Report on Ph.D. thesis by Khushwant Singh

Identification and Molecular characterization of the Putative Immunophilins (IMMs) in the Oilseed rape pathogens *Leptosphaeria maculans*, *Leptosphaeria biglobosa*, and *Plasmodiophora brassicae*

The submitted Ph.D. thesis deals with conserved family of proteins called immunophilins in three very important pathogens of oilseed rape – fungi *Leptosphaeria maculans*, *Leptosphaeria biglobosa* and protist *Plasmodiophora brassicae (Pb)*. This thesis fulfils all demands for the type of such publications and comprises all parts which are needed.

In **Introduction**, the main theme of thesis – putative immunophilins (IMMs) of common oilseed rape pathogens – is outlined properly and sufficiently. **Hypotheses, aims and objectives** are clearly defined and they were accomplished during the experimental work.

The first there parts of **Review of literature** deal with above mentioned pathogens from the point of view their biology, symptoms caused on the host, pathogenicity related genes and diagnostics methods. The next part is aimed at the main object of thesis - immunophilins. Whole genome analyses of IMMs, types of cyclophilins and their subcellular localization, roles of IMMs , IMMs in phytopathogens and the role cyclophilin in plant immunity are described, widely. All parts are written clearly and there are comprehensible. I have some comments and questions (underlined) to this part.

- there are not links to figures and tables in the text, usually
- p. 4 – the statement „bacterial and viral diseases have little effect on their yield (Abdel-Farid et al., 2009)" is arguable, there are many publications dealing with viruses on *Brassica* crops on WOS (e.g. Walsh, Spak etc.).
- p. 9 – chapter Host resistance – it is not clear to which species of *Leptosphaeria*. The term "resistance to stem canker" should not be used – the resistance is to pathogen, not to disease
- p. 21 – Can you define the term plant immunity?

The **Material and methods** are described almost adequately, there are some ambiguities and some information is missing:

- I could not find Fig. 1 giving location of *Leptosphaeria* isolates
- Table 5 – "origin" instead "properties" should be given in the legend

- There is no experimental design (How many plants were inoculated? Replication?) and statistical methods used in "Chapter 4.4. Plant material and fungal inoculation in oilseed rape" - in results the error bars are given
- the term day post inoculation and its abbreviation dpi are used in whole text, but in the legend of Table 7 dai (day after inoculation) is used
- p. 39 - there are some discrepancies in the amount of spores used for inoculation – in the text the concentration 10^6/ml is usually used, but on this page 10^7/ml is given
- In my opinion, Sequence submission to NCBI should be given in Results, not in MM. I cannot find the comment of Table 8, too.

The chapter **Results** reflects huge amount of author´s work and knowledge in studied field. The results are commented clearly, widely and properly. I have only some comments and questions:
- p. 42-44 There are no commentaries to Fig. 10-14
- Fig. 14 – the statistical methods used in result processing are given in the legend, but no experimental design – see my comment in MM
- p. 45 (and subsequent) – the designation of isolates should be the same as in the Table 5. I did not find description of isolate C40.
- Fig. 25 – were there any correlations between disease severity and amount of DNA?
- p. 57 – "DNA of *L. maculans* and *L. biglobosa*, respectively, was quantified in petioles of *B. napus* cv. Westar with species-specific primers" – which part of genome it was?

In the chapter **Discussion** the results are compared with the vast amount of publications from the different point of view. Author proved the ability to work with the literature and to comment obtained results, properly.

The Ph.D. thesis of Khushwant Singh has good quality, the results of his work was published in several journals. The comments and questions in my report are only aimed at elucidation of some ambiguities and discrepancies **I recommend this thesis to be accepted and to cap Ph.D. title to the applicant.**

Prof. Ing. Radovan Pokorný, Ph.D.   Brno 18.8.2016