

Justus-Liebig-Universität Giessen

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Evaluation of Doctoral Thesis of Mr. Piotr Kopeć ‘Genetic determinants of rapeseed (*Brassica napus* L.) resistance against *Plasmodiophora brassicae* Wor. infection.’

Overview

Thesis describes the identification and characterization of a genomic locus for resistance of *B. napus* cultivar ‘Tosca’ to clubroot. Clubroot is a disease of high economic importance causing severe yield losses. The exact gene/genes underlying resistance conferred by this locus were not previously identified.

The thesis is composed of four main sections. 1. Introduction, 2. Identification and characterization of the clubroot resistance locus, 3. Functional study of duplicated TNL gene. 4. Comparative analysis of the *Crr3Tsc* homologous loci. The chapters present a logical progression and provide a comprehensive answer to the scientific question. They reflect good theoretical knowledge of the discipline and ability to conduct high quality research.

1. Introduction. Introduction is comprehensive, presenting a good overview of the field for both the plant and fungal pathogen.
2. Identification and characterization of the clubroot resistance locus. First, a genetic map is constructed to narrow down the location of the locus. Then, long read Oxford Nanopore (ONT) sequencing in combination with RNA-seq data is used for detailed characterization. Analysis of ONT reads reveals Tosca-specific duplication covering gene functionally annotated as TNL (a group of genes known to be involved in resistance). The duplicated genes are also shown to be substantially different (on sequence level) from the homolog found in the susceptible line. No differential expression of TNL genes after inoculation was observed.
3. Functional study of duplicated TNL gene. Here candidate genes are transformed into *Arabidopsis* to confirm functional effect. Transformants carrying ‘Tosca’ TNL genes (CDSs) show resistance in comparison to controls. In addition, resistance effect is observed regardless of the transgene configuration, and both duplicates appear functional.
4. Comparative analysis of the *Crr3Tsc* homologous loci. Here candidate performs an *in silico* comparative analysis of the locus carrying TNL duplication described in the previous chapters. The analysis is quite interesting and points to a potential involvement of TEs and unequal crossing over in the initial gene insertion and subsequent duplication.

Novel contributions

While parallel work on resistance in 'Tosca' has been carried out by other groups, this is the only study which described the existence of the TNL-family gene duplication. The work therefore makes a novel contribution to the existing body of knowledge, answering an important scientific question. The methods used are consistent with state of the art, confirming high level of knowledge and research skill.

Potential suggestions

In the Introduction illustrations of the pathogen lifestyle and PTI, ETI pathway would perhaps make for easier reading, but the text is sufficiently clear without them.

In Chapter 4 it is mentioned that annotation quality/content of the TNL region is highly variable between assemblies. This is unfortunately a known issue, also because different genome projects used different annotation pipelines. The candidate gets around the issue using blastn and tblastx, which is one solution. I also think that this region would be an excellent candidate for comparative annotation (<https://bioinf.uni-greifswald.de/augustus/binaries/tutorial-cgp/>) or deep learning (https://www.plabipd.de/helixer_main.html) approaches, which could render the annotations more uniform/comparable. That being said this is something that could maybe be discussed, but need not be done ahead of the defence.

Overall Recommendation

The dissertation fulfils all requirements indicated in the currently binding regulations (art. 187 Ustawy z dn. 20.07.2018 r. Prawo o Szkolnictwie wyższym i nauce Dz. U. 2023 poz. 742). First, it provides an original solution of a scientific problem. Second, it confirms the general theoretical knowledge of Mr. Piotr Kopeć in the discipline of biological sciences and his ability to conduct research.

I therefore would like to put forward a motion to the Council of the Discipline of Biological Sciences of the Adam Mickiewicz University in Poznań to admit the PhD candidate, Mr. Piotr Kopeć, to further stages of the procedure.

I wish the candidate all the best for the future.

With best regards,



Dr. Agnieszka A. Golicz