June 21st, 2022

PhD Thesis Review

Candidate´s name: Alba Esteban Hernándiz
Department: Chemical Biology and Genetics. Laboratory of Growth Regulators. Palacký University Olomouc. Faculty of Science.
PhD Thesis title: Characterizing the mode of action of small molecule-based biostimulants
Examiner´s name: Andrés A. Borges; **E-mail**: aborges@ipna.csic.es

As opponent of the candidate **Alba Esteban Hernándiz**, under the supervision of Dr Nuria De Diego Sánchez, I want to state that the manuscript entitled ***Characterizing the mode of action of small molecule-based biostimulants,*** is **approved for its defence**, and according to the instructions and criterion provided for the PhD Thesis opponents at Program in Experimental Biology, hereby I present my review of this manuscript.
This thesis is organized in four units or chapters: the first one includes an introduction to the problems that modern agriculture face under climate change scenario, especially because of less precipitations and high temperature, where salt and drought stresses will be hard to combat. In this sense, the use of biostimulants, its positive effect against crop stress and the mode of action of small molecule-based biostimulants is a currently hot topic in science. This chapter, which also includes the aims and scope of this thesis (four objectives), is very well written and referenced by an updated state of the art about this research field.

The following chapter focused on the materials and methods used to perform all experiments described in the following chapter. The research methodology to conduct all the experiments carried out in the thesis are described in detail and the candidate demonstrates that she has a knowledge about the thesis topic and is able to address suitable experiments to study the effects of biostimulants and their mode of action both under normal and stress conditions.

Overall, the research methodology used is correct and meets well the requirements of a high quality experimental work.

Third chapter summarizes the more relevant results obtained. These results were already published except one, which is still under review but along this chapter, are presented and discussed in a form that the reader can understand the different objectives addressed in the thesis.

Last, the candidate presents the conclusions and future perspectives.

Assessment of the novelty and results obtained in this thesis

In the opinion of this opponent, this is an original work, as evidenced by its publication in various high-ranking impact journals. The hypotheses made for each of the objectives proposed in the work are correct, and the methodology used to prove the results obtained was appropriate. In addition, through her contribution in each of the aforementioned papers, the candidate has achieved a high level of skills and expertise to continue her scientific career.

This thesis deals with interesting aspects of small molecules-based biostimulants under controlled conditions and in the field. This work contributes to increase our knowledge about their efficacy, dosage, especially of polyamines, and about some of the possible mechanisms of action, all of which are necessary to understand their effective use for sustainable agriculture.

Several questions to discuss during the thesis defence

1. It is well known that plants under natural field conditions face several stresses simultaneously, and this combination is particularly difficult to combat, e.g., water and heat stress. It would be very interesting to use HTP platforms like Olophen for biostimulant screening under this complex combination of stress factors. What are your thoughts on this? Could it be possible to evaluate this?

2. As part of your thesis, you used polyamines (putrescine, ornithine, spermidine) as priming agents for both soil drenching as well as seed treatment. The latter type of seed treatment could mean that your treatment induces an epigenetic footprint capable to increase tolerance once the plant is mature and exposed to stress? Have you explored this hypothesis? What is your opinion?

3. Results obtained using putrescine (Put) and spermidine (Spd) in maize indicate different mechanisms/modes of action of these two polyamines. While Put performs better under normal (well-watered) conditions, Spd does not. The latter is more efficient under water deficit conditions. Could it be that Spd acts as mild stress agent and contributing to increase proline levels and additional plant defence responses under both normal and water deficit conditions, and that for this reason this treatment does not help to increase yield? What do you think about this?

After examining the manuscript of this PhD Thesis, I hereby recommend its defence

Signed: Dr. Andrés A. Borges
Chemical Plant Defence Activator group
IPNA-CSIC