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*

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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

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