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Review report on PhD Thesis of Mr. Piotr Koczorski
"Root microbiomes as controls of P use efficiency in woody crops"

Supervisors: Professor Katarzyna Hrynkiewicz – University of Nicolaus Copernicus in Toruń, Poland and Professor Christel Baum – University of Rostock, Germany

Piotr Koczorski submitted the doctoral thesis dealing with the testing of selected P solubilizing microbial strains on the growth of *Salix* spp. and P use efficiency. Moreover, the thesis concerning the assessment of the diversity and abundance of P solubilizing microorganisms in the rhizosphere and roots of *Salix* spp. in two sites of short rotation coppices.

The background and the scope of the thesis

Phosphorus is one of the most important nutrients essential for plants. However, P sources are nonrenevable, global phosphate deposits decrease and additionally P loss by leaching constitutes a problem connected with eutrophication. Therefore more effective phosphorus use in agriculture is urgently needed. To improve phosphorus availability for plants in a natural and environmental friendly way, in recent years P solubilizing microorganisms are more commonly applied to develop sustainable nutrient management strategies. It is known that these microbes can be used as component of biofertilizers, which can be good alternative for mineral fertilization. The applications of biofertilizers improve soil quality, provide biological control, promote symbiosis and increase nutrient fixation. However, there is still a knowledge gap concerning the interactions of bacteria and fungi with plants to develop effective biofertilizers containing P solubilizing microorganisms. Moreover, new bioproducts and selected strains of microorganisms must be tested to evaluate their influence on plant growth characteristics and other parameters important in the context of environmenal protection and climate change.

Taking into account 2030 Biodiversity Strategy it is worth to mention that these relations should be tested not only in monocropping but also in mixed cropping agronomic strategies. Short rotation coppices are one of the most popular cultivation strategy across Europe and due to ecological and economic benefits they started to develop also mixed cropping practices. However, there is still lack of deep knowledge on P mobilization in the soil and roots under such treatments.

Therefore, in this doctoral dissertation, in order to deepen knowledge and to find solution for improvement of P availability for plants, Piotr Koczorski performed experiments evaluating the influence of P solubilizing microbes on the growth of *Salix* spp. trees and characterized large number of bacterial and fungal strains with potential for P solubilization.

The scientific level as well as novelty of the thesis is very good, considering the importance of environmental protection by biofertilizers application, their beneficial effects on plant growth, market requirements and research subject. The thesis covers the problem with excessive mineral fertilization and especially shows mechanisms how to improve the P management to make this nutrient more available for plants. Good solutions will allow many applications for science and new practical bio-products. This is also one of the goals of European Commission to decrease mineral fertilization and chemical products use in agriculture. My opinion about doctoral dissertation background is therefore very positive and I find this topic very interesting and up to date.

General description and the structure of the thesis

The thesis presents original research results in the area of environmental protection and biological sciences. The content of the thesis includes four research publications, of which three are peer reviewed and one is so fare only a manuscript under review.

This PhD thesis is clearly presented and well structured. It consists of 13 main chapters. The thesis is written on 135 pages and enriched by figures and tables presented in particular research articles and at the end by 95 valuable references.

The presented thesis is organized in the following sections: list of publications that are a main part of the doctoral dissertation, abstracts in Polish and in English, introduction, aims of the study, research hypotheses, research methodology, publications, summary of results, discussion, final conclusions, future outlooks and references.

It is necessary to underline that dissertation is composed of three published papers and one unpublished manuscript of the research article. The research papers are presented below and all together have *impact factor* at 15.973 and 300 points of Ministry of Education and Science.

List of publications within doctoral dissertation:

- **P1** – **Piotr Koczorski**; Bliss Furtado; Katarzyna Hrynkiewicz; Michelle Breezmann; Martin Weih; Christel Baum, *Site-Effects Dominate the Plant Availability of Nutrients under Salix Species during the First Cutting Cycle*. ***Forests***, **2021**, 12(9), 1226, DOI: 10.3390/f12091226
- **P2** – **Piotr Koczorski**; Bliss Ursula Furtado; Marcin Gołębiewski; Piotr Hulisz; Christel Baum; Martin Weih; Katarzyna Hrynkiewicz, *The Effects of Host Plant Genotype and Environmental Conditions on Fungal Community Composition and Phosphorus Solubilization in Willow Short Rotation Coppice*. ***Frontiers in Plant Science***, **2021**, 12, 647709, DOI: 10.3389/fpls.2021.647709
- **P3** – **Piotr Koczorski**; Bliss Ursula Furtado; Marcin Gołębiewski; Piotr Hulisz; Dominika Thiem; Christel Baum; Martin Weih; Katarzyna Hrynkiewicz, *Mixed growth of Salix species can promote phosphate-solubilizing bacteria in the roots and rhizosphere*. ***Frontiers in Microbiology***, **2022**, 13, DOI: 10.3389/fmicb.2022.1006722
- **P4** – **Piotr Koczorski**; Bliss Ursula Furtado; Christel Baum; Martin Weih; Pär Ingvarsson; Piotr Hulisz; Katarzyna Hrynkiewicz, *Large effect of P solubilizing bacteria on growth and gene expression of Slix spp. under stress conditions induced by reduced phosphorus levels*. ***Manuscript under revision***.

In the first three chapters PhD candidate includes publication list and abstracts of the research. Research motivation, hypotheses, objectives and main scope of the study are clearly explained in chapters from 4 to 6. The previous works on short rotation coppices and P solubilizing microorganisms are presented in the introduction section as well as at the beginning of each research article of doctoral dissertation. Piotr Koczorski introduces reader to the dissertation topic and presents the objective of the study. PhD candidate underlined that the main goal of his research was to reveal site effects and management practices for the improved use of microbial P mobilization in biomass production with fast-growing tree species. The

objectives are clear and are connected with particular papers. Some of basic problems are discussed in each research article and solutions are presented in chapter 8 in each publication.

Chapter 7 includes the description of the used methodology of research in all presented research papers. Piotr Koczorski used many different techniques to confirm established hypotheses, including methods of P solubilizing microorganisms selection using various microbiological media, molecular identification of bacteria and fungi after DNA extraction from pure strains, biochemical analyses on enzymatic characterization of selected microbial strains, chemical methods used for soil and plant rhizosphere characterization, determination of bacterial and fungal microbiome in soil rhizosphere and plant roots based on next generation sequencing, total RNA isolation and sequencing. Results were elaborated bioinformatically and statistically using specialistic pipelines, workflows and software. The used not only basic but also deeper multiple data management is correctly provided and deliver relevant data, supporting formulation of proper conclusions.

The next part of dissertation (chapters 8-10) is focused on results and discussion presented in particular papers. The results obtained by Piotr Koczorski were described very carefully. The main motivation of the research is that the results will be in great importance for management the global P resources and understanding plant-microbe interactions under P deficiency stress. The discussion section of the thesis presents comparison of obtained results with literature. Author in this part of the thesis demonstrates and underlines that the mixed *Salix* plantations prevent nutrient loss and that P solubilizing bacteria alter the growth patterns and gene expression of willows. Moreover, he discusses the level of plant association as one of the main factor shaping bacterial and fungal diversity in willow short rotation coppices. At the end of dissertation the general conclusions basing on the performed research were described.

The thesis structure is well done, following logically topics to receive the results of all tasks. Very good critical analyses are made in this dissertation, in both separately for each article and in the chapters containing summary of results and discussion. Very relevant future outlooks presented at the end of dissertation become a base for future contributions.

The main contribution of the thesis

The most important achievements of Piotr Koczorski dissertation in my opinion are the following:

- mixed growth of *Salix* species promotes the alkaline phosphatases activity in P-deficient soil conditions;
- the fungal diversity at the same lavel of association was mainly driven by soil properties such as total nitrogen, total organic carbon and pH;
- cultivation of mixed willow species system increases the abundance of P solubilizing bacteria decreasing the problem of low P availability in agricultural soils and lowering the need of mineral fertilizers application;
- phosphate solubilizing bacterium of the Tora willow species significantly affected transcription in the leaves, affecting the upregulation of most genes, especially those related to photosynthesis, which are highly influenced by phosphorus.

Summary and Conclusions

The dissertation has contemporaty and very modern aspect described by 3 research papers and 1 manuscript under review. This thesis is well written and documented. The text is prepared in clear and concise manner. The conclusions confirm that the main objectives of the dissertation have been fulfilled. The experiments were well arranged and measurements methods and techniques were correctly applied. The results were elaborated and their interpretation was at very good scientific level.

The topic is very important not only for science but for many practical approaches, mainly in agricultural, horticultural and forestry sectors. The science and branches of industrial sector could use the results achieved in the thesis for future contributions and develoments, as author mentioned in the future outlooks section.

The following points can be stressed:

- The research topic is relevant for the development of bio-based product for agriculture, horticulture and forestry.
- The provided analyses fully correspond to all objectives of the thesis.
- The research subject, the methodology and methods are consistent, corrected and used appropriate.
- The results are analysed, interpreted and discussed in accordance with the scientific standards in the manner of environmental microbiologists community.
- The literature references are correctly selected and cited.

- The thesis clearly demonstrates that Piotr Koczorski can conduct independent scientific research and future research work carried out by him independently will meet the standards of his scientific community.

The following remarks and comments related to the thesis should be explained in details:

- Please clarify the main criteria of the selection of the used study sites in respect to their different properties and functions or other features. Are there such study sites in Poland that could be used for future research?
- Please justify the main reasons (besides P-solubilizing properties) of the selection of the tested bacterial and fungal strains that you used in this study.
- I would like to ask the PhD candidate if he can formulate more practical applications of the proposed achievements in the dissertation.
- Are there any bioproducts based on P solubilizing microorganisms used in agriculture sector?

The above comments don't affect the positive evaluation of the entire dissertation, which fulfills requirements set on PhD theses.

Piotr Koczorski PhD thesis covers interesting and very relevant topic, contains original and valuable scientific results and fits in coming time of multidisciplinary project developments. The rank of the results included in dissertation is highlighted by their publication in international Journals: *Forests*, *Frontiers in Plant Science* and *Frontiers in Microbiology*. It could be the base point not only for researchers but also for bio-based industry needs. The thesis meets the requirements imposed on a PhD dissertation and I recommend to admit Mr. Koczorski to public defense of his thesis. Because of the very high scientific level of results presented in doctoral dissertation I would like strongly recommend to the scientific committee to award the PhD degree to Piotr Koczorski and I request that Piotr Koczorski's doctoral dissertation be distinguished with an appropriate award.

Sincerely,

Magdalena Frąc

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Lublin, 14.07.2023