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Review on the dissertation

"Elucidation of genetic factors determining resistance or susceptibility to clubroot disease through GWAS, transcriptome profiling and functional genetics in natural *Arabidopsis* accessions"

by

Juan Camilo Ochoa Cabezas

Juan Camilo Ochoa Cabezas has worked on using a natural population of *Arabidopsis thaliana* accessions in a screen for clubroot resistance and has used GWAS, transcriptome profiling and molecular generation of mutants followed by their phytopathological and microscopical analysis. This is a very broad spectrum of methods and experiments that resulted in a well-structured and well written thesis.

The thesis consists of introduction, materials and methods, results and discussion as well as a summary. All parts are equally well written and balanced in their length and content. It consists overall of 158 pages, including references. The thesis is written in high quality English. The thesis is supported by 50 figures and 10 tables, all of them are very well to be understood. The introduction gives a balanced view on the problem of the clubroot disease including a short description on the biology of the pathogen together with its host including cell cycle regulation and plant hormones, the resistance factors that have been identified in different plant species as well as an introduction to some of the methods to be used, like GWAS. Finally, some more general remarks on plant immunity follow. Maybe for the part on the biology with the host an additional figure explaining the involvement of hormones and cell cycle might have been helpful. The research

objectives were clearly stated. The following parts of Materials and Methods and Results are equally well organized. In the Results the experiments are presented in straightforward manner. The figures and tables have been well chosen to illustrate the respective results. The quality of the presentation is very good and the pictures from the experiments have a high quality. There are few cases where the legibility could have been improved, mainly when the panels were taken from websites. For the first set of histological pictures some kind of orientation for the sections, i.e. where in the tissue are they to be found with a drawing in addition to the small insert with the complete hypocotyl would have been helpful. The final discussion follows the structure of the results section and is in general also a well written part.

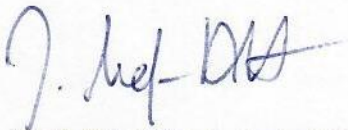
The thesis has dealt with the identification of resistance loci in Arabidopsis accessions using modern molecular methods. Thus, it clearly advances the current research field significantly. All methods used are scientifically sound and have been described in a manner so that other researchers can follow them. They were chosen in a suitable manner to progress with the research field that Juan Ochoa investigated.

The results independently led to the identification of a previously described, but not further characterized locus in resistant interactions. Juan Ochoa has done a huge amount of bioinformatic work together with the appropriate statistical analysis to come up with the highly significant loci RPB1 and RAC1. For both he could get mutants by CRISPR/Cas techniques and used these in phytopathological analyses. He could show that RPB1, but not RAC1 is involved in the resistance of this Arabidopsis ecotype and a specific isolate of the clubroot pathogen. Here, maybe in the future other isolates should be tested to find out more about the more general applicability of the resistance between several accessions and the clubroot pathogen per se. He is discussing a possible connection between the isolates that showed resistance in previous and his work by geographically near possibly locations, but that should and need to be tested. The description of the similarities between the ecotypes with respect of RPB1 are impressive. However, while the data support all findings except the promoter analyses. Here, the experimental findings with highly variable promoter regions are described in the text, but no figure shown as illustration. A second part of the thesis evaluated an effect observed on the accession Pro-0 that turned out to be most likely a slowed down development of the pathogen compared to the Col-0 accession. This is also done by molecular methods such transcriptome data.

In the discussion Juan Ochoa also shows a very good knowledge of the research field. This is demonstrated by including many aspects from the literature. It might read a bit in focus towards the second part of the thesis, albeit the identification of RPB1 is the major achievement. There is the impression that the RPB1 results are somewhat less and the results on RAC1 not at all discussed. The major part of the

discussion seems on the slower colonization phenotype of Pro-0. There is an experiment in NASC Affymetrix array on the interaction of Tsu-0 by Johannes Siemens at different time points in comparison to Col-0 after inoculation with isolate e as publicly available transcriptome data available that could have been exploited at least in the discussion.

Overall, the thesis demonstrates an appropriate understanding of the state-of-the-art in the research area as well as the capability of the author to deal with the most relevant and current literature. This very well written thesis contributes to a field that has still not been very often taken into consideration for clubroot research. Juan Ochoa has shown that he is able to create an independent scientific work. Based on the methodology established and the results generated I certainly recommend the thesis to be accepted and the candidate should be awarded the PhD degree.



Prof. Dr. Jutta Ludwig-Müller