The review of Ph.D. thesis: Mgr. Igor Koloniuk „Turnip ringspot virus: differentiation of new species in the *Comovirus* genus".

Mr. Koloniuk´s thesis is devoted to elucidation of inter- and intra- species variation of Radish mosaic virus and Turnip ringspot virus, probably two distinct species of *Comovirus* genus. It is composed from two parts. The first one contains a short literature review, description of material and methods, summarization of results and their discussion and summary and conclusions. The second one is composed from three articles published in journals – Virus genes (2010, IF=1,38), Archives of Virology (2009, IF=2,02) and European Journal of Plant Pathology (2008, IF=1,65) and four abstracts from conferences (two as oral and two as poster presentations.

In its first part, the literature review deals with characterization of *Comovirus* genus and Radish mosaic virus (RaMV) and Turnip ringspot virus description (TuRSV). The second part is aimed at problems of the virus species concept, some confusion in the Comovirus genus taxonomy and some future perspectives of the picorna-like virus taxonomy. With the regard to this Ph.D. thesis results it is important, that for the different virus species is less than 75 % amino acid (aa) sequence identity in the CPs, less than aa 80% (or 75%) sequence identity in the proteinase-polymerase region and the absence of cross-protection necessary .

The part Materials and Methods is written in the clear, accurate and concise manner with appropriate literature references.

The part Results and Discussion widens and specifies some results published in above mentioned publications. The huge part is aimed at the comparison of intra- and inter- species variability on the whole RNAs and polyproteins level. For the differentiation of RaMV and TuRSV virus it is interesting, that identity of large coat protein (CP-L) was 78,9%, for RNA dependent RNA polymerase (RdRp) 75,6 % and for small coat protein (CP-S) 65,1 %. The first two data are on the edge of virus species differentiation. These two viruses form distinct cluster in the Comovirus genus, when the phylogenetic analysis using above mention proteins was used. New approach, which include protease-polymerase region (ProPol) and combined CPs was used. RaMV and TuRSV shared 79,6% aa sequences in ProPol region and 73,3% in CPs. This was below the demarcation values of both genome region. As the cross protection between this two viruses was also absent, TuRSV can be described as species different from RaMV.

I have only one question. Is there any knowledge of serological traits of these viruses?

The Ph.D. thesis of Igor Koloniuk reflects modern approaches to plant virus systems, is compact and coherent and I propose to approve it.

doc. Ing. Radovan Pokorný, PhD.

Brno, 5.3.2010

Review of the PhD thesis of Igor Koloniuk: „Turnip ringspot virus: differentiation of new species in the *Comovirus* genus"

PhD thesis of Igor Koloniuk was submitted in the form of collection of his publications with a commentary having 28 pages and 53 citations. Almost half of citations are recent publications after the year 2000. Altogether thesis contains 6 publications from which there are 3 articles in relatively highly impacted journals and 3 presentations on conferences. The PhD student is the first author of two of these impacted publications and the second author of the last one. Other authors confirmed that Mr. Koloniuk was the main author of both results and publications themselves.

Differentiation and demarcation of new virus species inside of present genera is a topical theme in the frame of virus taxonomy. Thus, the main importance of the thesis is theoretical, but there are practical applications of results for virus determination as well. To obtain his results Mr. Koloniuk used the most modern existing methods. The quality of his results has international level. As far as I can judge the commentary is written clearly and also the English is good. As the publications have already been reviewed, there is only small space for the reviewer of the thesis to add some relevant remarks.

I have only several formal remarks:

- I miss the aims of the thesis,
- scientific names of viruses should consistently be written in italics with the first letter capitalized,
- two references Chen and Bruening, 1992 should be differentiated by letters a and b,
- if there are more than two authors, only first of them + et al. should be cited in the text,
- publications of the PhD student should not be used in literature review as it is an issue for his work and publications are its result,
- using two different names of an isolate within one chapter (p. 27 – TuRSV-B and TuRSV-CH210) is somewhat misleading for the reader.

Questions:

When in cross protection assay TuRSV had more than million times higher concentration than RaMV, isn´t it already the case of cross protection? What was the concentration (or titer) of RaMV in this case? Have you also tried other time span between the inoculations? In my opinion, two weeks is rather long time for so relatively short living plant as white mustard, especially in usually not very good conditions of pot trials. Lower concentration of the competitor may be a consequence of higher level of senescence–related resistance of older plants. Single inoculations by both viruses should have probably also be done to compare the concentrations.

Conclusion:

Mr. Koloniuk obtained internationally comparable results, published them in impacted journals and presented them on conferences. Thus he proved his capability of scientific work. Based on the above mentioned facts I recommend his PhD thesis for defence and afterward to confer him a scientific degree PhD.

Prague, March the 4th, 2010

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Peer review on the PhD thesis of Mgr. Igor Koloniuk

TURNIP RINGSPOT VIRUS: DIFFRENTIATION OF NEW SPECIES IN THE *COMOVIRUS* GENUS

Range of work: 35 pgs divided into List of Abbreviations, Introduction, Materials and Methods, Results and Discussion, Summary and Conclusions, References and Supplements (Reprints of author's publications described in the thesis)

Aims:

There were three major aims of this thesis. The first was the comparison of intra- and interspecies identity of comoviruses including the radish mosaic virus (RaMV) group and turnip ringspot virus (TuRSV) group. The second aim was to obtain complete genomic sequences of two TuRSV isolates and their molecular characterization and phylogenetic analysis. Last, but not least aim of the presented thesis was the research of the occurrence of TuRSV not only in Europe but also in America and evidence that RaMV and TuRSV are different virus species of the genus *Comovirus*.

Main results:

1. The complete genomic sequences of RaMV1 and TuRSV-M12, -CH210 were obtained and sequences of some genomic regions of other isolates were determined.
2. The comparison of intra- and interspecies identity of comoviruses was done and phylogenetic analysis was accomplished.
3. Absence of cross-protection between RaMV1 and TuRSV-CH210 isolates was proved.
4. On the basis of presented molecular and biological criteria TuRSV can be distinguished as a distinct species.
5. Specific Taqman probes were designed for further analyses of RaMV and TuRSV.

Reviewer comments:

1) To which part of the genome do the qPCR probes anneal? What were the criteria for the choice of these probes? How precise are the probes to discriminate between these RaMV and TuRSV viruses?
2) Are you sure that you always isolated and sequenced only one particular viral isolate (either RaMV or TuRSV)? How did you make sure that your isolates were not the mixture of different RaMV or TuRSV isolates?

Conclusion:

This work meets demands set to PhD thesis, because author proved his ability to work independently, to draw original conclusions from experimental data obtained by advanced scientific methods, and to summarize all in papers that were accepted in renowned international scientific journal. The thesis is written clearly and without errors, the proofs are clear and easily understandable. I think that the results will be surely of considerable interest to specialists working in the field.

Prague, 6.2. 2010

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