Supporting Information

Synergistic effect of nano-iron phosphide and wood vinegar on soybean production and grain quality

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Figure:

Figure S1. TEM images of FeP-NMs.

Figure S2. FeP-NMs Water and kinetic diameter.

Table:

Table S1. zeta potential and hydrodynamic diameter of FeP-NMs. Data given as mean ± STDEV (n = 3).

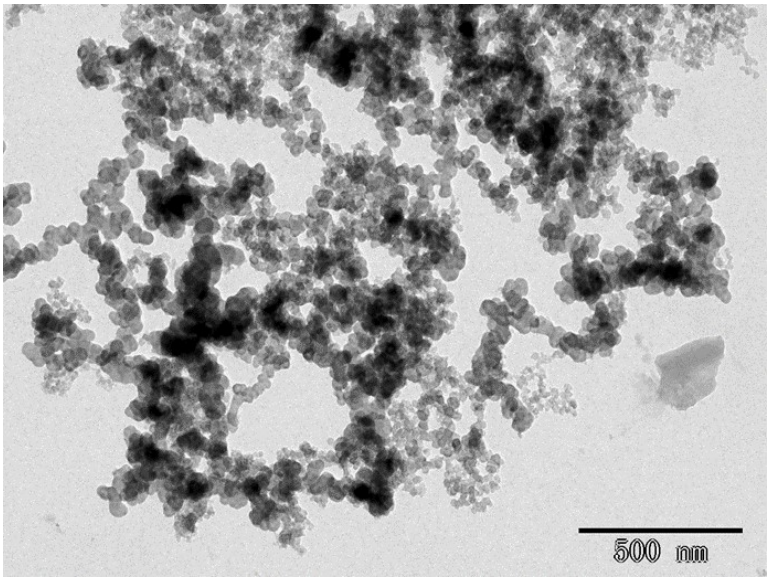

Figure S1. TEM images of FeP-NMs.

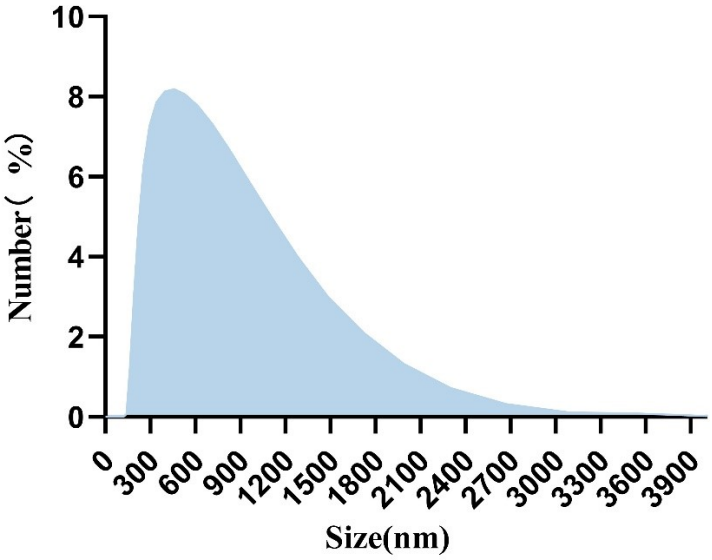

Figure S2. FeP-NMs Water and kinetic diameter.

Table S1. zeta potential and hydrodynamic diameter of FeP-NMs. Data given as mean ± STDEV (n = 3).

	FeP-NMs
Zeta potential (mV)	-0.06±0.25
Hydrodynamic diameter (nm)	486.6±13.7

Text S1. Gas Exchange and chlorophyll content

We measured net photosynthesis (Pn), intercellular carbon dioxide concentration (Ci), transpiration rate (E), and stomatal conductance (Gs) in the third fully expanded leaf of soybean on the 100th day after sowing using an open gas exchange system 110 (LI-6400XT). The third fully expanded leaf of each soybean plant was selected and the measurement points were taken at three points near the main leaf veins.

Text S2. Antioxidant systems

Sample pretreatment and quality control

At 80 days of age (DAS), we collected fresh root and stem samples from soybean plants and homogenized them in cold phosphate buffer (PBS, pH 7.4) containing 0.05 M Tris-HCl. Subsequently, after centrifugation at 4000 rpm for 10 min at 4°C, the supernatant was collected for subsequent assays. To ensure accuracy and linearity of the assay, standards of SOD, PDO, MDA, and CAT at known concentrations were prepared according to the procedures provided in the sample analysis kit[1]. Six replicate samples were tested for each data point and the experiments were performed in three replicates as a way to ensure the reliability of the experimental results.

Text S3. Organic nutrient content measurement in seeds

Starch

The starch content was determined using the method of Ma et al[2]. First, the residue after centrifugal extraction of total soluble sugars was resuspended using deionized water. Subsequently, perchloric acid was added and centrifuged at 2000 rpm for 20 min. The supernatant was diluted 10-fold with deionized water and then reduced in the same way as for total soluble sugars. We quantified the starch content using glucose as a standard and the coefficient of conversion of glucose to starch was 0.9. Each data point was tested with six replicate samples, and the experiments were replicated three times to ensure the reliability of the results.

Flavonoid

The flavonoid content in the grains was assessed using the method based on the formation of a red complex between flavonoids and aluminum ions in an alkaline nitrite solution. The determination was carried out using a kit from Suzhou Keming Biotechnology Co., Ltd. Soybean seed powder (0.02 g, passed through a 40-mesh sieve) was weighed and combined with the extract, then extracted for 2 hours at 60°C and 10,000 rpm. After centrifugation at 25°C for 10 minutes, the supernatant was collected. Sequential addition of other reagents followed by thorough mixing, allowing the mixture to stand for 15 minutes. The absorbance value was measured at 510 nm using a microplate reader (EPOCH-SN, BioTek, USA), with the red complex formation assessed at a wavelength of 620 nm. Each data point was tested with six replicate samples, and the experiments were replicated three times to ensure the reliability of the results.

Total protein

The total protein content in grains was determined by Coomassie brilliant blue method[3]. Weigh about 0.05 g of seed sample, add 1mL PBS, 8000g, centrifuge at 4 °C for 10min, take the supernatant and add reagent one. Absorbance values were determined at 620 nm using an enzyme meter. Six replicate samples were tested for each data point and the experiment was repeated three times.

Amino acid

The content of amino acids in grains was determined by α-aminohydrin ninhydrin reaction. Weigh about 0.1g of tissue, add 1mL of reagent I, homogenize at room temperature, centrifuge at 8000 g for 10min at 4°C, take 200μL of supernatant, add 200μL of reagent II, put it in a boiling water bath for 15 min, and wait for the test after it cooled down to room temperature, and then add reagent II, reagent III, and reagent

IV sequentially. The absorbance values were determined at 570 nm using an enzyme labeling instrument. Six replicate samples were tested for each data point and the experiment was repeated three times.

Reference

1. M. Li, P. Zhang, Z. Guo, W. Cao, L. Gao, Y. Li, C. F. Tian, Q. Chen, Y. Shen, F. Ren, Y. Rui, J. C. White and I. Lynch, Molybdenum Nanofertilizer Boosts Biological Nitrogen Fixation and Yield of Soybean through Delaying Nodule Senescence and Nutrition Enhancement, *Acs Nano*, 2023, **17**, 14761-14774.
2. Y. Ma, C. Xie, X. He, B. Zhang, J. Yang, M. Sun, W. Luo, S. Feng, J. Zhang, G. Wang and Z. Zhang, Effects of Ceria Nanoparticles and CeCl₃ on Plant Growth, Biological and Physiological Parameters, and Nutritional Value of Soil Grown Common Bean (Phaseolus vulgaris), *Small*, 2020, **16**.
3. P. Zhou, Y. Jiang, M. Adeel, N. Shakoor, W. Zhao, Y. Liu, Y. Li, M. Li, I. Azeem, Y. Rui, Z. Tan, J. C. White, Z. Guo, I. Lynch and P. Zhang, Nickel Oxide Nanoparticles Improve Soybean Yield and Enhance Nitrogen Assimilation, *Environmental Science & Technology*, 2023, **57**, 7547-7558.