourable and adverse environmental conditions, and are the justification of the thesis, this is an important formal requirement in accordance with the art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2023, item 742 with further amendments).

The list of articles are as follows:

1. 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.
2. **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.

**3. 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

The articles were published in the years 2021-2023 and the sum of impact factor according to JCR list equals to 15,718 (440 points according to the list of Ministry of Education and Sciences, Poland).

The articles were subject to review by experts in the field chosen by Editorial Boards of each journal, therefore I would like to focus on the structure of the PhD thesis, merits and integrity of the topic presented in each article as the solution of general scientific problem.

PhD thesis of Ms. Sena Turkan includes all together 104 pages, and possess following order:

List of papers

List of abbreviations

Introduction with subchapters:

Alarmones synthases and hydrolases in plants,

The physiological functions of RelA/SpoT (RSH) in plants

Cross-talk between the stringent response pathway and other signalling pathways

Canola (*Brassica napus* L.) growth and development

The aim of the study

Discussion

Summary of results and conclusions

References

Abstracts

Publication list

Publication I

Publication II

Publication III

Declarations of co-authors

## **2. Scientific merit of the thesis**

### **a. Originality of the research:**

The candidate's dissertation describes the regulatory role of nucleotides (p)ppGpp (alarmones), which were first discovered in *Escherichia coli*, as one of the mechanism to regulate a number of cellular metabolic processes with the involvement of *RelA/SpoT* genes. Ms. Sena Turkan based on wide and recent references explained that the stringent response is the name given to the alarmone-dependent regulation of cell metabolism. Plants' genes for alarmone production and degradation were discovered at the early twenty-first century, proving that the stringent response is a highly evolutionary conserved regulatory mechanism. Numerous plant species have been used to identify and describe the plant *RelA/SpoT* Homolog (*RSH*) genes. Alarmones have also been demonstrated to accumulate mostly in chloroplasts.

Overall presented study possessed strong scientific aspect which is very important from formal point of view to be in accordance with the art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2023, item 742 with further amendments), since the thesis has added a novelty to our understanding of the probable functions of RSH proteins in *Brassica napus*, especially in terms of plant growth and development, stress tolerance, and interactions with microbes. The study's findings based on a biological model

utilized in the experiments i.e. *B. napus* on the stringent response provide a substantial contribution to our understanding of general mechanisms which can be partially transferred to other plants mechanisms regulating growth and tolerance to stresses. Since stringent response is a highly evolutionary conserved regulatory mechanism it could be also considered as prospective tactics for the survival not only as a single organism but also the entire species, it is crucial to comprehend the methods by which RSH regulates plant metabolisms in variable environmental conditions. I would also like to stress that presented PhD thesis additionally possessed important applied aspect since better mechanism of mitigation the stresses in the group of crop plants might resulted in increasing agricultural yield and biologically controlling plant diseases. The Author with the team have also created a brand-new biocoating that might be utilized to enhance crop productivity and biocontrol phytopathogens. The concept of novel seed coat consisting of biodegradable materials and protective microorganisms presented in article no. 3 in (PeerJ journal) has been utilized to prepare patent application to Patent Office of the Republic of Poland (No. P.442362). This is certainly unique and extra applied value of presented by Ms. Sena Turkan PhD thesis.

b. Scientific merit of the chapters / articles:

Six comprehensive chapters serve as the dissertation's basis. Chapter I gives introduction and detailed background for studies arranged in subchapters. Subchapter 1 describes role of alarmone its chemical structure, alarmone sythases and hydrolases. Subchapter 2 gives the overview about physiological function of RelA/SpoT, RSH protein family in plants general but with the impact of the RSH composition in *Brassicaceae*. Subchapter 3 describes connections between the stringent response pathway and other alternatives of signal transduction in plants. Chapter 4 presents characteristics of canola (*B.napus* L) its growth and development and importance in crop production and human consumption. In the next chapter "The aims of the study" Author presents the PhD main objective and four specific subobjectives/questions. The aims of the study are clearly expressed showing the specific study tasks leading to the answers to these questions.

The core objective of PhD thesis is to determine the structure and function of *B. napus* proteins homologues to bacterial stringent response proteins, and the role of the plant stringent response in growth and development and adaptation to various environmental conditions which canola could face during the vegetation. Another applied aim of the candidate's actions was developing an novel seed coating with favourable properties e.g. protective against pathogenes and plant growth-promoting properties for canola seeds.

Chapter 3 "Discussion" serves a comprehensive analysis of Candidate's own findings against the background of abundant cited literature, which demonstrates that Ms. Sena Turkan is very involved in the topic and proves her deep knowledge of the role of alarmones in different organisms and induction of different RSH proteins as a results of various stresses. The next chapter "Summary of results and conclusions" gives an overview of major findings which could be presented in extremally compact form as follows: Overall, this study has added to our understanding of the probable functions of RSH proteins in *B. napus*, especially in terms of plant growth and development, stress tolerance, and interactions with microbes. In the subsequent paragraph I would like to present more detailed opinion about the articles aims and conclusions. The next chapter no. 5 is "References" consisted of 99 items. Chapter 6 is "Abstract" where we can find the condensed concept of the study in English and in Polish language. Then the next page (36) "Publication list" arranged as a table with the current IF and points granted by Ministry of Education and Science, Poland for each article and bottom row with summarized IF and MEiN points.

The next part with no numbered pages are the articles and at this point I would like to present the aims and conclusions in more specific way.

#### Publication I (page 37)

The main goal was to understand the function of the stringent response in the plant's reaction to abiotic (salinity stress) and biotic stimuli (*Serratia liquefaciens*, *S. plymuthica*, and *Massilia timonae*, PGPR bacteria for which the ability to promote the growth of canola has been confirmed), researchers analyzed the expression of *BnRSHs* in *B. napus* seedlings in response to salt stress, response to the presence of plant growth-promoting rhizobacteria (PGPR), and response to plant growth-promoting fungus (PGPF).

Based on the references the Candidate has presented recent information that in the polyploid plant *B. napus*' genome there are 14 *RSH* genes (including 2 pseudogens). According to an *in silico* examination of the *RSH* amino acid sequences from *B. napus*, there are three subgroups that are reminiscent of other plant species: *RSH1*, *RSH2/3*, and *CRSH*. These genes' promoter regions contain a number of regulatory components that react to a range of environmental signals, including light, hormones, and biotic and abiotic stressors. These findings highlight the possible functions of *RSH* proteins in *B. napus* and imply that they play a role in plant growth and stress tolerance. The discovery of these regulatory elements offers insightful knowledge on the processes by which these genes are regulated in various environmental contexts.

#### Publication II (page 60)

The main Author's goal was to determine if the calcium-dependent stringent response controls seed development in *B. napus*. Therefore Candidate examined the expression of the *CRSH* gene with RT-qPCR application and location of the *BnCRSH* transcript with fluorescence in-situ hybridization technique utilization.

The Author demonstrated the great conservation of the EF-hand calcium-binding motif in plant *CRSH* proteins. During *B. napus* seed maturity, calcium ions and *BnCRSH* transcript levels rose. In growing canola seeds' cotyledons and hypocotyl, *BnCRSH* transcripts are concentrated. The findings of this study clearly imply that seeds enter a dormant condition as a result of calcium-dependent stringent response during late stages of plant development by limiting the expression of both plastid and nuclear genes.

#### Publication III (page 70)

The Candidate's goals were as follows: the creation of a seed biocoating made of chitin, methylcellulose, and *Trichoderma viride* spores and subsequent assessment of its effect on canola seed germination and seedling growth as well as the impact of this seed coating on plant metabolism as measured by superoxide dismutase activity and expression of *RSH* genes.

The Author demonstrated that *T. viride* strains were efficient at preventing the growth of three significant plant diseases, notably *Fusarium culmorum* which facultative pathogen with ability to attack wide range of plants. The novel seed covering did not affect seed germination and also didn't generate stress reaction in *B. napus*. The new seed coat, which enhanced the length and biomass of shoots and roots, encouraged the development of *B. napus* seedlings. The novel seed covering prevents the growth of common canola diseases (*Botrytis cinerea*, *Fusarium culmorum*, and *Colletotrichum* sp.), is reasonably priced, and stimulate the growth of seedlings.

### 3. Substantial merit of the thesis

In the evaluated PhD thesis, Candidate presents study with the general goal to explain the mechanism of stringent response in *B. napus*, the role of alarmones and the probable functions of *RSH* proteins in alarmone synthesis and hydrolysis in *B. napus* in response to various favorable and adverse environmental condition (abiotic and biotic stress) and endogenous factors especially in terms of plant growth and development, stress tolerance, and interactions with microbes. The topic possess important theoretical aspect but also potential practical application. The Author proved that during seed

development, *BnCRSH* expression increased. It was found in the hypocotyl, inner, and outer cotyledons, the *BnCRSH* transcript was not present in seeds collected at early stages of development (35 days after flowering). These findings are very significant because they represent the first report of *CRSH* expression and transcript localization in growing seeds of *B. napus*. Novelty of this findings is also important from formal point of view and prove that Candidate's PhD thesis give contribution in the field of biological science. It clears up that the increased concentration of alarmones inhibits the expression of nuclear and plastidial genes, which is essential for adjusting the metabolism of developing seeds. The information possess also applied aspect since it is a promising target for plant breeders as modifications have the potential to improve seed production.

The dissertation aim and research questions are appropriate in considering recent advancements and element of novelty areas of plant gene expression regulation in response to various factors. The methodology used in the study are very advanced to recall few techniques like (chronological order according to the list of articles): in silico analysis of *B. napus*, *B. olearacea*, *B. rapa*, *C. sativa*, and *R. sativus RSH* genes and proteins, semi-quantitative RT-PCR (sqRT-PCR), flow cytometry, RT-qPCR, in situ hybridisation, spectrophotometric methods. To summarize the research methodologies used are appropriate for the aims in the work. The techniques used are quite well suited to the purpose of the research and thoroughly documented. Proper statistical methods were applied e.g. expression data were assessed using one-way ANOVA, also two-way ANOVA was utilized, when appropriate. Therefore I can state that statistical analyses were accurately carried out throughout the experiments.

The topic of the thesis is presented in full clarity. It summarizes the knowledge that is now known about the individual areas of the research that was done and draws attention to the knowledge gaps and ambiguities. The Author outlined the planned aim in a logical manner and included research questions as support. The results are described correctly. The final findings are in agreement with the put out hypotheses. It is clear from these that the candidate is capable of critically analysing and presenting research results.

#### **4. Layout and register**

The dissertation is not organized in the same way as a typical doctoral thesis (Introduction, Materials and Methods, Results, Discussion, Conclusions). There are six chapters: Introduction (with four subchapters: Alarmones synthases and hydrolases in plants, The physiological functions of RelA/SpoT (RSH) in plants, Cross-talk between the stringent response pathway and other signalling pathways, Canola (*Brassica napus* L.) growth and development), The aim of the study, Discussion, Summary of results and conclusions, References, Abstracts. After the Abstracts the three articles are located and subsequently the Declarations of co-authors.

The quality of the candidate's presentation and editing is very good. The PhD thesis is prepared reliably. The English language used in the dissertation is generally correct, and it is well written.

In the PhD thesis main body we can find only three figures and one table, they are adequate to the content of the chapter, possessed good quality, and they are informative. At first someone might think it is not much, but when we look at the attached separate articles we can find figures and tables very well prepared and very helpful in understanding the whole problem. In my opinion there were no need to duplicate all the graphical part of thesis in main body of the thesis since all can be found in the attached separate articles. This is justified from substantial point of view, and also environmentally friendly point of view, less pages duplicated means more nature and trees saved.

## 5. Critical notes

The Author resigned from the chapter Materials and methods, which in my opinion is not necessary since there are attached all publications with detailed methodology. The chapter Results is also missing, but in this case I would like to see some summarized results from each experiment/article gather and presented in one paragraph. But of course we can't say that the information is missing since the Author attached all articles, but reader have to check it separately. Another think I would suggest is formal chapter with the summarized contribution of Ms. Sena Turkan in the whole process of each article preparation. We can find that in each article separately, but put it together would benefit in better understanding of her role in the scientific achievement.

In my opinion almost all subchapters of Introduction is really well written. The only think I would like to consider is the last subchapter of Introduction: Canola (*Brassica napus* L.) growth and development). I would like the Candidate to expand the topic connected with the importance of *B. napus* as a crop in the genetic/breeding aspect to fill the applied aspect. This species was substantially improved according to its value as industrial crop for oil production. To be more specific I would like the Candidate to comment changes in fatty acid composition during breeding process. What was the reason why oil from the classical *B. napus* cultivars could be consumed with significant limitations? What fatty acid composition possessed modern *B. napus* cultivars, and what are the types of cultivars? The question is also connected with high genetic plasticity of this species, it revealed high susceptibility to artificial selection pressure made by breeders. Maybe we can combine this phenomenon with the highest number of RSH gene family among all *Brassicaceae* family? Please comment this phenomenon.

The expression of nuclear and plastidial genes is inhibited by the elevated alarmone concentration, which is crucial for regulating the metabolism of growing seeds. In literature there are interesting reports about the correlation of the level of DNA methylation in seeds and aging process.

Do you think what could be the possible role of alarmones in seed longevity?

Do you think the regulation of alarmone synthesis and hydrolysis could be a prospective opportunity in plant germplasm conservation (seed banks)?

The last question is connected with publication 3. You listed serious diseases affecting crop production, one of the most important is *Fusarium culmorum*. In what way the infection could affect the yield? What effect on the cellular level possessed mycotoxins of *F. culmorum*?

The comments included in the review do not reduce the substantive value of the study, which in my opinion is very high. The doctoral thesis covers a very extensive range of fully original research and possess strong scientific and applied aspect.

## 6. Final grade

In my opinion, the PhD thesis of Ms. Sena Turkan represents an important scientific contribution to the understanding of the role of stringent response of plant to various favourable and stressful conditions. It should be noted that all of the results of the candidate's research have been published in well-known and highly rated scientific journal possessed high impact factor.

The presented research is extensive enough for a doctoral thesis. All the experiments utilized advanced techniques and the results obtained are of very high quality. The presentation and interpretation of the findings, proved the PhD student's expertise in the field of study.

The PhD thesis indicated that the candidate has achieved the intended objectives. The thesis meets all the criteria for a doctoral dissertation and is ready for defence and evaluation by the appropriate scientific committee.

I, hereby, declare that the reviewed PhD thesis by Ms. **Sena Turkan** meets the criteria pursuant to art. 187 of Act of 20 July 2018 The Law on Higher Education and Science (Journal of Laws of 2023, item 742 with further amendments) and request that the Research Discipline Council of Nicolaus Copernicus University in Toruń, Faculty of Biological and Veterinary Sciences accepts Ms. **Sena Turkan** further stages of doctoral proceedings in the field of exact and biological sciences, in the discipline of biological sciences.

I, hereby, request that the thesis is accepted with distinctions.

Justification:

In my opinion the very strong scientific aspect of the presented PhD thesis proved big achievement of the Candidate. The Author performed laborious experiments with very advanced molecular techniques application combined with conventional methods to solve the problem of stringent response in plants to various favourable and adverse environmental condition. I would like to recall publication II (in journal *Industrial Crops & Products*, 2023) where we can find report on *CRSH* genes expression and localization in developing seeds, that is the first report of any plant RSH. That's strong justification of scientific importance of the evaluated PhD thesis. Additionally the results presented in publication III (in the journal *PeerJ*, 2023) with novel seed coat more environmentally friendly, including PGPF factor possessed strong applied aspect and the seed coat is subject to patent application to Patent Office of the Republic of Poland (No. P.442362).

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Reviewer's signature