

Doctoral thesis review

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

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Reviewer: Doc. RNDr. Michaela Sedlářová, Ph.D.
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The doctoral thesis of ing. Ondřej Zelba presented on 127 pages focuses on pathosystem *Triticum* spp.-*Puccinia graminis* which stands in the centre of human attention for centuries as the rust disease threatens cereal yield and thus nutriment. The Ph.D. applicant continued the work of leading rust experts from the Crop Research Institute in Prague-Ruzyně (Ing. Pavel Bartoš, DrSc., Mgr. Alena Hanzalová, Ph.D. and RNDr. Veronika Dumalasová, Ph.D.) using molecular markers. Information on resistance of wheat cultivars recently grown in the Czech Republic to races present in local *P. graminis* population, esp. those newly emerging in Europe, which arose from his doctoral study at CULSP is of great importance to agriculture and science. Interesting are esp. data on resistance of some wheat cultivars in greenhouse and field trials that could not be explained by the range of available molecular markers. The text represents rather well-developed compendium of recent knowledge in this problematics, supported by student's co-authorship of four papers.

Comments to the text of thesis (version which I obtained for review):

- Abstract - rewrite 8th sentence to „Quantification of disease severity is done ... learning, and assessment of fungal biomass based on chitin quantification“
- p. 14 (I am using total paging of *pdf file) – do not use “enemy” but “pathogen” or “disease causal agent”
- p. 15 – better 2. Literature overview; insert taxonomical position of rust in 2.1 or 2.2 – do not use “under“ basidiomycota or 2.3. - use standard taxonomical classification (Pucciniaceae, Pucciniales, Pucciniomycetes, Pucciniomycotina, Basidiomycota, Fungi)
- it would be useful to show *Pgt* lifecycle (and highlight phases (types of spores) important for epidemics during growing season and those playing role in global *Pgt* spread)
- p. 18 – 2.3.1. - significant variation - of what? + Use „host range“, not „host-range“.
- Comment to text on p. 19 which I suggest to rewrite. – Recent taxonomy and identification of fungi (esp. those microscopic) is based on so called polyphasic approach = combination of morphological together with molecular (+ sometimes other) characters. Both forma specialis as well as physiological race represent intraspecific categories, but physiological race (biotype or group of biotypes) is not officially recognized as a taxonomy unit while f. sp. is allowed by International Code of Botanical Nomenclature (and can of course include several races). All taxonomic categories could be found on <https://www.indexfungorum.org/names/Names.asp> - where also other *Pg* subspecies and formae speciales are listed
- p. 34 – I would not use “immunity” but “resistance” if speaking about breaking by new races; update information in the last sentence as EU approach to NGT is changing recently - <https://www.europarl.europa.eu/news/en/press-room/20240202IPR17320/new-genomic-techniques-meps-back-rules-to-support-green-transition-of-farmers>
- p. 42 – “AUDPC” from title is not explained in following text
- p. 43 – rewrite “Such trials need not to be isolated from the influx of new inoculum from the atmosphere”
- p. 48 – in hypotheses - No. 2 is listed twice
- p. 51 – citation “Dumalasová et al., currently under review” – any updates? Can you put provisional citation in the list?
- p. 55 – do not use APR in name of chapter, give full text
- p. 62 – “data...were” (not was)
- p. 68 – “moderate effect ... resistance”- replace text to previous page; Fig. 9 – “Number of observations is indicated above each group.” – I cannot see it in graph? The following text (contrasting reactions in greenhouse tests) is more discussion than results
- p. 72 – do not use “x” but “-“ Michigan Amber – *Pgt* TRTF interaction
- p. 75 – Fig. 10 – how do you mean „This graph includes data for both flag and flag-1 leaves.“ – is it an average value? Rewrite

- p. 78 – do not use “Germany’s outbreak” but “disease outbreak in Germany”; rewrite “races TTRTF and TKTF in recent samples from Slovakia and Austria that are both virulent to *Sr38*.” - Race is not virulent to a gene! Please, speak about “cultivar/line/genotype bearing the gene ...” – also in the following text of discussion... „Results shown here indicate it is not present in current day cultivars in Czech Republic and it would not be effective against today’s important races (notably TKTF and TTRTF).” – better „Our results indicate that wheat cultivars recently grown in the Czech Republic do not contain *Sr36* and it would not be effective against currently important *Pgt* races (notably TKTF and TTRTF).“
- p. 85 – please rewrite „It appears that the results from the two methods correspond less with higher disease severity“ ?
- Figs. 14 and 15 – the axis Y should start with 0% - you cannot use negative percentages! Fig. 16 – I would name it “Image segmentation in infected leaves photographs of wheat cultivars Evina and Rivero“
- p. 88 – “and virulence to those races”? Term virulence / avirulence refers to pathogen, not to the host!
- p. 89 – Chapter 9 – better use “Future perspectives”
- Do not use in names of chapters Annex together with its synonym Appendix – I recommend to keep “12. Appendices” (or “Supplementary data”) for presentation of figures and to rename chapter 11 e.g. to “Information about PhD candidate” starting with 11.1. Author’s name and identifiers” (normally those in details are part of separate Summary of Ph.D. thesis or CV of a Ph.D. applicant which are sent to reviewers), 11.2. List of author’s papers – I miss statement of authors’ contribution which is essential to judge input of a PhD candidate – please add!!!, 11.3. List of conference contributions – authors are missing, do use standard citation, 11.4. Internship
- Part 11.5 – the text should be replaced to page 3 - it has no sense to have 2 acknowledgement parts!
- Chapter 12. . be more careful in captions to , do not use general terms, be specific!!! 12.1. does not show mycelium (which is hidden within tissues) but pycnidia and aecia; fungal colonies? I suggest to use standard description under figure, avoid repeating “Following image...”

What I really miss is citing four student’s own papers in the text – please add those citations to relevant parts of results and discussion!

Questions to stimulate discussion during defence by which the applicant demonstrates his professional erudition:

- With global climate change do you expect that *P. graminis* ssp. *graminis* would meet in CZ conditions favourable for overwintering? This would increase genetic variability in *Pgg* populations.
- Are there information on mixed infections in countries where both *Pg* ssp. (*graminis* and *graminicola*) are present? In case when both subspecies would be present on *Berberis* to complete their sexual process – what are the consequences? Are you aware of studies to assess gene flow in *Pg* or other rusts?
- Physiological races of biotrophic fungi and oomycetes have been designated according to various systems over the time. With increasing number of host differential lines used to identify pathogen races the number coding has become popular (instead of letter code nomenclature used traditionally in *Pgt* research) – what do you see as their advantages?

In conclusion, the doctoral student Ondřej Zelba gathered valuable experimental data, mastered a number of methods and demonstrated his ability to interpret own results in the context of current scientific knowledge. In accordance with § 47, paragraph 4, Act No. 111/1998 as well as Regulation of the dean No. 4/2019 Specification of the Study and Examination Regulations for study in the doctoral study programs of the CLSU Prague at the Faculty of Tropical Agriculture, **I RECOMMEND the acceptance of the submitted doctoral dissertation for defence and**, in the case of successful public defence, **awarding degree of Ph.D.**

In Olomouc, 12. 2. 2024



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