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REVIEW REPORT

on the PhD 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"

prepared base on invitation letter from the Dean of the Faculty of Biological and Veterinary Sciences Nicolaus Copernicus University in Toruń
Supervisor:
dr hab. Grażyna Dąbrowska prof. NCU,
Co-supervisor:
dr Agnieszka Mierek-Adamska.

1. The Context and the Scope of the Thesis

The stringent response is a stress signalling system mediated by the alarmones (pp)pGpp in response to nutrient deprivation. Research into the stringent response has exploded both in bacteria and plant species in recent years, with new insights highlighting the complexity and broad range of functions that these alarmones control.
The PhD dissertation by Sena Turkan, MSc is devoted to the investigations of the stringent response in plants homologous to bacterial stringent response as the plant RelA/SpoT Homolog (*RSH*) genes have been identified and characterized in various plant species.
In this context this thesis provides an update on our current understanding of the molecular changes invoked upon the accumulation of alarmones which can play a very important role in *Brassica napus* growth, development and adaptation to the environmental changes.
Several important issues affecting the stringent response performance are presented with the focus on the relationship between the alarmones and both - the biotic and abiotic stress as well as during seed development.
The work was focused on *B. napus*, which is the third most important source of edible vegetable oils in the world, a crop of great economic importance for food, feed, fuel, and other important commodities.
To develop new crop cultivars via traditional breeding or transgenic techniques that can produce high yields even in adverse environmental conditions the molecular processes underlying plant stress response have to be thoroughly understood. Based on the literature data it could be concluded that stringent response might be of crucial importance to obtain those stress-resistant cultivars of crop plants. In this context, the PhD thesis addresses the highly relevant and vital areas of current agriculture research.

2. Layout and the content of the Thesis

The reviewed PhD thesis of Sena Turkan is based on the coherent collection of 3 highly-impacted scientific articles published in reputable, international journals:

1. Dąbrowska G.B., Turkan S., Tylman-Mojżeszek W., Mierek-Adamska A. (2021). **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, 22(19): 10666.
   IF – 6.208, MNiSW – 140; Contribution: 35%;
2. Turkan S., Mierek-Adamska A., Głowacka K., Szydłowska-Czerniak A., Rewers M., Jędrzejczyk I., Dąbrowska G.B. (2023). **Localization, and expression of CRSH transcript, level of calcium ions, and cell cycle activity during *Brassica napus* L. seed development**. Industrial Crops & Products, 195: 116439.
   IF – 6.449, MNiSW – 200; Contribution: 70%;
3. Turkan S., Mierek-Adamska A., Kulasek M., Konieczna W.B., Dąbrowska G.B. (2023). **New seed coating containing *Trichoderma viride* with anti-pathogenic properties**. PeerJ, 11: e15392.
   IF – 3.061, MNiSW – 100; Contribution: 65%.

The total Impact Factor of those publications is high and equal to IF = 15.718, which gives the average value per publication IF=5.239, while summarized MNiSW points are equal to 440.

The PhD student has proving her excellent analytical skills and high quality of her work which is demonstrated by two first-authored peer reviewed publications in reputable journals, in addition to one co -authored manuscript - altogether attesting very good performance and writing skills. The other co-authors of the papers provided statements, which show that the involvement of PhD student in the preparation of these articles is in the range of 35-70%, which indicates that her participation in the development of the research plan, its implementation, conducting studies, interpretation of the results and preparation of the above-mentioned articles was significant. All publications included in the dissertation are characterized by a very good scientific level and care for appropriate presentation, documentation and discussion of the presented results and conclusions. They were reviewed by a group of selected exerts, so their high scientific level was confirmed.

The PhD dissertation has 107 pages altogether and is well written in English in clear and concise manner.

This PhD thesis is well structured and correctly presented. It consists of introductory part which is divided into 6 short chapters written in total on 35 pages. At the beginning of this dissertation lists of abbreviations is presented followed by Introduction, The aim of the study, Discussion, Summary of results and conclusions, References as well as Abstract in English and Polish.

The main part of this dissertation comprise of three original publications, attached in the form of reprints of articles chosen as the basis of PhD procedure, moreover statements of co-authors of these publications are included.

I consider the layout of the doctoral dissertation and the order of chapters appropriate. In my opinion the dissertation itself constitutes a logical and closed whole.

3. Substantive assessment

The main aim of this doctoral thesis was 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, development and adaptation to environmental stress in canola. Moreover, another purpose was also to develop an innovative seed coating with antimicrobial and plant growth-promoting properties for *B. napus* seeds.

In order to solve the stated research problem, a compilation of experiments were designed to study the potential function and mechanisms underlying the stringent response in canola. As a consequence, a broad spectrum of methods and the most advanced not only genomic but also proteomics and transcriptomics based approaches were used.

The PhD thesis of Ms. Sena Turkan is thematically homogeneous and begins with the presentation of the current state of knowledge related to the study. The first chapter, provides basic information about genes encoding alarmone synthases and hydrolases in plants, the physiological functions of RelA/SpoT (RSH) in plants, the correlation between the stringent response pathway and other signalling pathways as well as about canola (*Brassica napus* L.) growth and development stages. The aim of the study is indicated as a second chapter of this thesis. In the third chapter which is entitled Discussion, the main results previously published in three original papers are presented.

The literature for this part is carefully referenced (99 references) and a comprehensive bibliography contains all the relevant papers for the discussed field. It is worth mentioning here that most of the references are from the last decade, showing the topical issues. This part is well written and provides a very good background for understanding the rest of the thesis. In the final section the general conclusions of the work are summarized.

The three published papers (P1 – P3) are the most important component of the PhD dissertation, and each of them contains an analysis of research issues related to the plant stringent response and their impact on different function in *B. napus*, including analysis of a newly developed seed coat influence on canola seeds germination and antifungal properties.

The first paper (P1) entitled " *In silico* study of RSH (RelA/SpoT homologs) gene family and the expression analysis in response to PGPR bacteria and salinity in *Brassica napus*" focuses on determining the complexity of the plant RSH groups in chosen representatives of the *Brassicaceae* family.For this purpose, *In Silico analysis of B. napus, B. oleracea, B. rapa, C. sativa, and R. sativus RSH* genes and proteins were carried out, followed by the phylogenetic analysis with use the Plant Care datebase and BLASTN (Basic Local Alignment Search Tool) using the NCBI nucleotide database.

In this publication *B. napus* RSH gene expression in response to salinity was examined. Moreover, what is more important the expression of BnRSHs was analyzed in the presence of 3 species of Plant growth-promoting bacteria (PGPR) bacteria *i.e. Serratia liquefaciens, S. plymuthica, and Massilia timonae*, for which the ability to promote the growth of rape has been confirmed.

To evaluate the effects of NaCl and/or the presence of PGPR on mRNA level of *RSH* genes semi-quantitative RT-PCR(sqRT-PCR) assays were performed.

One of the significant conclusion is the fact that in plants belonging to the *Brassicaceae* family the stringent response is coordinated by numerous isoforms of RSH proteins. There is a high level of conservancy between the respective orthologs of RSH genes and proteins analyzed in

the study plant species. The wet-lab expression analysis of selected *B. napus* RSH genes in response to salt stress supported the idea of different physiological roles of plant RSH isoforms. Another noteworthy result is the demonstration that plant stringent response might be one of the pathways via which PGPR bacteria promote plant growth and development, however this seems to be bacteria species-dependent.

The second work (P2) entitled " Localization, and expression of *CRSH* transcript, level of calcium ions, and cell cycle activity during *Brassica napus* L. seed development" concerns the relationship between the possible role of the Ca2+-dependent RSH and *B. napus* seed development. According to the Authors the involvement of stringent response in regulating plant growth and development is now widely accepted however the possible role of this mechanism in seed development remains elusive. Therefore, in this paper, the level and localization of *BnCRSH* mRNAs, cell cycle activity, and the level of calcium ions were examined in developing canola seeds in five different periods after flowering (DAF). Those observations were possible through the use of modern techniques like for example: flow cytometric analysis, *in silico* analysis of plants *CRSH,* analysis of gene expression using RT – qPCR reaction, analysis of calcium levels in *B. napus* seeds with a cryo-electron microscopy technique, as well as *in situ* localization of *BnCRSH* transcripts during seed development with fluorescent *in situ* hybridization technique (FISH).

The results obtained are of a high importance especially because of the fact that it is the first report on *CRSH* expression and localization of *CRSH* transcript in developing seeds.

During canola seed maturation, an increase in calcium ions and the amount of *BnCRSH* transcripts was observed.

The increased concentration of alarmones inhibits the expression of nuclear and plastidial genes, which is essential for adjusting the metabolism of developing seeds.

From that point of view, the results of this study are valuable, strongly suggest that calcium-dependent stringent response during late stages of plant development, by inhibiting the expression of both plastid and nuclear genes, bring seeds into a dormant state.

The last paper (P3) entitled " New seed coating containing *Trichoderma viride* with anti-pathogenic properties " contains much more applied research comparing to the results presented in two previous publications.

Although, this paper aims at designing a new seed coating composed of chitin, methyl cellulose, and *Trichoderma viride* spores and assessing its effect on canola (*Brassica napus* L.) growth and development, this is a subsequent continuation of the research described in P1 and P2. The Authors hypothesized that the newly developed seed coating would be inert to seed metabolism, and thus they assessed the expression of the *RSH* genes and the activity of SOD as markers of stress in plants.

To develop a new seed coat, the antifungal activity of *T. viride* against common canola pathogenic fungi (*Botrytis cinerea, Fusarium culmorum, and Colletotrichum* sp.) was analyzed. Moreover, the effect of seed coating on germination ratio and seedling growth was evaluated. To verify the effect of seed coating on plant metabolism, super oxide dismutase (SOD) activity and expression of the stress-related RSH (RelA/SpoT homologs) were determined.

The results showed that the *T. viride* strains used for seed coating significantly restricted the growth of all three pathogens, especially *F. culmorum*, for which the growth was inhibited by over 40%.

As a results of conducted research, a cost-effective and environmentally responsible seed coating was successfully developed, which will be easy to exploit on an industrial scale.

Additionally, the obtained results confirm that the new seed coating promotes seedling growth limits the growth of plant pathogens and does not affect the stress-related markers in plants. It is worth to notice that the test results described in Publication III were used to prepare a patent application (Patent no: P.442362).

Taking into account the obtained results, as the most valuable achievements of Miss Sena Turkan dissertation I would like to highlight:

- Identification 14 *RSH* genes in the genome of the polyploid plant *B. napus* and indicating that they could be divided into three subgroups similar to other plant species: *RSH1, RSH2/3* and *CRSH.*
- Identification of various regulatory elements within the promoter regions of these genes which definitely provides valuable insights into the mechanisms by which these genes are controlled under different environmental conditions (Publication I).

- Indicating that the EF-hand calcium-binding motif is highly conserved in plant CRSH proteins and that the level of calcium ions and *BnCRSH* transcripts increased during canola seed maturation.
- Suggesting that calcium-dependent stringent response during late stages of plant development, by inhibiting the expression of both plastid and nuclear genes, bring seeds into a dormant state (Publication II).

- Showing that the expression of *BnRSH* genes is influenced differently by biotic and abiotic factors, suggesting that they have diverse functions in plants (Publication I and Publication III).

- Developing the new seed coat and demonstrating the effectiveness of *T. viride* strains in inhibiting the growth of three important plant pathogens, particularly *Fusarium culmorum* (Publication III).

Specific comments/ questions addressed to the doctoral dissertation are given below. I expect the candidate refer to them during the public defense:

1. Because of the fact that the developed seed coating is currently being tested in field conditions, it is not easy to predict the level of success i.e. the percentage of increased resistance to tested pathogens. I wonder whether it will be possible thanks to the new seed coat also influence on inhibiting another fungal pathogens in the future, especially those which are more common for *Brassica napus*?
2. What do you think about the prospect utility of stringent response in agriculture, especially in canola resistance breeding?
3. Why have you selected and tested two different forms of *B. napus* i.e. winter cultivar "Harry" and spring cultivar "Karo"? What was the reason of conducting one part of research on *B. napus* cv. Harry and another part on cv. Karo?

4. Final conclusion

In the assessment of the work, I conclude that Ms. Sena Turkan has done a huge and important work contributing to a better understanding of the plant stringent response role in growth, development and adaptation to environmental stress in canola. In 3 articles included in

this dissertation, PhD student demonstrates the knowledge of the analysis and interpretation methods of experimental data concerning genes encoding alarmone synthases and hydrolases. The method of planning the research, its execution, the form of presenting the results and their analysis prove the high scientific and research maturity of the Author of the dissertation.

The presented dissertation is an original and innovative solution to a scientific problem and significantly enriches our knowledge about the role of stringent response in controlling plant metabolism in changeable environmental conditions. The results of this research may accelerate work on the possibility of increasing canola resistance to biotic and abiotic stresses.

I am convinced that the reviewed PhD dissertation by Sena Turkan entitled „The role of plant stringent response in *Brassica napus* L. in response to biotic and abiotic factors and during seed development„ meets the requirements for doctoral thesis (art. 190 of the Act of July 20, 2018 Law on Higher Education and Science (Journal of Laws 2018, item 1668, as amended) and I request the Discipline Board of Biological Sciences of the Nicolaus Copernicus University in Toruń to admit the PhD student to further stages of the doctoral dissertation.

Taking into account the scope, level and importance of the research as well as a very high impact factor of the publications, I apply for the distinction of the reviewed doctoral dissertation because:

1. It shows a very good quality of the PhD thesis confirmed by three publications in highly recognized research journals (IF from 3.061 to 6.449; average value per publication IF=5.239).
2. It introduces elements of novelty in the scope of the discussed subject.
3. It contains extensive and original experimental material based on reliable results obtained with the use of state of the art methodology.
4. The obtained results are of practical importance and contribute to the progress in modern agriculture, especially in resistance plant breeding, which is in line with the integrated pest management.

Poznań, 04.09.2023