

## A new biotechnological-based iron-fertilizer formulation for environmental sustainable correction of chlorosis of soybean plants grown in calcareous soils

Carlos M.H. Ferreira.<sup>1,2,3</sup>, Sandra Lopez-Rayó<sup>2</sup>, Juan Lucena<sup>2</sup>, Eduardo V. Soares<sup>3,4</sup>, Helena M.V.M. Soares<sup>1,\*</sup>

<sup>1</sup>REQUIMTE/LAQV, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, Portugal

<sup>2</sup>Departamento de Química Agrícola y Bromatología, Facultad de Ciencias, Universidad Autónoma de Madrid, Spain

<sup>3</sup>CEB-Centro de Engenharia Biológica, Universidade do Minho, Braga, Portugal

<sup>4</sup>Laboratório de Biengenharia-CIETI, Departamento de Engenharia Química, ISEP, Porto, Portugal  
[\\*hsoares@fe.up.pt](mailto:hsoares@fe.up.pt)

Iron (Fe) is an essential element for the proper development of life. Plants require Fe for chlorophyll synthesis. However, due to the lack of Fe, plants grown in alkaline calcareous soils are very susceptible to a symptomatic array named iron-deficiency induced chlorosis (IDIC). The inappropriate chlorophyll production results in the reduction of crop yields with major implications in many agricultural regions worldwide. Organic chelating agents, namely aminopolycarboxylic acids (APCAs), are adequate for IDIC amending. However, the low biodegradability of such compounds raises several environmental concerns. Therefore, the replacement of APCAs by new more environment-friendly alternatives is needed.

The main objective of this work was to develop environmentally-friendly alternatives to current APCAs used in agriculture to amend IDIC. As an alternative, in this work, a new green freeze-dried iron fertilizer containing siderophores [high Fe(III) affinity chelators produced by several microorganisms in Fe-deficient conditions] able to bind Fe at pH 9 was produced biotechnologically from a culture of *Azotobacter vinelandii* in Fe-deficient conditions and its potential for correcting Fe deficiency of soybean plants grown in calcareous soil conditions was studied [1, 2].

For this purpose, the ability of the freeze-dried product for maintaining Fe in solution, without being displaced by other cations or being retained by soil surfaces was evaluated in alkaline soils or some of its relevant constituents; lower amounts of Fe were lost to soil in the three day experiments. Then, the efficiency of the freeze-dried formulation in mending IDIC in soybean plants grown in calcareous soil was studied by measuring plant development (dry mass), chlorophyll production (measured by the SPAD index), and plant tissue Fe content. For comparison, *o,o*- ethylenediaminedi(*o*-hydroxyphenylacetic acid (*o,o*-EDDHA) was used as positive control and no Fe treatment was used as negative control. Plants treated with *A. vinelandii* iron fertilizer developed a dry mass comparable to that of *o,o*-EDDHA and a significant increase of the SPAD levels when compared to the negative control plants. On average, iron content was also greater on green iron-fertilizer treated plants than on negative control treated ones but lower than on positive control.

In conclusion, the overall results pointed out that the freeze-dried product prepared from *A. vinelandii* has potential for application in IDIC amendment in calcareous soils.

### Acknowledgments

This work was financially supported by: Project PTDC-AGR-TEC-0458-2014 - POCI-01-0145-FEDER-016681 - funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES and project RTI2018-096268-B-I00 by the State Research Agency, Ministry of Science, Innovation and Universities of Spain. Carlos Ferreira would like to thank the support from his grant with reference SFRH/BD/95490/2013 from FCT.

### References

- [1] HMVM Soares, CMH Ferreira, EV Soares, JJ Lucena, S Lopez-Rayó, "Freeze-dried fertilizing compositions containing siderophores, their preparation processes and their uses for treating plants". Provisional patent submitted (December 2018).
- [2] CMH Ferreira, S. López-Rayó, JJ Lucena, EV Soares, HMVM Soares. Evaluation of the efficacy of two new biotechnological-based freeze-dried fertilizers for sustainable Fe deficiency correction of soybean plants grown in calcareous soils. *Frontiers in Plant Science* (accepted for publication).