FRONTIERS IN PLANT SCIENCE

Evaluation of the efficacy of two new biotechnological-based freeze-dried fertilizers for sustainable Fe deficiency correction of soybean plants grown in calcareous soils

*Supplementary Materials*

Carlos M.H. Ferreira1,2,3, Sandra López-Rayo4, Juan J. Lucena4\*, Eduardo V. Soares2,3, Helena M.V.M. Soares1\*

1-REQUIMTE/LAQV, Departamento de Engenharia Química, Faculdade de Engenharia, Universidade do Porto, rua Dr. Roberto Frias, 4200-465, Porto, Portugal

2-Bioengineering Laboratory-CIETI, Chemical Engineering Department, ISEP-School of Engineering of Polytechnic Institute of Porto, rua Dr António Bernardino de Almeida, 431, 4249-015 Porto, Portugal

3-CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

4-Departamento de Química Agrícola y Bromatología, Facultad de Ciencias, Universidad Autónoma de Madrid, 28049-Madrid, Spain

**\*Corresponding authors:**

[hsoares@fe.up.pt](mailto:hsoares@fe.up.pt) (Helena M.V.M. Soares);

[juanjose.lucena@uam.es](mailto:juanjose.lucena@uam.es) (Juan J. Lucena)

1. Culture media composition

*Bacillus subtilis* was kept in minimal medium (MM) agar while *Azotobacter vinelandii* waskept in Burk´s medium (BM) agar (Newton et al., 1953), at 4º C. MM agar contained per liter: 10 g glucose, 1.47 g glutamic acid, 3.0 g potassium hydrogenophosphate (K2HPO4), 1.0 g potassium dihydrogenophosphate (KH2PO4), 0.5 g ammonium chloride (NH4Cl), 0.1 g ammonium nitrate (NH4NO3), 0.1 g sodium sulphate (Na2SO4), 10 mg magnesium sulphate heptahydrate (MgSO4.7H2O), 1 mg manganese(II) sulphate tetrahydrate (MnSO4.4H2O), 0.5 mg calcium chloride (CaCl2) and 20 g agar. BM agar was prepared as previously described (HiMedia Laboratories, 2015) replacing sucrose by glucose; the medium contained per liter: 10 g glucose, 0.8 g K2HPO4, 0.2g KH2PO4, 0.20 g MgSO4.7H2O, 0.253 mg sodium molybdate (Na2MoO4), 0.13 g calcium sulphate (CaSO4) and 20 g agar.

Liquid media were of the same composition, without agar addition. The final pH of the media was set to 7.0 ± 0.1. For iron-replete media, 29 mg of iron(III) chloride (FeCl3) was also added.

Table S1 –Nutrient solutions used to balance the macronutrients.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Nutrient concentration (mol L-1)** | | | |
| Solution | Ca(NO3)2 | KH2PO4 | K2HPO4 | MgSO4 |
| A | 0.57 | - | - | - |
| B | - | 0.16 | 0.04 | - |
| C | - | - | - | 0.04 |
| D | - | 0.22 | 0.10 | - |

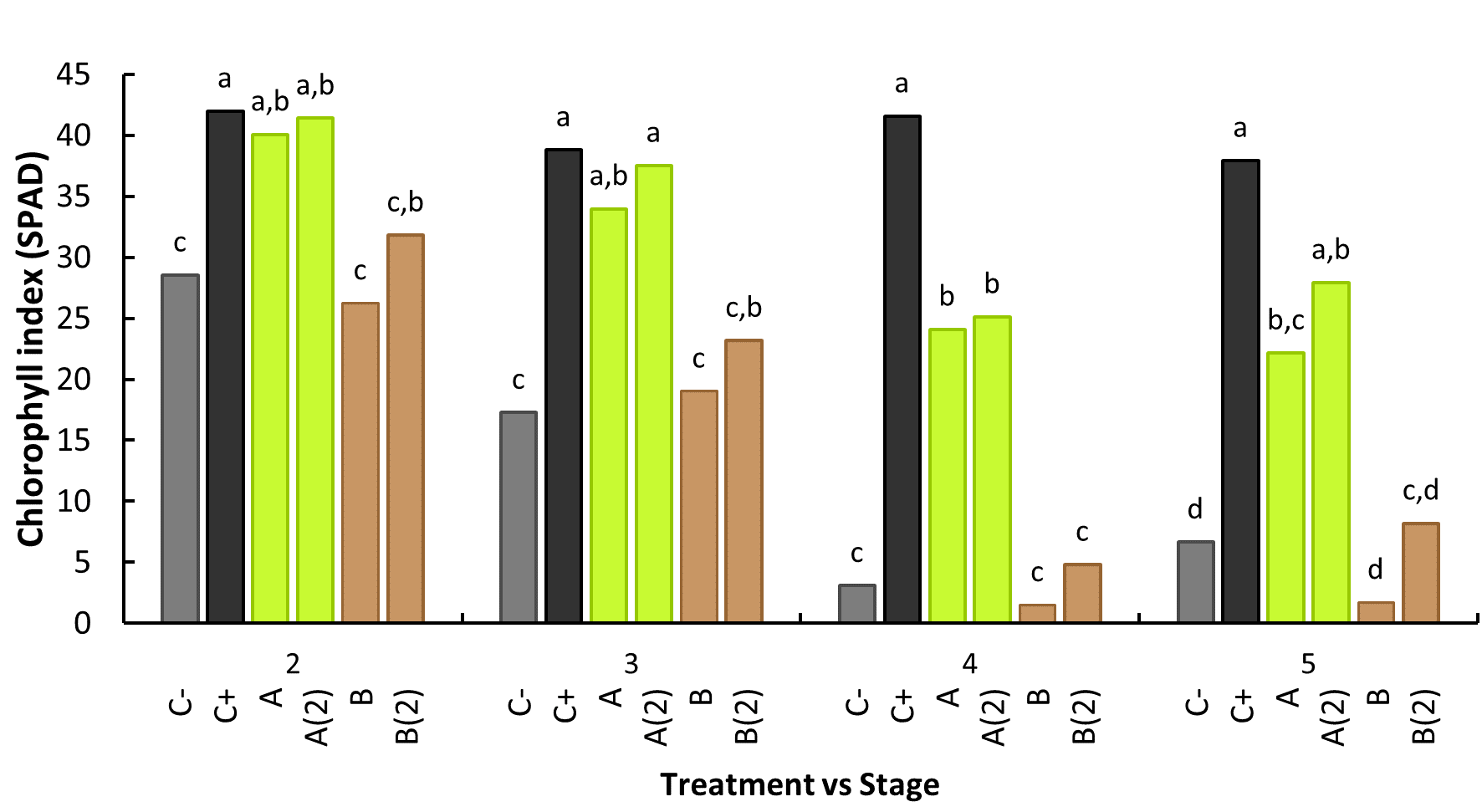


Figure S1. Intra-leaf stage (from 2nd to 5th stage) treatment comparison of average SPAD read at DAT 21. Different letters denote a significant difference of SPAD levels within each leaf stage within 95% confidence interval, as shown by Tuckey HSD test. C-: no iron treatment (negative control); C+: EDDHA (positive control); A: A. vinelandii ISS; B: B. subtilis ISS. Treatments with (2) represent plants with a second application performed 15th day after the first treatment (n=5).

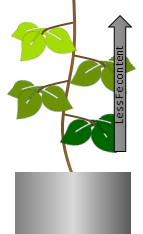


Figure S2. General model of leaf SPAD and Fe content progression on Azotobacter vinelandii ISS treated plants.

1. Bibliography

HiMedia Laboratories, 2015. Burks Medium - M707. Mumbai, India. http://himedialabs.com/TD/M707.pdf. Accessed 15 May 2015

Newton, J.W., Wilson, P.W., Burris, R.H., 1953. Direct demonstration of ammonia as an intermediate in nitrogen fixation by *Azotobacter*. J. Biol. Chem. 204, 445–451.