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Review
of the PhD thesis entitled
**“Biodiversity of symbiotic nitrogen fixing rhizobia isolated from arable and virgin soils from
Huambo province, Angola”**
elaborated by André Loução Bongo

The presented PhD thesis was done at the Laboratory of Agricultural Microbiology of the Department of Plant Protection, Faculty of Natural Sciences and Technology, University of Life Sciences in Wrocław under supervision of promoter Prof. dr hab. Stanisław Pietr and auxiliary promoter dr Małgorzata Patrycja Oksińska

The evaluated thesis addresses the questions of fundamental importance to soil microbiology and agriculture – biological nitrogen fixation (BNF) and the interactions between plant and nitrogen-fixing bacteria (NFB). Studies on BNF are of great importance since this process play a key role in crop productivity. Nitrogen-fixing bacteria can supply up to 90% of plant's requirement for nitrogen and have the beneficial effects on plant growth and development which can resulted in the higher crop production and increased plant resistance to the various environmental stress conditions. Moreover, considerable enhance in a crop yield have been reported in the response to plant and soil inoculation with nitrogen-fixing bacteria. This provides an opportunity to engineer closer associations between NFB and food crops, in which fixed nitrogen is delivered to plant more effectively. Symbiotic nitrogen-fixing bacteria form symbiosis with plants from the family *Fabaceae* that are a valuable resource of vegetable protein. The representative of this family common bean (*Phaseolus vulgaris* L.) is an important food crop for its edible seeds and pods as well as major source of protein throughout the world. It also contains important minerals (iron and zinc) and many vitamins. It has been calculated that common bean is the basic nutritional food for over 100 million people living the countries in Sub-Saharan Africa as well as the primary source of dietary protein providing over 65% of the protein, and 35% of the caloric intake. Therefore, the studies on nitrogen-fixing bacteria that can enhance the yield of common bean are of great significance for the agriculture over the world.



Structure of the dissertation

Dissertation of André Loução Bongo was prepared in the traditional PhD thesis format and contains all needed chapters. The subsequent parts of his thesis consist: introduction displaying the literature review, an description of the materials and methods, a presentation of all the results, a general discussion, conclusions and list of references. In typical form the chapter presenting aim of the study is usually a separate chapter, in this case is a part of the introduction. The work also contains a list of acronyms and abbreviations, lists of tables, figures, maps, schemes, photos as well as abstracts in English and Polish. Abstract provides a summary of the dissertation and its key results, however some information should be presented more accurate. For example, instead of saying “most of plant growth promoting bacteria” their exact number should be given. Moreover, in the Polish version the title of the thesis should be written in Polish language. The presented dissertation is organized into coherent and logical work. Author collected a huge number of references (470) and all important papers related to the scientific field of the thesis are included in. They were properly used and correctly cited. The work of André Loução Bongo is written in accurate English with correct grammar, spelling and punctuation. There are only few language mistakes.

Methodology

All presented experiments were properly designed and conducted. In the section “Materials and methods” Author detailed described plants and methods used for the isolation and characteristics of isolates. The same can be said about the pot and field experiments.

The indigenous rhizobia were isolated from the soil samples and from two species of trapping plants i.e. common bean (*Phaseolus vulgaris*) and adzuki bean (*Vigna angularis*). All soil samples taken up from natural forest, fallow, and cultivated fields at different regions of Huambo province, and one soil sample collected from the desert of Namibe province were subjected to analyses of their physico-chemical properties. The isolation of rhizobia directly from soil was performed by traditional plate method on YMAA medium and from crushed nodules collected from the trapping plants on YEMA medium. The selection of putative rhizobia from isolated from soil and nodules was carried out on the basis of colony morphology formed on YEMA-CR medium, their inability to grow on the S1 and D1 media and Gram staining. One hundred and fifty eight isolates considered to be rhizobia were used in the pot experiment in order to check their ability to nodulate common bean cv. Basta. Additionally, the effect of these isolates on plant biomass was determined.



Isolates that stimulated the growth of the common bean (cv. Basta) in the pot experiment, at the primary screening, but did not induce the nodule formation were tested for the ability of indole-3-acetic acid production. Apart from phenotypic characterization the selected isolates were subjected to genetic analyses. This involved the analysis of sequences of the following genes 16S rDNA, *nifH* and *NodC*. The selected nineteen isolates were used in the experiment for their authentication. For this, in common bean treated with isolates the number of active nodules, the nodules dry mass, the shoot dry mass, root dry mass, and shoot dry mass to root dry mass ratio were measured. The next step of the research included the evaluation of the symbiotic interaction with four common bean cultivars. The important part of the study involved the field experiments carried out in Angola. Such approach allowed to check if the selected strains may be a useful tool to increase the productivity of common bean in Angola.

I would like to stress that André Loução Bongo did a huge work analysing all isolates by microbiological and genetic techniques as well carrying out the pot and field experiments. The organization of the experiments ensured that the right type of data is available to answer the questions of interest as clearly and efficiently as possible. Information presented in sections “Materials and methods” are sufficient to replicate the experiments.

Substantive assessment of the dissertation

In the well-written “Introduction” the Author presented the background of his study and provided the reader with the essential context needed to understand the research problem and its significance. In the first parts of this chapter the environmental conditions of agricultural production in Sub-Saharan Africa with particular emphasis on agricultural production in Angola and the social-economic importance of the common bean production and their direct impact on the diet of people were described. In the successive sub-sections the Author demonstrated the current state-of-the art of the biological nitrogen fixation, symbiotic rhizobia, mechanism of nitrogen fixation and problems connected with the assessment of rhizobia diversity. André Loução Bongo paid also attention on the practical aspects of his research related to the use of selected rhizobia as a tool to increase the common bean productivity in Angola.

The review of the literature presented in the Introduction led the readers to the clearly stated aims of the study. The main goal of MSc André Loução Bongo study was to characterize the rhizobia diversity present in the different soils of Huambo province, Angola and select the most effective

indigenous rhizobia nodulating common bean.

During the study PhD student tried to verify the hypothesis that soils of Huambo are colonized by indigenous rhizobia species, that are able to establish an effective symbiotic relationship with *Phaseolus vulgaris* and improve common bean production by higher level of nitrogen fixation. Additionally, André Loução Bongo verified the second hypothesis assuming that trapping plants will be more effective for the isolation of a greater variety of rhizobia as compared to the isolation of rhizobia from soils.

To achieve the goals André Loução Bongo used a spectrum of well-chosen methods that allowed him to isolate, characterize and identified native rhizobia. Moreover, allowed him to assess the impact of selected rhizobia on the common beans nodulation and biomass.

The experiments carried by the Author brought many interesting results that are presented on 50 pages of the dissertation in the form of 31 tables, 11 figures and 5 photos. All the results are clearly presented, described and documented. The strong point of the study is the statistical analysis of data. They were subjected to the different statistical tools that made the results valuable and reliable. Some of the important results of André Loução Bongo's studies show that: (1) the biodiversity of rhizobia nodulating the common bean in the studied regions of Huambo is low; (2) the dominating native rhizobia nodulating common bean are closely related to *R. miluonense* or *R. aegyptiacum*/*R. bangladeshense*/*R. binae*/*R. miluonense* group; (3) several isolates from the nodules of common bean and adzuki bean could be considered as the plant growth promoting bacteria for common bean and these bacteria are closely related to *Burkholderia diffusa*, *Beijerinckia fluminensis*, *Herbaspirillum huttiense*, *Enterobacter ludwigii*, *Enterobacter wuhouensis* and *Rhizobium puesense*; (4) isolates named as *R. miluonense* strain HBA15a, *R. aegyptiacum* strains HCC321 and HLo8 showed high symbiotic effectiveness with the common bean and used as an inoculum of seeds significantly increased the plant biomass in comparison with the control plants. The last outcome proved that the most effective rhizobia can be a potential candidate for the production of inoculants for common bean in Angola. Enhancing nitrogen fixation in target crops promises substantial benefits for worldwide agriculture, while decreasing the use and environmental impact of industrial nitrogen fertilizers.

The Authors assumed that strains, HEC1 and HC4 related to genus *Paraburkholderia*, are probably new species because they are not clustered with the any of recognized species. In my opinion it is risky to say this. To confirm that one have new species the multilocus sequence analysis using a



number of housekeeping genes, followed by a concatenated tree should be performed. Another approach involve the whole genome sequencing followed by homology studies.

The general conclusions as well discussion were well laid out and presented in a logical sequence. MSc André Loução Bongo carefully draw the conclusions and the claims are supported by the results. The Author well discussed the contribution of other researchers in the field of his study.

Final conclusion

I have found Mr André Loução Bongo dissertation to be a very good one. It presents new interesting results that enhance our knowledge on the biodiversity of rhizobia inhabiting the soil in Angola. It should be again underlined that his studies apart from the basic scientific value have also applicable aspect and the obtained outcomes may be useful for the improvement of crop productivity of common beans not only in Angola but also in other countries.

In my opinion the doctoral dissertation “Biodiversity of symbiotic nitrogen fixing rhizobia isolated from arable and virgin soils from Huambo province, Angola” by André Loução Bongo fulfils all requirements set for PhD theses in science as specified by Polish law, Regulation of the Minister of Science and Higher Education of 19 January 2018, (Dz.U. 2018 poz. 261) and Regulations introducing the Act - Law on higher education and science, 3 July 2018 - (Dz.U. 2018 poz. 1669).

The PhD thesis contains new valuable data, proposes original solutions to important scientific and practical problems. Moreover, it proves candidate’s general knowledge and demonstrate profound familiarity with the field and demonstrate mastery of research methods and their application.

Based on the above, I conclude that André Loução Bongo MSc should be admitted to the next stages of the PhD procedure. Taking into account the wide range of the study and methods applied, novelty and the high scientific level of the evaluated PhD thesis André Loução Bongo should get award for the best doctoral dissertations.

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