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Supporting Information

Enhanced Legume Root Growth with Pre-Soaking in α-Fe_2O_3 Nanoparticle Fertilizer

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Electronic Supplementary Information (ESI) available: Experimental details: XEDS image of Pt-iron oxide NPs; XRD and FT-IR plots of iron oxide NPs; Table showing size of different seeds; Plots showing the growth rate of green pea, green gram, black bean, and red bean seedlings in iron oxide and hybrid NPs; images showing first and second generation legume plants in potted soil; SEM-XEDS image of green gram roots grown in low concentration of iron oxide NPs and high concentration of Pt-decorated iron oxide NPs; FT-IR plots of black bean roots grown in low and high concentration of iron oxide NP growth solution. See DOI: 10.1039/x0xx00000x

Fig. S1 shows the x-ray energy dispersive spectroscopy (XEDS) characterization of hybrid Pt-iron oxide NPs conducted on a TEM. Pt nanoparticles (NPs) were successfully attached on to iron oxide seeds, as suggested by the peaks for Fe, O, and Pt. The Cu peak is from the TEM holder, while the Cl peak is attributed to the unreacted chloride ions of the Pt precursor, H_2PtCl_6.

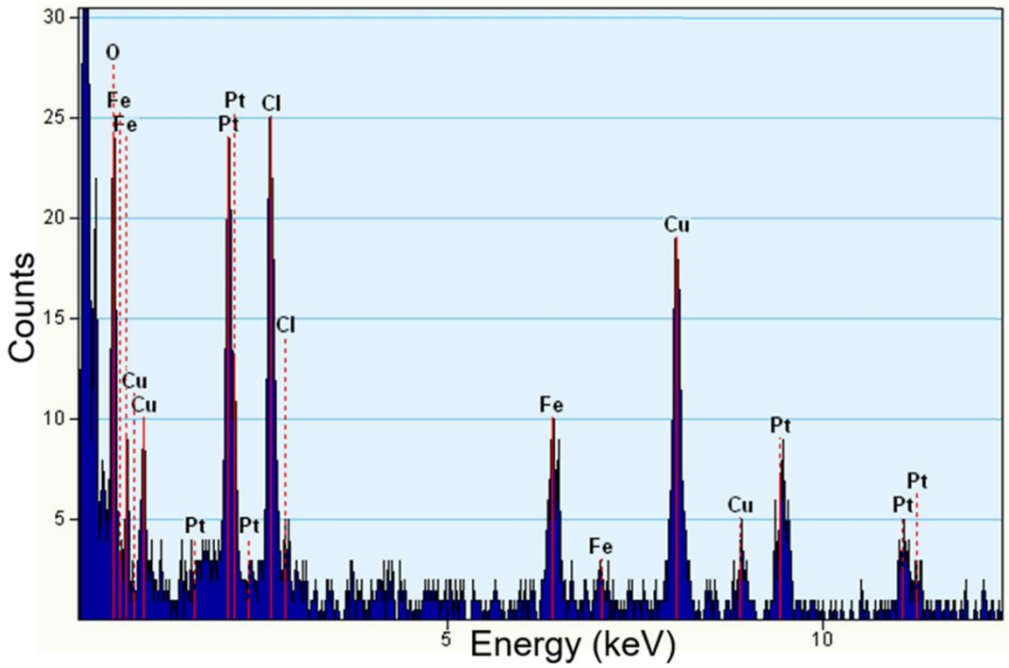

FIG. S1 TEM XEDS plot of hybrid Pt-iron oxide NPs.

Fig. S2 shows the x-ray diffraction (XRD) plot of iron oxide NPs used in this study. Powdered NP samples for XRD measurements were prepared via multiple washes in DI water, centrifugation, and air-drying. The 2θ peaks at 21°, 33°, 35°, 41°, 49°, 55°, 58°, 63°, 65°, and 72° matched well with the (012), (104), (110), (113), (024), (116), (122), (214), (300), and (1010) crystal planes of hematite crystal structure.

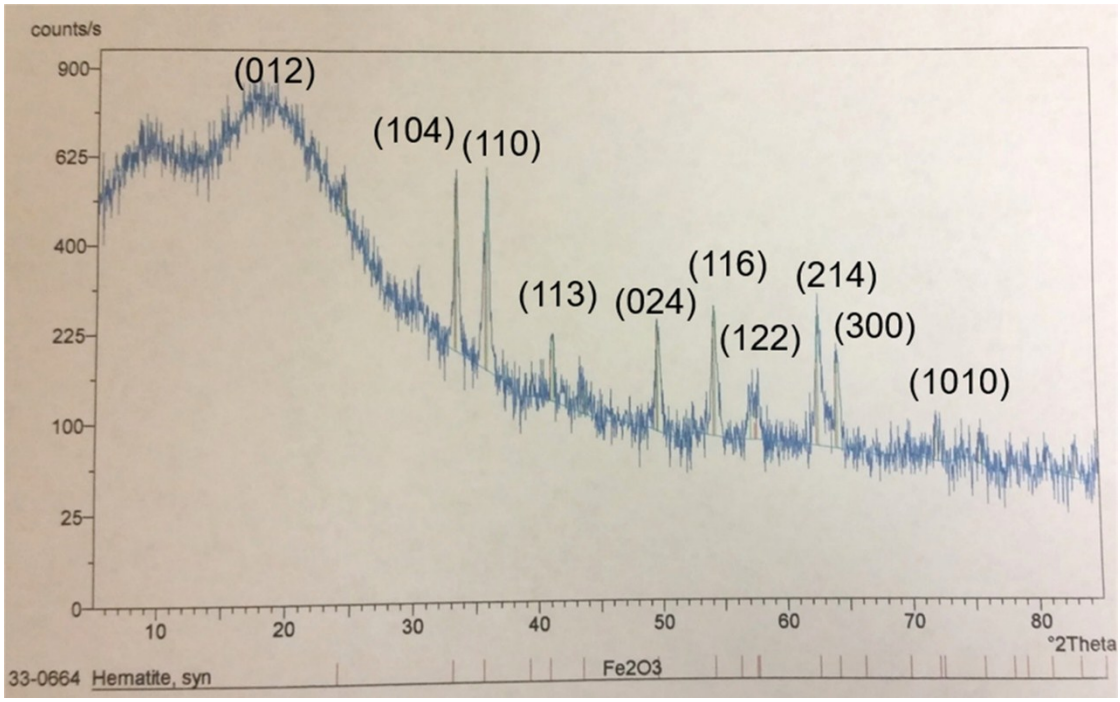

FIG. S2 XRD scan of iron oxide NPs.

Table S1 shows the sizes of each type of seeds used for our experiments.

TABLE S1. Sizes of edible legume seeds used in our experiment

Type of seed	Length of dry seed (mm)
Green gram	4.755±0.236
Black bean	8.505±0.694
Chick pea	9.21±0.654
Green pea	9.225±0.558
Red bean	16.815±0.734

Fig. S3 shows the plots summarizing the influence of iron oxide NP soak solutions at different concentrations on the growth of different legume seeds.

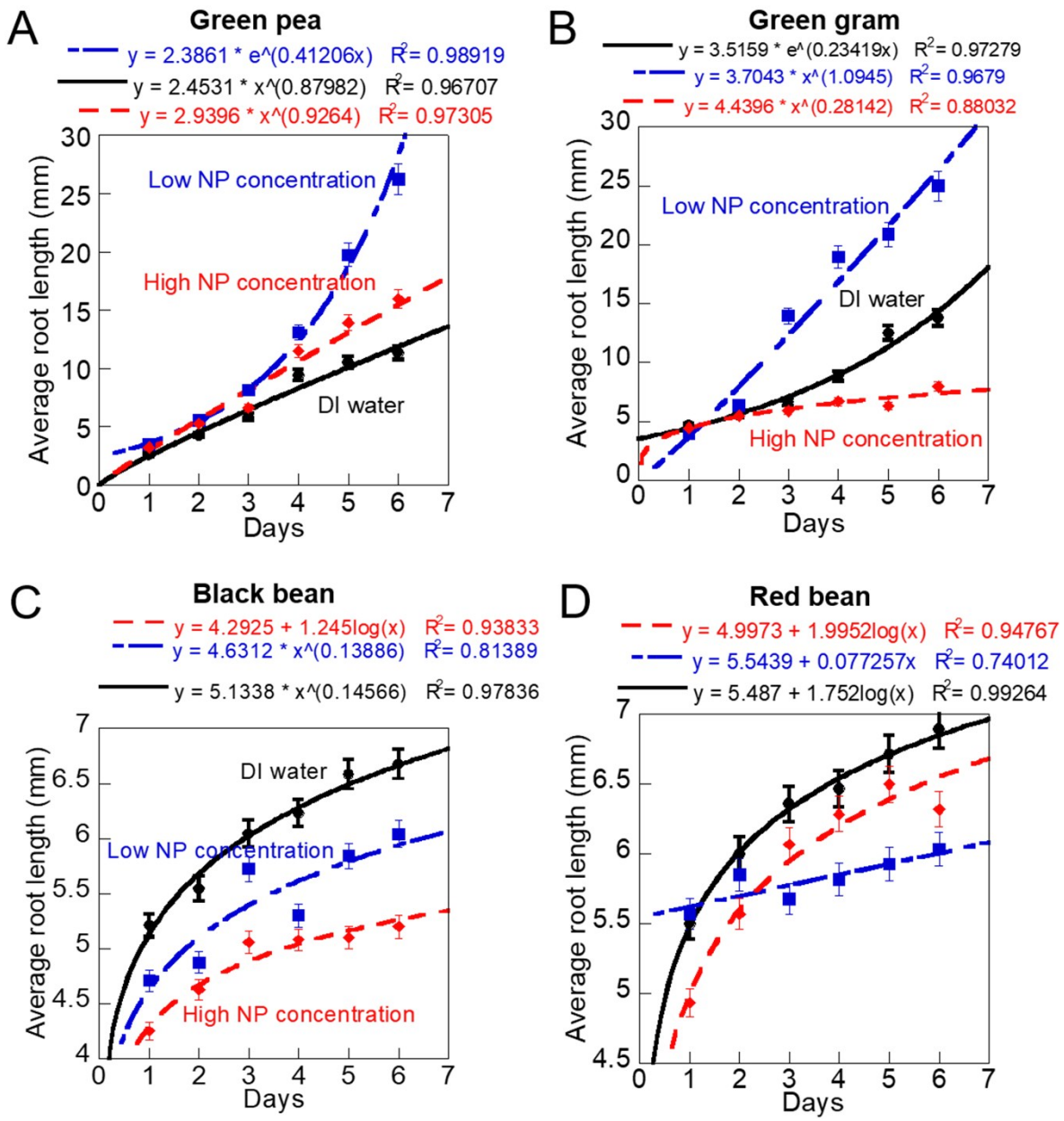

FIG. S3 Plots showing growth rate of different embryonic roots in iron oxide NP growth solution. (A) green pea, (B) green gram, (C) black bean, and (D) red bean.

Fig. S4 shows the effect of hybrid Pt-iron oxide NPs on the growth of different seedlings.

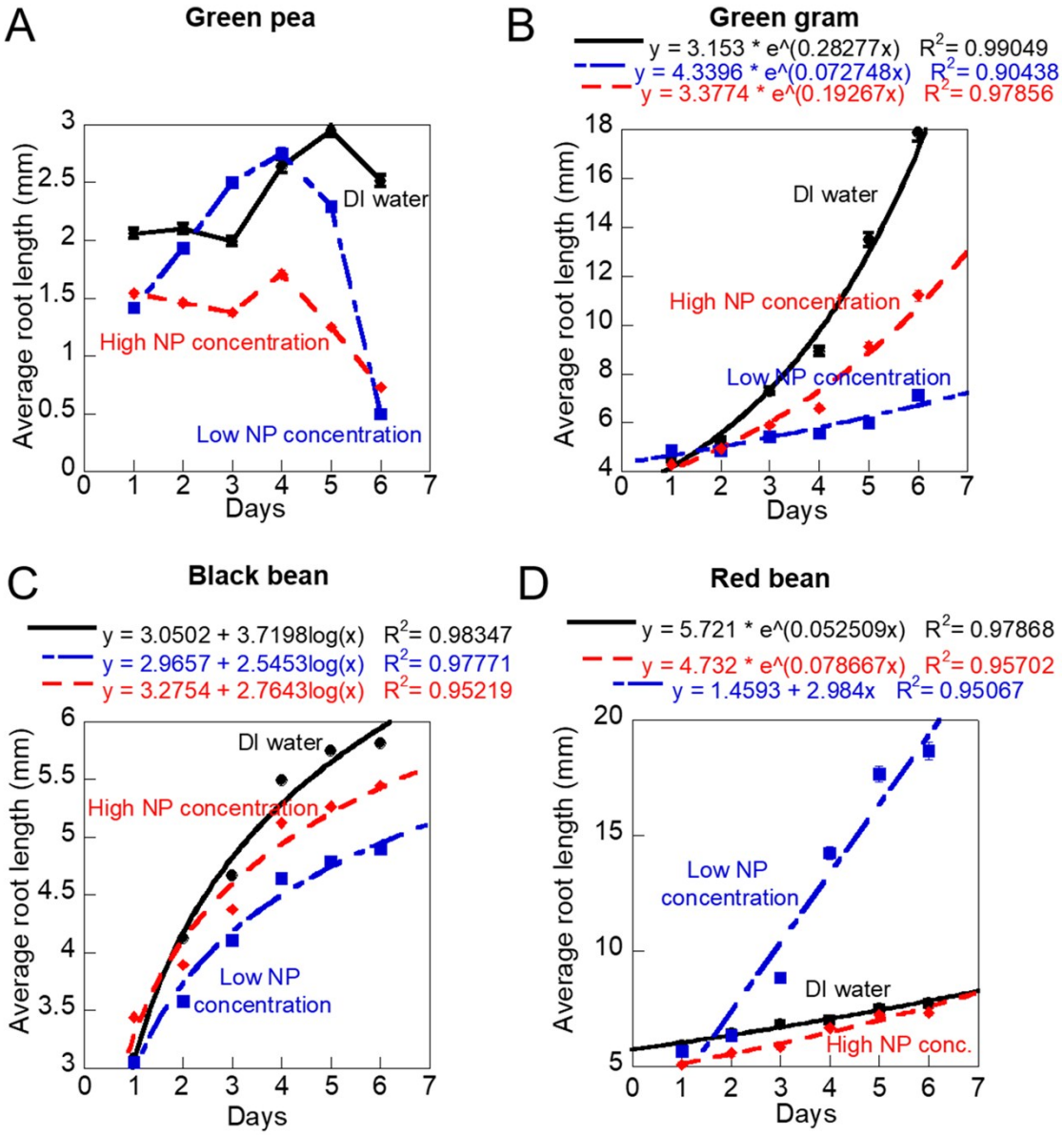

FIG. S4 Plots showing growth rate of different embryonic roots in hybrid Pt-iron oxide NPs. (A) green pea, (B) green gram, (C) black bean, and (D) red bean.

To further understand the effect of NPs on seedling, we conducted comprehensive statistical analysis on the embryonic root growth results of different seeds for both iron oxide and Pt-decorated iron oxide NPs. Differences among the treatments (Water, Low NP, and High NP) were tested using two-factors repeated measures analysis of variance (ANOVA) and all computations were performed using MATLAB 2015b version. First, we compared the effects due to three treatments on growth of root length. For this we tested the null hypothesis of all mean root length are equal at 0.05 level of significance for different treatments. The ANOVA p-values for each seed for iron oxide NPs treatments are given in row 1 and that for hybrid NPs treatments are given in row 5 in Table S2. We noticed that p values for all seeds except red beans were less than 0.05 due to treatment with iron oxide, as shown in Table S2. Therefore, we rejected the null hypothesis of all mean root length were equal and concluded that there was a significant difference among the mean root length for chick peas, green gram, green peas, and black beans due to treatment of iron oxide NPs at different concentrations. In addition, we observed that p-values corresponding to hybrid treatments were always greater than 0.05 for all seeds and it implied that there is no significant difference in root length in comparison to the control DI water when using hybrid treatment. As a conclusion, Table S2 shows that iron oxide NP soak solution enhanced root growth.

In order to study which concentration of iron oxide NP treatment showed the maximum difference in mean root length in comparison to the control DI water growth solution, we performed multiple comparisons in repeated measures using pairwise comparisons with Bonferroni size adjustment.[1] Consequently, the 95% confidence interval for differences of mean root length was

constructed for each pair (water and Low NP, water and High NP, Low NP and High NP) of treatment. If 0 is not included in the confidence interval, we concluded that there is a statistically significant difference in root length between the pair. In Table S2, row 2 shows the confidence interval for water and Low NP pair, row 3 shows the confidence interval for water and High NP pair, and row 4 shows the confidence interval for Low NP and High NP pair. The cell with the label 'difference' implies that there is a significant difference in mean root length between the two treatments and the label 'no difference' implies that there is no significant difference due to the treatment. For example, the p-value for chick pea is 0.01 and it implies that there is an effect due to the treatments. Thus, we conducted a pairwise comparison to obtain confidence interval. The confidence interval corresponding to (Water and Low NP) and (Low NP and High NP) does not include zero and it implies that there is a significant difference in mean root length between water and Low NP treatment, and Low NP treatment and High NP treatment. Meaning that Low NP has enhanced the growth rate of the chick peas root length. The confidence interval corresponding to water and High NP treatment contains zero and it implies that there is no significant difference in mean root length between water and High NP treatment. Thus, from column 2 of Table S2 we concluded that Low iron oxide NP treatment enhanced the growth of the chick pea root. This can also be observed by looking at Fig. 4.

TABLE S2. Results of two-factors repeated measures ANOVA

	Chick pea	Green gram	Green pea	Black bean	Red bean
P-value: α-Fe_2O_3	0.01	0.04	0.02	0.04	0.78
Water and Low NP	[– 6.36 – (different	[– 21.56 – no difference	[– 8.95 – 1. different	[– 0.28 1.53] no difference	[– 1.06 2.08] no difference
Water and High NP	[– 2.16 3.26] no difference	[– 9.55 10.72] no difference	[– 5.66 1.44] no difference	[0.26 2.08] different	[– 1.20 1.94] no difference
Low NP and High NP	[1.49 6.91] different	[1.87 22.14] no difference	[– 0.30 6.83] no difference	[– 0.37 1.45] no difference	[– 1.70 1.44] no difference
p-value: Hybrid	0.93	0.10	0.53	0.45	0.46

The legume seeds pre-soaked in low and high concentration of iron oxide NP growth solution of pH 7 were potted to investigate plant growth rate and any potential adverse effect of the NPs. Each type of legume was grown in the same pot to keep all conditions the same. Fig. S5A shows the first generation chickpea plants grown from seeds pre-soaked in low and high concentration iron oxide NPs. The low concentration iron oxide NPs induced enhanced growth rate as compared to the higher concentration. Chick pea seeds from the plant grown with low iron oxide NP solution was further potted in soil to investigate the effect on second generation. Fig. S5B shows the healthy growth of the second generation of chickpea plant from seeds pre-soaked with iron oxide nanofertilizer. One whole generation of healthy growth of chickpea plants from seeds soaked in low concentration iron oxide NP solution at pH 7 rules out any possible adverse effect of these NPs at this particular concentration ($5.54x10^{-3}$ mgL^{-1} Fe). The healthy growth of first generation plants from other legume seeds pre-soaked with both low and high concentration of iron oxide NPs (pH 7) is shown in Fig. S6. Further potting experiments with legume seeds pre-soaked in iron oxide NP growth solutions of different pH are currently being conducted.

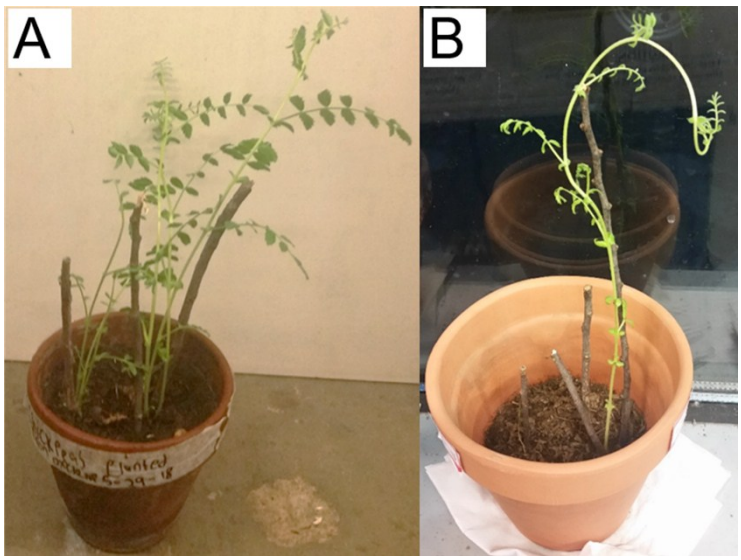

Fig. S5 Images of chickpea plants from seeds pre-soaked with different concentration of iron oxide NP growth solution of pH 7 (A) first generation plants and (B) second generation plant from seeds pre-soaked in low concentration iron oxide NP growth solution.

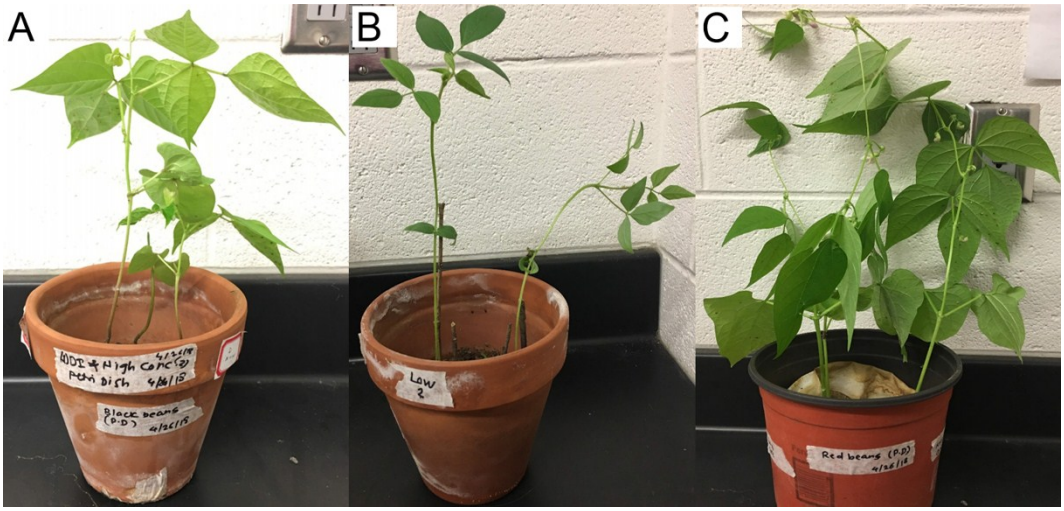

Fig. S6 Images of different legume plants grown from seeds pre-soaked with different concentration of iron oxide NP growth solution (pH 7) (A) black beans, (B) green gram, and (C) red beans.

Fig. S7 shows the chemical composition of green gram roots grown with high concentration iron oxide NP soak solution (27.7 mgL^{-1} Fe), as characterized by a scanning electron microscope (SEM) equipped with x-ray energy dispersive spectroscopy (XEDS).

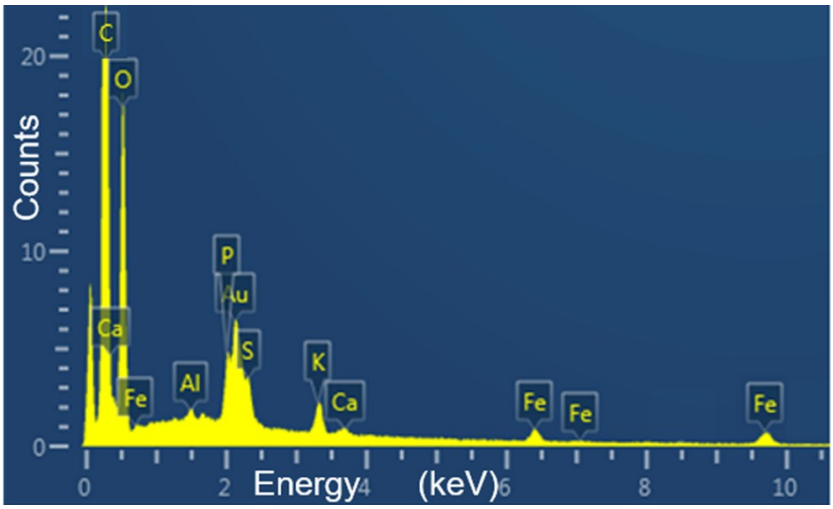

FIG. S7 XEDS data of green gram root surfaces grown in high concentration iron oxide NPs.

The chemical composition of green gram roots grown in low concentration iron oxide NPs ($5.54x10^{-3}$ mgL^{-1} Fe) was characterized via a SEM equipped with XEDS as shown in Fig. S8.

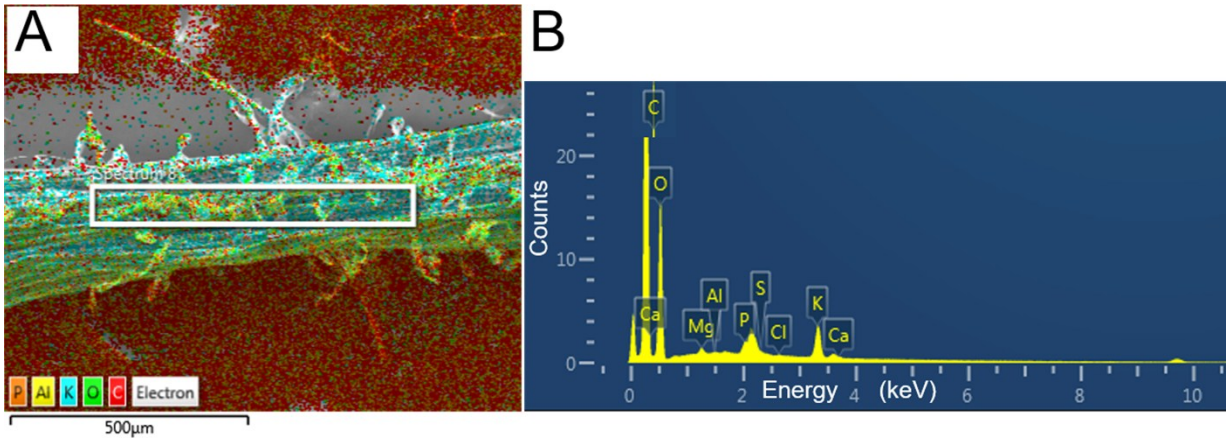

FIG. S8 XEDS image of green gram root surfaces grown in low concentration iron oxide NPs. (A) layered XEDS image and (B) XEDS mapping.

Fig. S9 shows the XEDS plot of green gram root surfaces for seeds soaked in high concentration hybrid Pt-attached iron oxide NP growth solution.

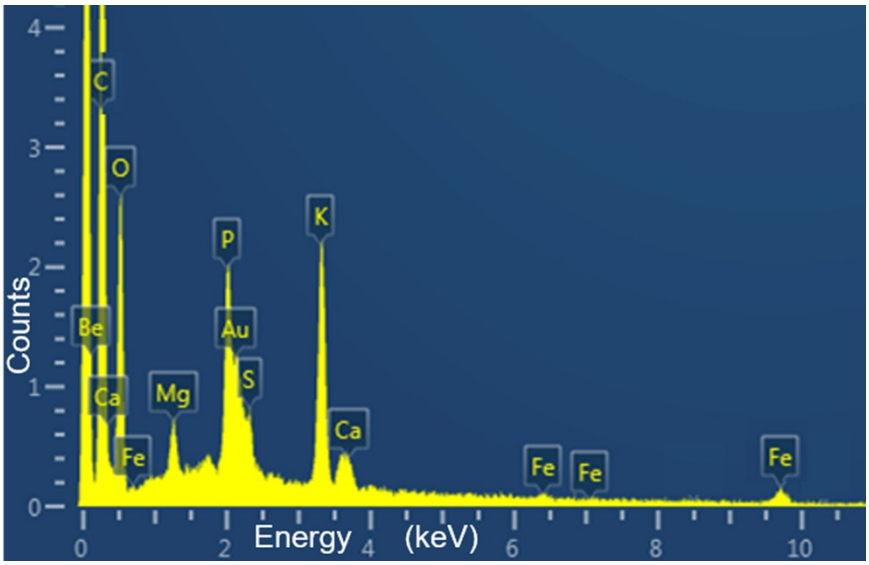

FIG. S9 XEDS image of green gram root surfaces grown in high concentration hybrid Pt-attached iron oxide NP growth solution.

Fig. S10 shows the FT-IR characterization of embryonic black bean roots from seeds soaked in iron oxide NP growth solution at neutral pH.

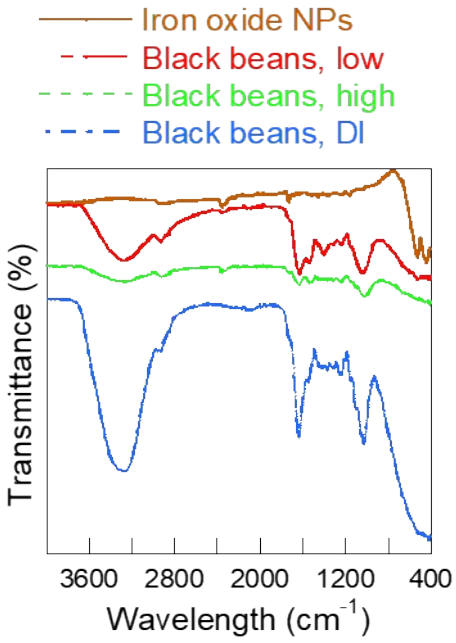

Fig. S10 FT-IR plots for iron oxide NPs and embryonic roots of black bean seeds grown in different concentrations of iron oxide NPs.

References

1 A. Minke, Conducting repeated measures analyses: experimental design considerations. In Annual Meeting of the Southwest Educational Research Association, Austin, TX, 1997, **17**.

