

Kraków, September 11, 2023

## **REVIEW**

**of the doctoral thesis of Mr. Piotr Kopeć, MSc  
titled “Genetic determinants of rapeseed (*Brassica napus* L.) resistance against  
*Plasmodiophora brassicae* Wor. infection”**

### **Introduction**

Clubroot is a devastating disease seriously affecting yield of crops from the Brassicaceae family, including oilseed rape *Brassica napus*, the most economically important species from that group. The disease is caused by a soil-borne obligate endobiotrophic microorganism *Plasmodiophora brassicae*, a member of the clade Rhizaria. It is difficult to control agronomically, thus introduction of genetic resistance to new cultivars is critical and a range of resistance genes has been reported in the literature and utilized in breeding programmes. Nevertheless, thorough characterization of those resistance genes still remains a challenge. In the PhD dissertation of Piotr Kopeć, such a comprehensive structural and functional analysis of a region on chromosome A03 in *B. napus*, governing resistance to clubroot in the cultivar ‘Tosca’, is provided.

### **Structure of the dissertation**

The dissertation of Mr. Piotr Kopeć comprises 103 printed pages. The structure of the thesis can be defined as ‘hybrid’. It includes one multi-author scientific report published in a reputable journal (*Frontiers in Plant Science*), where the PhD candidate performed a leading role, as documented both by the fact that he was the first author of the article, as well as the included statements of all co-authors on their roles in the course of experiments and paper writing. The remaining scientific content of the thesis comprises unpublished results on the functional and comparative analysis of the clubroot resistance locus in ‘Tosca’.

The dissertation starts with acknowledgements and a table of contents, followed by general abstracts in Polish and in English, the bibliographic record of the published report

included in the thesis and enumeration of sources of funding, including two external projects financed by the National Science Center; one lead by prof. Wojciech Karłowski (Harmonia 2016), the supervisor of the thesis, and the other awarded to the PhD candidate (Preludium 2021), along with four internal (University funded) projects, all of them also awarded to Mr. P. Kopeć. It is important, as it shows that the tasks realized in the course of the PhD studies were positively reviewed both at the stage of proposals and the final outcome. Following the list of abbreviations, the main body of the dissertation starts with a chapter titled ‘Introduction’ spanning 17 pages, in which the Author describes the origin and significance of *B. napus*, characterizes *P. brassicae* and the clubroot disease, presents state-of-the-art knowledge on mechanisms of plant resistance against pathogens, describes *P. brassicae* pathotypes, sources of resistance to clubroot and their use in crop improvement, concluding the introduction with a sub-chapter presenting novel opportunities provided by recently emerged genomic technologies. I found the ‘Introduction’ chapter very informative and well-conceived, it was also very readable (as was the rest of the dissertation).

In the following one-page chapter the Author descriptively presented the aims of the thesis. The subsequent chapter included the full text of the *FPLS* 2021 paper, the corresponding supplementary materials, co-author statements, and a one-page comment pointing at a US patent and a parallel report published in *Sci. Rep.* 2021. Importantly, in none of those resources the existence of the gene duplication reported by Kopeć et al. (2021) was revealed.

The subsequent two chapters present results of the functional (chapter 4; 18 pages) and comparative (chapter 5; 16 pages) analyses. Finally, Chapter 6 provides overall conclusions presented on one page followed by a list of ca. 150 references which constitute a comprehensive collection of available reports on the subject of the dissertation.

### **Evaluation of the scientific merit of the dissertation**

As stated in Chapter 2 of the thesis, the research aimed primarily at the identification of loci associated with clubroot resistance in *B. napus* ‘Tosca’. As described in the paper titled “Local duplication of TIR-NBS-LRR gene marks clubroot resistance in *Brassica napus* cv. Tosca”, it was achieved through genetic linkage mapping of a doubled haploid population segregating with respect to resistance to *P. brassicae*. The mapping population was derived from a cross between ‘Tosca’ and the BRH-1 line and genotyped with Brassica 60K SNP array and additional SSR and

SCAR markers. A region spanning over 0.4 cM (corresponding to ca. 91 Kb physical distance) on the A03 chromosome, overlapping with a previously described resistance locus *Crr3*, was revealed. Subsequently, Oxford Nanopore reads allowed for structural characterization of the resistance locus, later in the dissertation referred to as *crT*, containing a duplicated TIR-NBS-LRR gene. In the report, a robust and comprehensive evidence on the functional impact of the ‘Tosca’-derived resistance locus was presented. The research was methodologically sound despite the complexity of the experiment, especially at the phenotyping stage, where appropriate measures were undertaken to standardize the scoring for the severity of the clubroot symptoms.

Most interestingly, a duplication of a functional resistance gene was documented in the ‘Tosca’ background. It was shown to be present in all clubroot-resistant DH lines, as verified by a PCR assay. Here, a minor critical remark; the precise design of the PCR assay was missing in Materials and Methods, primer sequences and coordinates of anchor sites in the reference genome were not provided in the report, and neither was the nature of the segregating indel and its position relative to the genes.

The authors also investigated expression levels of genes within the resistance locus and concluded that the two resistance gene candidates did not show any changes in expression upon inoculation with *P. brassicae*, while the difference in gene copy number was directly and constitutively reflected by a twofold increase of the cumulative transcript level as compared to susceptible lines carrying one gene copy. In my opinion, upregulation of a resistance gene is not an essential prerequisite for its function. In fact, as NBS-LRR genes are involved in sensing the pathogen, the protein has to be present in the cell before the infection takes place. Possibly, the observed sequence variants differentiating the duplicates might provide better recognition for specific *P. brassicae* pathotypes resulting in a more effective reaction to infection in ‘Tosca’. Certainly, other explanations are also conceivable, as proposed in the dissertation.

In the additional comments to the report (section 3.5 of Chapter 3) Mr. P. Kopeć pointed at studies published in parallel with the *FPLS* 2021 paper. In a US patent, the same resistance locus was reported, as well as in a *Sci. Rep.* 2021 report. In both sources the region spanned across a much larger genetic distance and no structural characterization of the locus revealing the presence of the gene duplication was provided, which makes the paper of Kopeć et al. (2021) the most detailed resource regarding the structure and function of ‘Tosca’-derived resistance to

clubroot and the only one revealing the existence and potential significance of the gene duplication for the reaction of *B. napus* to infection with *P. brassicae*.

In the following chapter Mr. P. Kopeć presented results on the functional analysis of the duplicated TIR-NBS-LRR genes at the *crT* locus which was based on the genetic transformation of *A. thaliana* Col-0 with constructs comprising each of the two genes separately or both genes in tandem. He concluded that in Arabidopsis any of the two genes increased resistance to *P. brassicae* and there was no further benefit from having both gene copies. The experiment was correctly carried out and validated, despite the fact fusing the genes in question with fluorescent proteins was not successful. Several hypotheses on the mode of action of the resistance genes and the role of the duplication were presented in the discussion. I wonder which one is the most compelling from the PhD candidate standpoint.

The final chapter describing results of the research on the *crT* locus, hereon more broadly referred to as *Crr<sup>Tsc</sup>*, deals with the evolutionary aspects of the locus and presents comparative analysis of homologous sequences across Brassicaceae. It was based on a bioinformatics approach and revealed a complex evolutionary history of the locus, including the presence of three lineages resulting from a whole genome triplication event, experiencing frequent gene losses and harboring highly divergent paralogues. Interestingly, the resistance gene(s) were acquired more recently by the L3 lineage, likely as an insertion, possibly co-transposed with a *Helitron* transposable element. While these results should be considered as preliminary, they undoubtedly provide an interesting perspective to the evolution of resistance gene clusters in plants. Regions harboring resistance gene analogs are known to be among the most dynamically evolving, as exemplified by the presence of the recent duplication in *crT*. It would be interesting to hear an opinion of Mr. P. Kopeć on possible mechanisms involved in the structural evolution of resistance gene clusters and their significance.

## **Conclusion**

The research presented in the doctoral dissertation of Mr. Piotr Kopeć is valued for the basic research aiming at the elucidation of genetic mechanisms of resistance to biotic stressors and the evolution of resistance loci. At the same time, the reported results can be applied in resistance breeding of *B. napus* and other Brassicas. The theoretical section of the thesis clearly shows his deep knowledge on the subject of investigation. Methodology-wise, the PhD candidate

competently used a range of ‘wet’ laboratory protocols combined with extensive bioinformatics-based investigations. He is able to plan his research, formulate hypotheses, provide sound conclusions and propose future directions.

**The dissertation fulfills all requirements indicated in the currently binding regulations (Ustawa z dn. 20.07.2018 r. Prawo o szkolnictwie wyższym i nauce; Dz. U. 2023 poz. 742). In particular, it provides an original solution of a scientific problem (art. 187 ust. 2), it confirms the general theoretical knowledge of Mr. Piotr Kopeć in the discipline of biological sciences and his ability to conduct research (art. 187 ust. 1).**

**Thus, I 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.**

A handwritten signature in black ink, appearing to read 'Dariusz Grzebelus', written in a cursive style.

prof. dr hab. inż. Dariusz Grzebelus