Opponent statement of doctoral thesis

Title:	**Genetic analysis of resistance in *Triticum* spp. to newly emerging races of wheat stem rust**
Author:	**Ing. Ondřej Zelba**
Supervisor:	Ing. Iva Viehmannová, Ph.D.
Co-supervisor:	RNDr. Veronika Dumalasová, Ph.D.
Reviewer:	Ing. Jana Mazáková, Ph.D.

The doctoral thesis presented by the candidate Ondřej Zelba focuses on the analysis of resistance of wheat cultivars currently grown in the Czech Republic to the fungal pathogen *Puccinia graminis*, using various types of molecular markers and phenotyping methods. The topic of the thesis is considered very relevant in the context of current wheat-*P. graminis* pathosystem research. The biotrophic species *P. graminis* is a high-risk pathogen by having a mixed reproduction system, polycyclic behaviour resulting in a large effective population size, high potential for gene flow, and high mutation rate. Therefore, the focus of the thesis is related to the problem of the newly emerging physiological races of *P. graminis* with respect to their ability to break down the efficacy of resistance genes present in currently cultivated wheat cultivars. Having accurate and up-to-date information on the genetic nature of resistance in wheat cultivars and the presence of local physiological races of the pathogen is very important, as the management of wheat stem rust heavily relies on the cultivation of resistant cultivars and the application of fungicides.

The thesis has the standard structure usual for works of this type. It is compiled in the extent of 77 text pages, formally divided into 12 main chapters, supplemented with 16 figures, and 17 tables. The thesis is structured clearly and comprehensibly, with each chapter and subchapter having a clear and logical function and order.

The first chapter provides an introduction and explains the motivation for the problems solved in the rest of the thesis.

The chapter "Literature Review" shows the broad knowledge of the candidate in the field of his research area and is supported by adequate cited literature sources. The chapter consists of subchapters that describe the biology of wheat rust pathogens, the classification and virulence evolution of *P. graminis*, and its impact in the tropics, subtropics, Czech Republic, and Europe. More than half of the pages of the literature review are focused on stem rust resistance in wheat and phenotyping methods evaluating plant resistance to stem rust in wheat.

The aims and hypotheses of the thesis are clearly and specifically formulated.

The chapter "Materials and Methods" provides information about panels consisting of the large number of wheat genotypes used for analyses. The set of molecular markers used for the detection of resistance genes was selected based on the availability of markers, the importance of resistance genes, and knowledge of their previous usage in breeding programs. Results obtained from molecular screening are compared to field trials and greenhouse phenotypic tests. Furthermore, adult plant resistance (disease severity) of selected wheat cultivars was evaluated in more detail using visual assessment, image analysis with machine learning, and quantification of chitin in samples. The methods used reflect the methodology for this research area and are sufficiently described and suitably applied.

Extensive results are described in detail and documented in particular subchapters, tables, and figures. The author clearly and appropriately interprets and discusses the obtained results with the results published so far in the works of other authors. The author has achieved original information on the local level. However, these are needed to effectively identify and incorporate resistance into agronomically desirable cultivars and should be helpful for farmers, breeders, and future research on a global scale.

The chapter "Conclusion" is written briefly and accurately.

Ing. Ondřej Zelba is the main author of one journal paper and the co-author of three journal papers related to the thesis topic and listed in WoS/Scopus databases.

I have the following comments and questions on the doctoral thesis:

Page 3, chapters 2. 1. or 2. 2.: I miss whole taxonomical classification of rusts. I mean main taxonomical categories.

Page 3, chapter 2. 2.: Is it necessary for *Pt* and *Pst* to complete the entire life cycle in conditions of the Czech Republic? How do *Pt* and *Pst* overwinter in the Czech Republic?

Page 4, chapter 2. 2.: I would recommend to add graphical life (disease) cycle of *Pgt* (e. g. use it from Agrios: Plant Pathology).

Page 7, chapter 2. 3. 2.: Physiological races are not taxonomical units, but are still intraspecific categories that differ in virulent pattern.

Page 9–10, chapter 2. 3. 3.: There is no information on how many phylogenetic clades of *Pgt* (genetic groups) have been identified so far.

Page 21, chapter 2. 6. 5.: I would prefer to use "resistance to different pathogens" instead of "resistance to different diseases".

Page 21, chapter 2. 6. 5.: I would prefer to show the split Table 3 on one page.

Page 22, chapter 2. 6. 5.: The acronym CRISPR is not explained in the text.

Page 22, chapter 2. 6. 5.: Could you explain the difference between "immunity" and "resistance"? I know that in English-written papers it is common to use "plant immunity" as PTI, ETI; however, I would prefer to use "resistance" instead of "immunity".

Page 30, chapter 2. 8. 1.: AUDPC is not explained in the text.

Page 33, chapter 2. 8. 4., Table 5: I would prefer to add "5´ to 3´" to the cell called primers. There is no explanation of the abbreviation "F:/R:"

Page 35, chapter 2. 9. 2.: Rewrite "15 samples with gained virulence" to "Fifteen samples with gained virulence" (beginning of the sentence).

Page 40, chapter 5. 4.: There is no information regarding the concentration of DNA, ethidium bromide, agarose gel and primers.

Page 42, chapter 5. 4. Change units of volume "0.14 µl of primer mix, 2 µl of DNA sample" to units of concentration.

Page 55, chapter 6. 1., Figure 8: There is no information on the molecular marker that was used for agarose electrophoresis.

Page 66, chapter 7. 1.: Rewrite "all of them were virulent to Sr38" to "all of them were virulent to wheat cultivars with *Sr38* gene".

Page 66, chapter 7. 1.: Rewrite "they are both virulent to Sr38" to "they are both virulent to wheat cultivars with *Sr38* gene". Check the other sentences with "virulent to gene".

Page 76, chapter 9: Rewrite "virulence to those races".

I would prefer to move tables and figures from chapter "Discussion" to chapter "Results", because they collect and describe the obtained results of the candidate; however, in the tables and figures, there is no comparison with the research of other studies.
I would recommend using a dash instead of a hyphen to specify numerical ranges.
Generally, I would recommend to check legends and captions of figures and tables to improve the text. Some of the sentences in the table and figure legends are in bold, others are not. Regarding *Pgt* name, I would recommend unifying figure legend. Readers can read: stem rust, *Puccinia graminis*, *P. graminis*, *Puccinia graminis* f. sp. *tritici* or *Pgt*.

Based on the high quality of the thesis, I can conclude that the author proved the theoretical and practical knowledge, very good orientation, and ability of scientific work in the thesis discipline.

From the above-mentioned facts, I state that the submitted thesis of the candidate Ing. Ondřej Zelba meets all the requirements given on doctoral thesis, and therefore I propose to accept the thesis for defence, and after successful completion, I recommend awarding him the doctorate degree Doctor of Philosophy (Ph.D.).

Prague, 19 March 2024

Mazáková Jana

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