

## **OPPONENT'S OPINION OF THE DOCTORAL THESIS**

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Doctoral thesis title: **Genetic analysis of resistance in *Triticum* spp. to newly emerging races of wheat stem rust**

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The assessed doctoral thesis of Ing. Ondřej Zelba on the title "Genetic analysis of resistance in *Triticum* spp. to newly emerging races of wheat stem rust" was focused on the study of resistance in wheat varieties grown in the Czech Republic, especially in relation to newly detected pathotypes. As the author reports, resistance genes were detected in a set of varieties using molecular markers and the results were compared with infectious resistance tests under field conditions and phenotypic resistance tests of key plants using the described races of the pathogen.

The submitted doctoral thesis totals 107 pages of text and appendices consisting of a list of published articles and poster presentations. It also lists participation in a foreign internship, author identifiers, acknowledgements and the method of funding from the grant of the Czech University of Life Sciences Prague and the international project HORIZON 2020. The thesis is divided into 10 main chapters and supplemented with 17 tables and 16 figures. The text of the thesis is very appropriately and efficiently supplemented with a Glossary of abbreviations and technical terms used. Its scope and usual structure corresponds to the general requirements for the formal organization of a doctoral dissertation.

A short and concise introduction is followed by a rather extensive literature review, which is comprehensive, includes citations of available literature and shows the author's good orientation in the topic. In the Czech abstract, I would recommend replacing the term "rez travní" with "černá rzivost trav" in order to comply with the Czech nomenclature of plant diseases and pests, which was approved in 2010 by the plenary of the Department of Plant Protection of the Czech Academy of Agricultural Sciences as binding for use in Czech scientific and professional publications. In both versions of the abstract I would then add the Latin name *Puccinia graminis* Pers. The literature review of almost 30 pages of text provides a comprehensive view of the knowledge related to wheat stem rust in general, the biology of the pathogens, the classification of the genus *Puccinia graminis*, the evolution of virulence, the significance of the disease in the tropics and subtropics, but also in the Czech Republic and in the context of Europe, and clearly describes the resistance genes of wheat stem rust. How significantly would climate change affect the occurrence of wheat stem rust? Last but not least, the review presents the possibilities of resistance phenotyping and genomics of the pathogen in cereals. I would like to ask the author what is the situation with the occurrence of wheat stem rust in the Czech Republic (after all, the more harmful pathogens in our country are leaf rust of wheat and yellow (stripe) rust of wheat) and what can be expected trends in the occurrence and harmfulness of this disease in the coming years?

The aims of the thesis and the hypotheses, which are listed on page 36, give a general overview of the breadth and intentions of the problem addressed. They are clearly and clearly stated and fully capture the focus of the dissertation.

The following chapter "Material and methods" provides basic data on the characteristics of the wheat genotypes analysed. As stated in the text, the seed for testing and analysis was obtained from the Gene bank of the Crop Research Institute Prague - Ruzyně and then from the Central Institute for Supervising and Testing in Agriculture Brno. From this perspective, how does the author evaluate the benefits of gene banks for research work and breeding programmes? In the tabular overview of 90 wheat varieties I missed some passport data that the author could have included, e.g. ECN (national accession number) if the sample was from Gene bank, year of registration in the Czech Republic or first registration in the EU, name of the maintainer and country of origin or pedigree. Although I understand that pedigrees of varieties have not been published by most companies for some time to protect their know-how.

The methodology also describes the isolation of plant DNA, the selection of a set of markers based on their availability, the importance of the gene or knowledge of its previous use in breeding and the availability of genotypes - varietal standards. For the description of the field resistance trials, I lack at least basic data on agrotechnics (forecrop, soil preparation, sowing date, pesticide use) and information on how many years of testing were used for the purpose of the work (if necessary, indicate the years of testing). For the evaluation of disease symptoms, a scale from 1 to 9 was used, with 1 indicating minimal symptoms and 9 indicating the most extensive disease symptoms. Would it not be preferable to standardise the rating of disease incidence and give the rating in the standard way, as is done in the trials by the Central Institute for Supervising and Testing in Agriculture in accordance with its methodology or by the Gene bank, which uses crop descriptor list for the rating? That is, 1 - susceptible and 9 - resistant variety. The Gene bank Prague, which takes over the results of infectious nursery rust tests in wheat from Mgr. Hanzalová, Ph.D. presents the rating in this way and these results can also be found in IS GRIN Czech.

Among the varieties selected for phenotyping of adult plants, winter wheat LG Mokka from the French company Limagrain Europe, S.A.S. was chosen. The author states that it was the most grown in 2022 (5.5%), but was also the most grown in the previous year, when it accounted for 5.8% of the multiplication area. Why do you think that a variety which is feed type and also suitable for the production of wafers and biscuits (category CK), which has a low level of falling number and lower bulk density, which has less resistance to yellow rust and which is a late variety, had the largest multiplication area in the Czech Republic for 2 years? The author further

states that this variety is highly susceptible to wheat stem rust under field conditions and that this is unpublished data. Here I would like to correct the author, the database of varieties of the Central Institute for Supervising and Testing in Agriculture on its website provides information that according to provocation tests the LG Mocca variety is less resistant to wheat stem rust attack i.e. rating 5-4 ([https://eagri.cz/public/app/sok/odrudyNouVF.do?lang=en\\_US](https://eagri.cz/public/app/sok/odrudyNouVF.do?lang=en_US)). In the Gene bank field trials, in the artificial infected nurseries, the variety LG Mocca was scored as susceptible to grade 1 over two years of testing (<https://grinczech.vurv.cz/gringlobal/accessiondetail.aspx?id=73157>).

Two Pgt isolates that had different virulence patterns were selected for phenotyping and used to inoculate 58 wheat cultivars previously tested with molecular markers to verify the presence of some of the genes tested. The methodology is followed by descriptions of seed establishment and germination, subsequent plant springing and inoculation using an atomizer. Disease symptoms were then assessed 12 days after inoculation. The flag leaves and top 2 leaves were then used for image analysis using machine learning. The leaves that were scanned were then used for quantitative and statistical analysis.

The results of the analyses show that the *Sr38* resistance gene is present in the Czech varieties most frequently (63.8%) and although in field trials the degree of infection was lower in varieties carrying this gene, the races present in 2020 are already predominantly virulent to it. I would like to ask from which grass was the *Sr38* gene derived, which many of our registered varieties are said to carry, and whose resistance is surpassed by the Digalu race? A significant finding is that the *Sr31* and *Sr24* genes are present in 10.3% and 13.8% of varieties respectively and continue to be effective against the current races.

To further describe their resistance, phenotypic tests were performed on a small sample of cultivars on mature plants. As expected, varieties with the combination of two of the resistance genes tested were the best performing under field conditions. What level of resistance rating did they achieve? Phenotypic tests on adult plants showed that adult resistance is present in some varieties and cannot be determined by other tests. The author notes that machine learning image analysis has been successfully performed with both germplasm and adult plants and is demonstrated here as a simple method of quantifying rust symptoms in wheat. Several KASP markers have been tested on Czech cultivars for use in detecting the *Sr8a* and *Sr11* genes. However, these markers used, as the author states, show inconclusive results and follow-up tests must be performed for their further use. What tests does the author have in mind?

The final part of the dissertation gives a concise, comprehensive and objective picture of the intentions, methodological procedures and results achieved, and also presents future

perspectives. The contribution of the doctoral thesis is also that its results can be applied in breeding, but also in practice and subsequent research projects.

In conclusion, I can say that the assessed doctoral thesis of Ing. Ondřej Zelba entitled "Genetic analysis of resistance in *Triticum* spp. to newly emerging races of wheat stem rust" is, despite minor reservations and inaccuracies, as a whole, in terms of professional and formal aspects, excellently prepared and meets all the requirements set for a doctoral thesis. In conclusion, I can state that the aims and objectives of the submitted doctoral thesis have been fully fulfilled and after answering the above questions and comments, **I recommend the committee of the Faculty of Tropical Agriculture, Czech University of Life Sciences in Prague, the doctoral thesis of Ing. Ondřej Zelba to defend and award the academic-scientific degree of Ph.D. I would like to congratulate Mr. Zelba and wish him good luck and success in the field of science and research.**

Prague, 12th February, 2024

  
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