

## Citrate-coated cobalt ferrite nanoparticles for the nano-enabled biofortification of wheat

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**Table S2** Iron fixed in alkaline soil through fixation assay.

| Fe added<br>(mg L <sup>-1</sup> ) | Final Fe<br>concentration<br>added<br>(mg L <sup>-1</sup> ) | Iron Source                          |   |   |   |  |   |
|-----------------------------------|---|--------------------------------------|---|---|---|--|---|
|                                   |   | FeSO <sub>4</sub> 7 H <sub>2</sub> O |   |   | Citrate-coated CoFe <sub>2</sub> O <sub>4</sub> NPs |  |   |
|                                   |   | Adsorbed Fe<br>(%)                   | Fe extracted<br>with CaCl <sub>2</sub><br>0.01M<br>(mg kg <sup>-1</sup> ) | Fe extracted with<br>DTPA-TEA-CaCl <sub>2</sub> (mg<br>kg <sup>-1</sup> ) | Adsorbed Fe<br>(%)                                  | Fe extracted with<br>CaCl <sub>2</sub> 0.01M<br>(mg kg <sup>-1</sup> ) | Fe extracted with<br>DTPA-TEA-CaCl <sub>2</sub> (mg<br>kg <sup>-1</sup> ) |
| 0                                 | 0   |                                      | ND  | 2.77 ± 0.29   |   | ND   | 2.77 ± 0.29   |
| 10                                | 9.09  | 100                                  | ND  | 4.24 ± 0.28   | 100   | ND   | 2.38 ± 0.32   |
| 25                                | 22.73   | 100                                  | ND  | 5.76 ± 0.46   | 100   | ND   | 2.05 ± 0.15   |
| 50                                | 45.45   | 100                                  | ND  | 9.52 ± 0.16   | 100   | ND   | 2.30 ± 0.20   |
| 75                                | 68.18   | 100                                  | ND  | 13.05 ± 1.81  | 100   | ND   | 2.03 ± 0.02   |
| 100                               | 90.91   | 100                                  | ND  | 17.85 ± 1.84  | 100   | ND   | 2.33 ± 0.44   |
| 200                               | 181.82  | 100                                  | ND  | 40.20 ± 6.46  | 100   | ND   | 2.26 ± 0.32   |
| 400                               | 363.64  | 100                                  | ND  | 73.50 ± 5.70  | 100   | ND   | 2.14 ± 0.12   |
| 800                               | 727.27  | 100                                  | ND  | 144.95 ± 16.21  | 100   | ND   | 2.75 ± 0.44   |
| 1600                              | 1454.55   | 98.77                                | ND  | 170.56 ± 13.98  |   |  |   |

ND, No detected

Mean value and standard deviation, n=3

### The Fe fixation assay

A sample of 5 g of soil was placed in a Falcon tube. The weight of the tube and soil was recorded. Then 20 mL of Fe solution or suspension of NPs with a known and increasing concentration of Fe (0-1600 mg kg<sup>-1</sup>) was added. In addition, 2 mL of CaCl<sub>2</sub> 0.1 M solution was added to maintain a base ionic strength. The samples were shaken at 120 rpm for 2 h. Afterward, the samples were centrifuged for 10 min at 1200 rpm. The supernatant was recovered, filtered, and stored for further analysis. In parallel, the weight of the tube containing the soil was recorded, and then extraction with 20 mL of CaCl<sub>2</sub> 0.1 M solution was carried out. If Fe was detected in the CaCl<sub>2</sub> extract, then the extraction was repeated. But if Fe was not detected then extraction with DTPA-TEA-CaCl<sub>2</sub> solution was performed.