PhD School on Agriculture, Environment and Bioenergy
(http://sites.unimi.it/dottorato_aab/)

(XXXV cycle, 2019-21)

Project draft

1. **Field of interest**

*Indicare **il/i** settore/i scientifico disciplinari:*

AGR13: biomass re-use, circular economy.

2. Project title

Biostimulant from Biomasses

3. Tutor (Fabrizio Adani)

- Eventually: co-tutor/s

4. Relevance of the topic and state of the art:

The regulation of plant growth and the development and alleviation of the negative effects of environmental stresses during ontogenesis, are important factors determining the productivity of cultivated plants. While it is well recognized that biotic and abiotic stress prevents essentially all crop systems from achieving their yield potential, current understanding of the mechanisms involved, and the strategies to mitigate these effects are limited. Abiotic stresses may be prevented by optimizing plant growth conditions and through provision of water and nutrients and plant growth regulators (PGRs—auxins, cytokinins, gibberellins, strigolactones, brassinosteroids). In addition to these traditional approaches, biostimulants are increasingly being integrated into production systems with the goal of modifying physiological processes in plants to optimize productivity. Plant biostimulants are defined as substances or microorganisms whose functions, when applied to plants or the rhizosphere, are to stimulate natural growth processes by enhancing nutrient uptake, nutrient efficiency, tolerance to abiotic stress and crop quality (European Biostimulant Industry, 2016a). During recent years there has been a progressively increasing demand for biostimulants and it is estimated that a provide a global market increase will reach 2.91 $ billion by 2021 (Anonymous, 2016), for which the EU is the largest market (European Biostimulants Industry Council, 2016b). The definitions of biostimulant substances and their associated effects are still changing, since they are dependent, on one hand, on market interest and on the other, on research results. At the moment, only five categories of substances, namely microbial inoculants, humic substances (humic and fulvic acids), aminoacids and seaweed extracts are identified commercially as biostimulants, as they have shown the capacity to stimulate plant growth and yield, improve nutrient uptake and resistance to exogenous stresses and to stimulate plant metabolism (du

Jardin, 2012; Ertani et al., 2009; Calvo et al., 2014). The recent interest in developing more sustainable and resilient agriculture has led to the identification of naturally occurring substances with plant biostimulant effects which can be used instead of synthetic ones (Calvo et al., 2014; European Biostimulant Industry, 2016a). <u>In this perspective, the identification of new biostimulant fonts has a great interest for the future application instead of synthetic ones and their production.</u>

5. Layout of the project (draft):

At the moment, only five substance categories, namely microbial inoculants, humic substances (humic and fulvic acids), aminoacids and seaweed extracts are identified commercially as biostimulants, having shown the capacity to stimulate plant growth and yield, improving nutrient uptake and resistance to exogenous stresses or stimulating plant metabolism (du Jardin, 2012; Colla et al., 2014; Ertani et al., 2009; Calvo et al., 2014). In order to expand and improve the biostimulant sector, the possibility to identify wastes or by-products as sources for the same biostimulant molecules or discovering new bioactive mixture, thereby saving on raw materials, is a recent development (Ugolini et al., 2015; Pane et al., 2016). Moreover, biomass transformation process such as anaerobic digestion improved biostimulants properties linked to the anaerobic digestion transformation done. In addition to the waste re-use microalgae are known to be reach in biostimulant fraction which quali-quantitative composition depended by the algae typology and growing condition.

5.1. Materials & Methods:

<u>**1. Materials sampling and biomass cultivation 1-6 months**</u>

Algae and biomasses will be characterized as potential feedstock for the biostimulant extraction; following parameters will be assessed: pH, redox potential, total and volatile solids, carbohydrates (quali-quantitative analysis), protein, lipid fraction, dissolved organic fraction quali and quantitative characterisation, ammonia, nitric and total Kjeldahl nitrogen, soluble and total phosphorous, micronutrients and contaminants (e.g. heavy metals).

<u>**2. Biomasses and algae pre-treatment to improve biostimulant effects. 6-12 months**</u>

Biological treatment and chemical separation will be performed to obtain biostimulant fraction. With the aim to develop sustainable process enzymatic hydrolysis, chemical separation will be considered.

<u>**3. Screening test for the biostimulant activity 12-20 months.**</u>

Biostimulants fraction will be tested for the hormone-like activity such as auxin-Like Activity and gibberellins-like activity by using lab screening test based on the seed epicotyl and hypocotyl growth (Audus, 1972).

4. Vegetative biostimulant tests. 12-20 months

The best results obtained of the previous Gibberellins and Auxine like activity, will be tested in a vegetative biostimulant assays. The biostimulant effect could be tested following a protocol published by Trinchera et al. (2010), and modified as follows: Zea mays (cv FAO 300) seeds were primed overnight with water, and germination was then obtained at 28 ◦C for 48 h until roots were 3.5–5.5 cm long. Two seedlings were then put in pots of 6 cm of diameter filled with sand and SMH was added at 0.9, 9, 90 and 900 ppm (mg kg^{-1} of freeze dried product in sand) solubilized in 150 mL of water or 50% Hoagland's nutrient solution (HS) (Hoagland and Arnon, 1950). ANOVA and post hoc Fisher's LSD statistical tests were carried out by Statistica® software (StatSoft Inc., USA).

5. Biostimulants extract chemical composition 20-30 months

Effective biostimulant fraction were chemically characterised by using a GC MS metabolomic approach (Scaglia et al., 2018).

6. Evaluation of the results and data elaboration. 24-36 months

Basing on the preliminary data, the Partial least square chemometric (PLS) approach will applied to identify the most important chemical classes involved into biostimulant effect. Thus a minimum dose will be looked for.

5.2. Schedule and major steps (3 years):

In order to obtain a broader vision about the Biostimulant effects from different kind of biomasses and from micro algae nutrients, the work-plan of this PhD project could be as follow:

1-6 months: 1.Materials sampling and biomass cultivation

6-12 months: 2. Biomasses algae pre-treatement to improve biostimulant effects.

12-20 months: 3. Screening test for the biostimulant activity

12-20 months 4. Vegetative biostimulant tests.

20-30 months 5. Biostimulants extract chemical composition

24-36 months 6. Evaluation of the results and data elaboration.

6. Available funds (source and amount): Fondi Fabrizio Adani (100.000 €)

6. Literature: max 10 citazioni

1. Abbas S. M. (2013). The influence of biostimulants on the growth and on the biochemical composition of *Vicia faba* CV. Giza 3 beans. Rom. Biotech. Lett. 18, 8061–8068. Available online at: http://www.rombio.eu/vol18nr2/1%20Salwa%20Mohamed.pdf

2. Abd El-Baky H. H., Hussein M. M., El-Baroty G. S. (2008). Algal extracts improve antioxidant defense abilities and salt tolerance of wheat plant irrigated with sea water. Afr. J. Biochem. Res. 7, 151–164. Available online at: http://www.academicjournals.org/journal/AJBR/article-abstract/82070DD10085

3. Abdalla M. M. (2013). The potential of Moringa oleifera extract as a biostimulant in enhancing the growth, biochemical and hormonal contents in rocket (*Eruca vesicaria subsp. sativa*) plants. Int. J. Plant Physiol. Biochem. 5, 42–49.

4. Adani F., Genevini P., Zaccheo P., Zocchi G. (1998). The effect of commercial humic acid on tomato plant growth and mineral nutrition. J. Plant. Nutr. 21, 561–575.

5. Adholeya A., Tiwari P., Singh R. (2005). Large-scale production of arbuscular mycorrhizal fungi on root organs and inoculation strategies, in In vitro Culture of Mycorrhizas, eds Declerck S., Strullu D. G., Fortin J. A., editors. (Heidelberg: Springer;), 315–338.

6. Aguado-Santacruz G. A., Moreno-Gómez B., Rascón-Cruz Q., Aguirre-Mancilla C., Espinosa-Solís J. A., González-Barriga C. D. (2014). Biofertilizers as complements to synthetic and organic fertilization, in Components, Uses in Agriculture and Environmental Impacts, eds López-Valdes F., Fernández-Luqueno F., editors. (New York, NY: Nova Science Publishers Inc.), 155–180.

7. Aguirre E., Leménager D., Bacaicoa E., Fuentes M., Baigorri R., Angel M. Z., et al. . (2009). The root application of a purified leonardite humic acid modifies the transcriptional regulation of the main physiological root responses to Fe deficiency in Fe-sufficient cucumber plants. Plant Physiol. Biochem. 47, 215–223.

8. Aharoni A., Galili G. (2011). Metabolic engineering of the plant primary–secondary metabolism interface. Curr. Opin. Biotechnol. 22, 239–244.

9. Aitken J. B., Senn T. L. (1965). Seaweed products as a fertilizer and soil conditioner for horticultural crops. Bot. Mar. 8, 144–148.

10. Albregts E. E., Howard C. M., Chandler C., Mitchell R. L. (1988). Effect of biostimulants on fruiting of strawberry. Proc. Fla. State Hort. Soc. 101, 370–372.