



Universidad Nacional de Río Cuarto
Facultad de Ciencias Exactas, Físico-Químicas y Naturales
Departamento de Ciencias Naturales

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Characterizing the mode of action of small molecule-based biostimulants

Ing. Alba Esteban Hernández

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PhD Thesis Review

Examiner: **Fabricio D. Cassán**

As an opponent of the candidate **Ing. Alba Esteban Hernández**, under supervision of **Ing. Nuria De Diego Sánchez**, PhD, I want to state that the manuscript entitled: "**Characterizing the mechanism of action of small molecule-based biostimulants**" is **approved** for its defense. According to the instructions and criterion provided for the PhD Thesis opponents at program in Experimental Biology, I would like to present my review of this manuscript.

The **aims and scope** of this thesis are clearly detailed in the first section (or chapter) of the manuscript and summarize the most representative experimental activities developed by the PhD candidate along this process. Some minor considerations are suggested for this chapter: page 12, Objective 2: Is it necessary to include a justification and reference for this objective in this section? (Please consider...To evaluate the effect of the polyamines putrescine and spermidine on the production, yield, and quality yield parameters of maize subjected to water deficit). page 12, Objective 3: Is it necessary to include a description of these molecules in the objective? (Please consider...To characterize the mode of action of the selected metabolites, the non-proteinogenic amino acid ornithine (Orn), putrescine (Put) and 1,3- diaminopropane (Dap), using *Arabidopsis* (*Arabidopsis thaliana* L.) as a model plant).

The **second section** is a **literature review** and summarizes the impact of global warming in the



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planet and how the current abiotic stresses conditions affect the higher plants. The chapter offers a clear idea of the impact of saline stress on crops and shows biotechnological alternatives to maintain productivity under environmental limiting conditions. It highlights the use of biostimulants as a technological alternative to counteract this problem and emphasize those biostimulants used to improve the plant's response under stress conditions. In this context, the candidate makes clear the importance of having precise tools for the evaluation of the effectiveness and mode of action of different biostimulants using phenotyping approaches and how this fact correlates with a growing demand in the use of alternative technologies to increase crop productivity in a sustainable way. The section or chapter is well organized, the information is accurate and provides a detailed overview of the context of the thesis. The bibliography is current and the information adequate. It is a chapter well achieved by the candidate.

The **third section** summarizes the methodology used in the **supplements I to V** related with the evaluation of the behavior of different plant species grown under abiotic stress conditions and modified by addition of small molecule-based biostimulants: *Arabidopsis* (supplements I and V); pea (*Pisum sativum* L.) (supplement II) and maize (*Zea mays* L.) (supplement III and IV), plus the experiments for the evaluation of ornithine as a foliar spray to enhance the tolerance to water deficit in barley (*Hordeum vulgare*). The methodology is described in a precise and clear way which ensures the reproducibility of whole experiments in the future. The experimental design and statistical analysis used by the candidate are adequate and have been clearly detailed in the text. In relation to the use of different small molecule-based biostimulants as priming molecules (i.e. N⁹-Substituted cytokinin derivatives, putrescine, spermidine, 1,3-diaminopropane and ornithine), it would be interesting to calculate an effective concentration for each compound based on the concentration of the solution used to soak the seeds and the volume of liquid absorbed by them. This will give a more precise idea of the effective concentrations of each molecule to induce changes on the plant metabolism.

The **fourth section** summarizes the results obtained in experiments related to **supplements I to V**. These results were already published (supplements I, II, III and V) or submitted for its publication (supplement IV). The last supplement in particular, despite it is still under revision has been clearly presented and discussed in relation to the objectives and scope of this thesis.



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This is a solid work, as evidenced by its publication in high-ranking impact journals. In addition, through her contribution in each of the aforementioned papers, the candidate has achieved expertise and criteria along this process.

The main **conclusions and perspectives of this thesis** are considered of high value by the reviewer. The reviewer also considers these would help to generate a concrete contribution in the field of study and biostimulants technology. The conclusions are clear, precise and summarize the most important achievements of this thesis. There is a strong coherence between the proposed objectives, results and the conclusions generated throughout the manuscript. Regarding perspectives, the candidate should consider the adequacy of the system for the evaluation of biostimulants according to the treatment (i.e soil, seeds or plant) where the active substance is affected by application procedure as well as physical and chemical properties of the target plant tissue.

In base of this revision the opponent's questions for the thesis defense are:

1. The candidate mostly used NaCl as the main effector of abiotic stress; however, under natural conditions plants are simultaneously exposed to different stress effectors (i.e. temperature, light, salinity by different salts, etc.) Have you evaluated these high-throughputs methodologies under a combination of stress effectors?
2. One of the most studied responses of plants to stress is related to their capacity to reduce growth during alarm phase, mainly in early developmental stages; however, in late stages the plant response could modify both its vegetative growth and the reproductive development. Have you evaluated the plant response to stress at reproductive developmental stages with the same biostimulants proposed in this thesis? Have you correlated these results with those obtained at early developmental stages?
3. Plants treatment with exogenous biostimulants help them to alleviate abiotic stress conditions. How long is this treatment effective after application? Have you evaluated some effective strategy to maintain plant responses for long periods?



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After examining the above mentioned PhD thesis, I hereby recommend its defense.

Sincerely,

Prof. Fabricio Dario Cassán

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