

Supporting information

**Selenium nanomaterials promoted ferredoxin and iron-sulfur protein synthesis,
and Acetyl CoA carboxylase activity to improve photosynthesis and fatty acid of
soybean**

Number of pages: 8

Number of texts: 4

Number of figures: 8

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Text S1. Method for preparing release profiles of Se ENMs in ultrapure water and soybean leaf extract solution.

Experimental on the release of Se ENMs in ultrapure water were conducted by the following steps: Take 2 mg Se ENMs in 1 L ultrapure water and shake it at 4 °C at 250 rpm. Take 30 mL mixture at certain intervals (6, 12, 24, 48, 72, 96, 120 h) and add 50 mg polyaluminum chloride ($\geq 98\%$, Sinopharm Chemical Reagent Co., LTD), and slowly add 1 mL 2M of NaOH to precipitate, centrifuge at 4°C at 4000 rpm for 10 min, and take supernatant to be measured by ICP-MS. Soybean leaf extract was prepared as follows: 5 g fresh soybean leaves were ground in liquid nitrogen, diluted with 500 mL ultrapure water, and filtered with gauze. The experiment on the release of Se ENMs in soybean leaf extract solution mirrors that conducted in ultrapure water, with the primary difference being the conversion of ultrapure water into soybean leaf extract solution. The specific operations of ICP-MS are as follows: 3 mL concentrated nitric acid (HNO_3 , AR, $\geq 60\text{-}65\%$, Sinopsin group chemical reagent Co., LTD) and 3 mL sample solution (obtained by the formula method in Text S1) were digested (190 °C, 30 min) by using a microwave accelerated reaction system (CEM corp, Matthews. NC). The digested solution was filtered (0.22 μm filter) and diluted to 50 mL. The release content of Se in the ultrapure water and soybean leaf extract solution were determined by ICP-MS..

Text S2. Methods for determination of elements in different samples.

Elements (Se, S, and Fe) content determination in different samples including

soybean leaves, thylakoid membranes (Text S3), and grains: 3 mL concentrated nitric acid (HNO₃, AR, ≥60-65%, Sinopsin group chemical reagent Co., LTD), 3 mL ultrapure water, and 25 mg dried sample were digested (190 °C, 30 min) by using a microwave accelerated reaction system (CEM corp, Matthews. NC). The digested solution was filtered (0.22 μm filter) and diluted to 50 mL. The content of Se, S, or Fe in the different samples were determined by ICP-MS.

Text S3. Extraction method of thylakoid membrane.

5 g plant leaves and 5 mL separation buffer (400 mM sucrose, 50 mM HEPS-KOH, pH=7.8, 10 mM NaCl, 2 mM MgCl₂) were ground evenly in a ceramic mortar and filtered with 2 layers of gauze. The filtrate was centrifuged at 5000 ×g for 10 min and the sediment was collected. The precipitation was suspended with separation buffer and centrifuged again (5000 ×g, 10 min) to obtain thylakoid precipitation. All operations are performed at 4 °C.

Text S4. The computational details of Se substitute for Fd.

To test the possibility of this substitution relationship, the geometry optimization and density functional theory (DFT) chemical description for the molecular structures of all title compounds were performed using Gaussian09 program package with B3LYP exchange-correlation functional and 6-311+G** basis set. Electron localization function (ELF) and Orbital composition analysis employed by Multiwfn program. The substitute energy E was calculated using the following equation:

$$E_{\text{sub}} = \{E_{\text{total}} - (E_{\text{Se}} - E_{\text{Fd}})\}$$

Where E_{total} , E_{Se} and E_{Fd} corresponds to the total energy of the Se substituted Fd, Se atom, and Fd, respectively.

Table. S1 List of genes primers used in this study.

Genes	Forward Primer	Reverse Primer
<i>GmEF1b</i>	GTTGAAAAGCCAGGGGACA	TCTTACCCCTTGAGCGTGG
<i>GmSUT2</i>	GGCCAAGGTTTATCTTTGGGAGTC	CAAGTTGCCACCACCAAACAAAG
<i>GmpsA</i>	AGCAACTCCCTTTTTACC	GACCCGCTATCAAGAAAAGAAT
<i>GmpsB</i>	TGGTGTTTATCAGTGGTGGT	TGATGATTGAGGCGGGATT
<i>GmpsB</i>	GCAAACCTATAGCCGCAGA	GGATGGTTTGGTGTTTTGATGA
<i>GmpsB</i>	CCCTCTGACCCTGTTCCT	ATATTCCAACCGCCCCAC
<i>GmACCse-A</i>	TGTGATCCTCGTGCTGCCATTTTC	TCTTGCCCATCCCTCTAGTGTCTC
<i>GmACCB-1</i>	TACCAGCATCTACTCCAGCACCTAC	GGGGCTTTTAAGAGGCGGAAGTG
<i>GmFAD2-1A</i>	AATGGGAGGTAGAGGTCGTGTGG	CAGTGAATGGTGGCTTTGTGTTGG
<i>GmFAD2-1B</i>	CCACCACCTACTTCCACCTCCTC	AATCACCCACACGCCAGTAAGAATG
<i>GmFAD3B</i>	GCAATTCACTTCGACAACCTGGCTTC	TCCAAGAACAAGAGAGCCCAGAAC
<i>GmDGAT</i>	ACTGCCTTCCAATGCTCTTCCAAG	CAATATGCCACTCTCACCAGACCTG

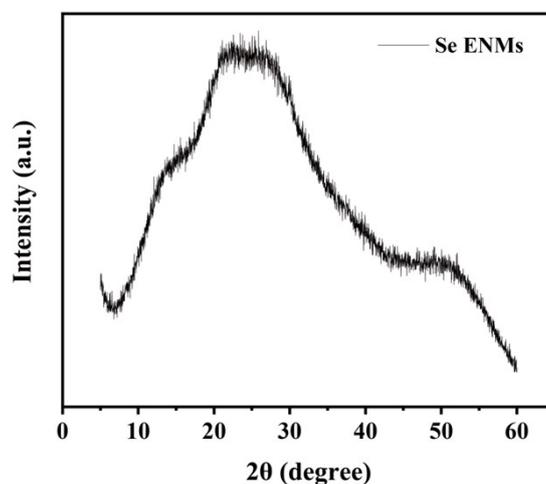


Fig. S1 XRD diffraction pattern of Se ENMs.

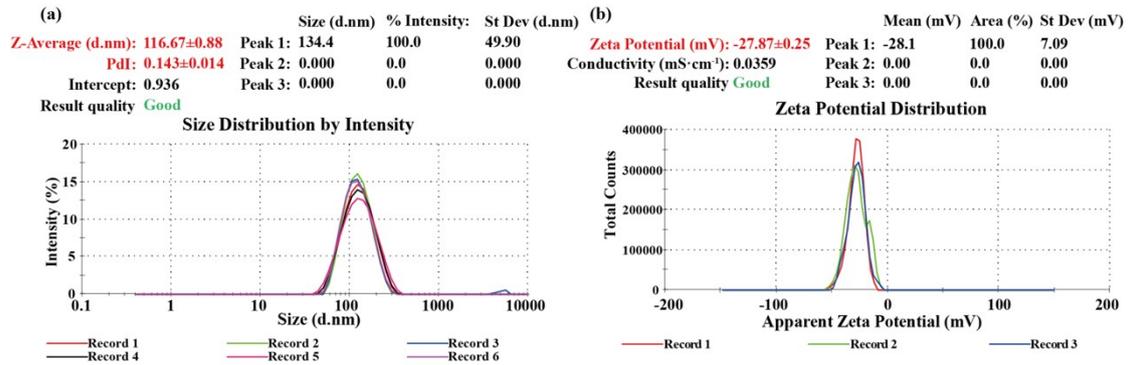


Fig. S2 The hydrodynamic diameter, PDI (a) and zeta potential (b) of 10 mg·L⁻¹ Se ENMs.

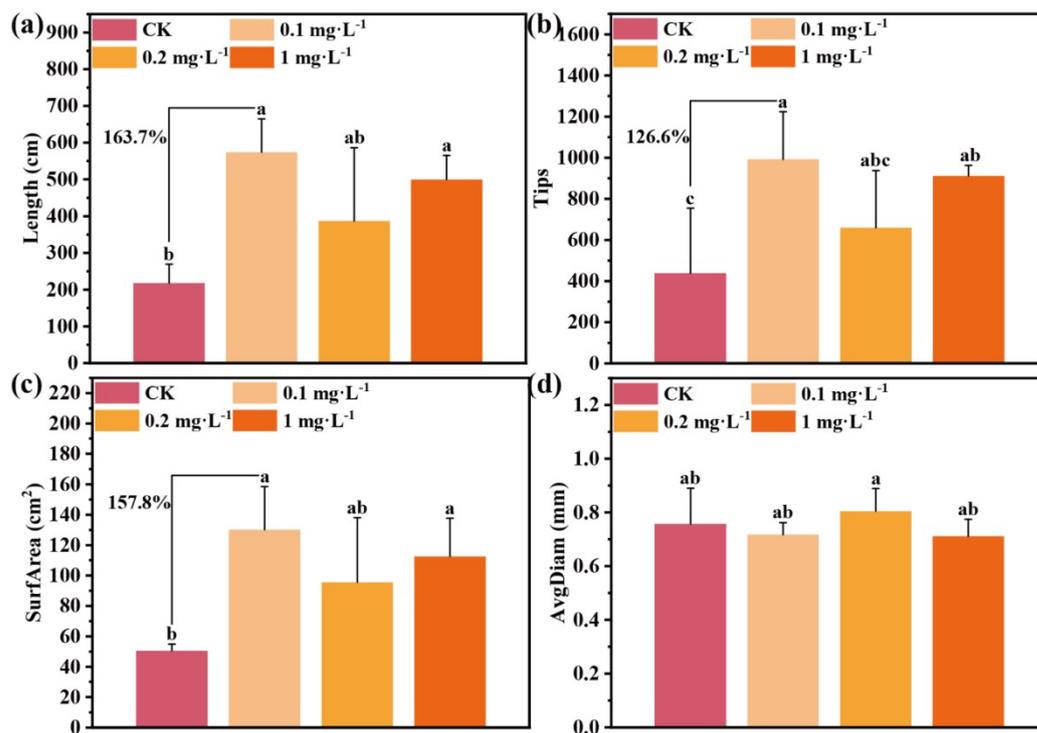


Fig. S3 Root growth index by treated with Se ENMs (0.1, 0.2, and 1 mg·L⁻¹). (a) Root length; (b) root tips; (c) root surface area; (d) root average diameter.

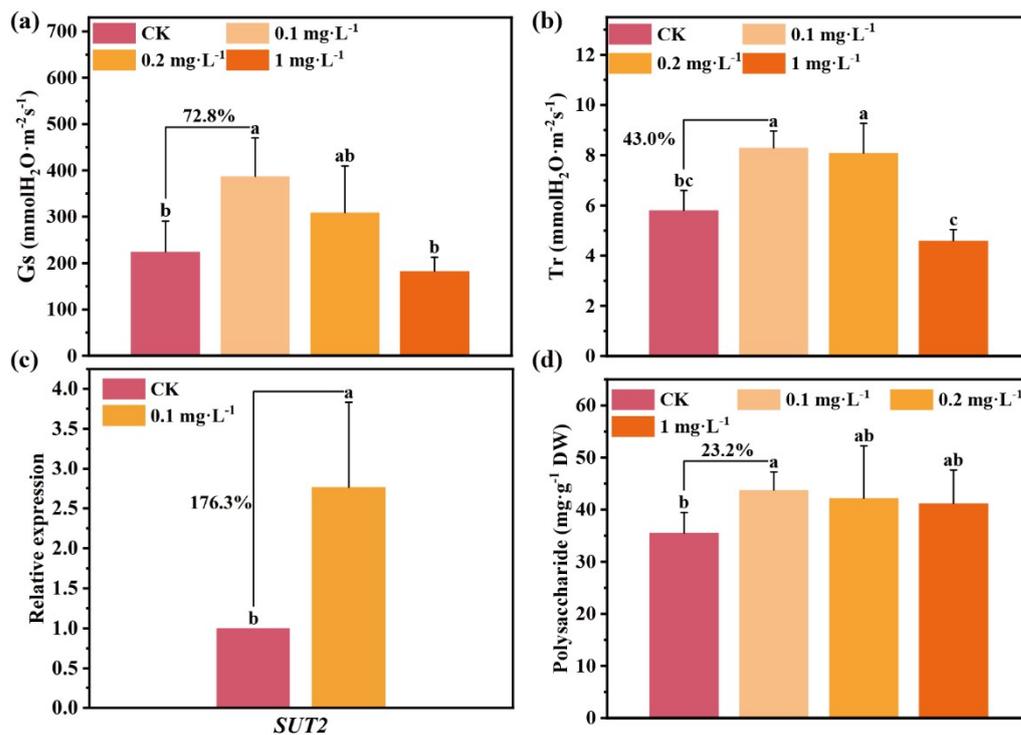


Fig. S4 Photosynthesis parameters of soybean leaves and polysaccharide transport in soybean under the treatment of Se ENMs (0.1, 0.2, and 1 mg·L⁻¹). (a) Stomatal conductance (Gs); (b) transpiration rate (Tr); (c) expression of *SUT2* gene in soybean shoots; (d) polysaccharide content in roots.

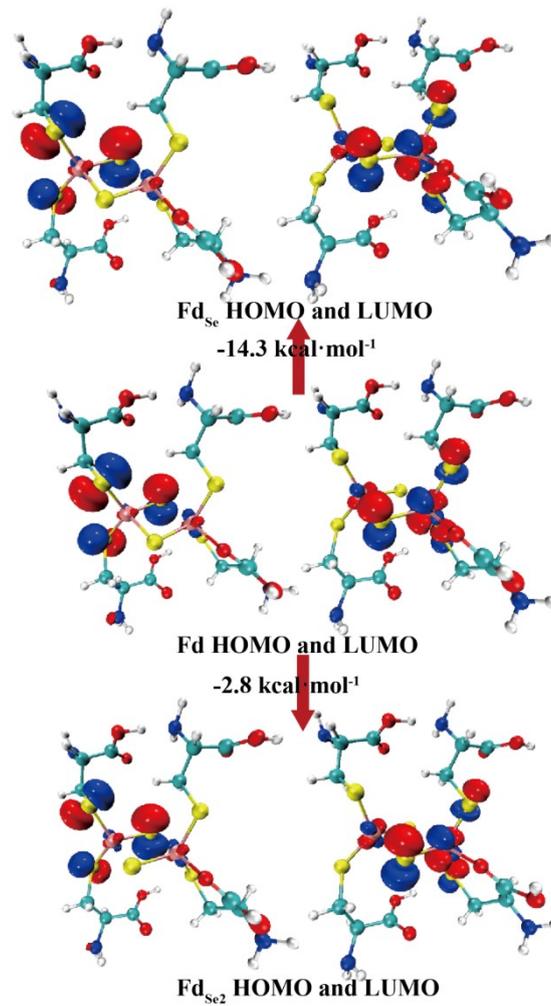


Fig. S5 HOMO and LUMO of Fd, Fd_{Se} and Fd_{Se2}. The calculated substitution energies to form Fd_{Se} and Fd_{Se2} are calculated to be 14.3 kcal·mol⁻¹ and 2.8 kcal·mol⁻¹, respectively.

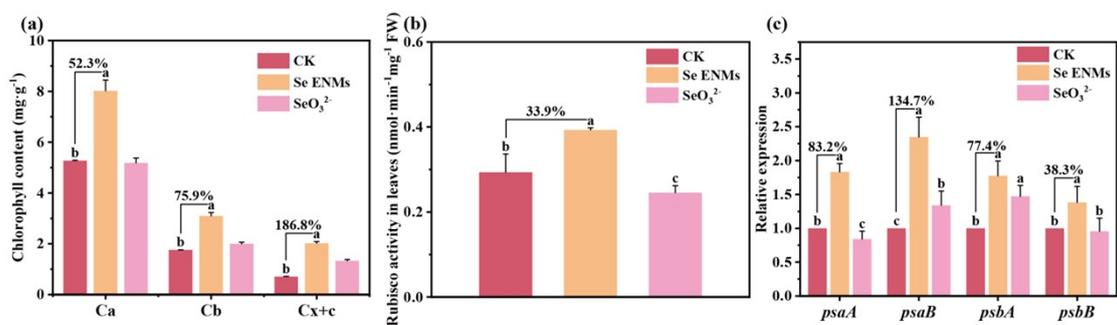


Fig. S6 Leaf pigment content and photosynthetic parameters upon exposure to Se

ENMs ($0.1 \text{ mg}\cdot\text{L}^{-1}$) and SeO_3^{2-} ($0.1 \text{ mg}\cdot\text{L}^{-1}$). (a) the content of chlorophyll a (Ca), chlorophyll b (Cb), carotenoid (Cx+c); (b) Rubisco enzyme activities in leaves; (c) the expression of photosynthetic genes *psaA*, *psaB*, *psbA* and *psbB* in leaves.

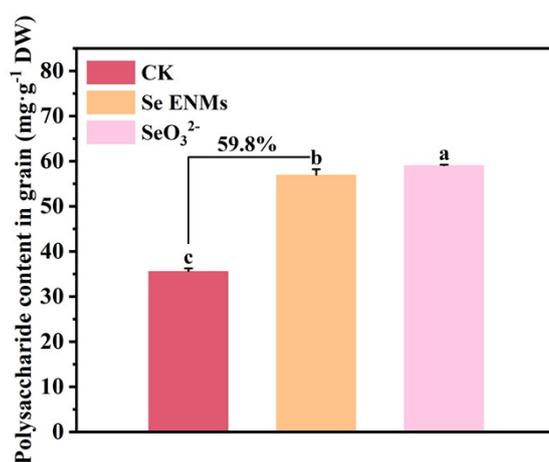


Fig. S7 The polysaccharide content in soybean seeds by treated Se ENMs ($0.1 \text{ mg}\cdot\text{L}^{-1}$).

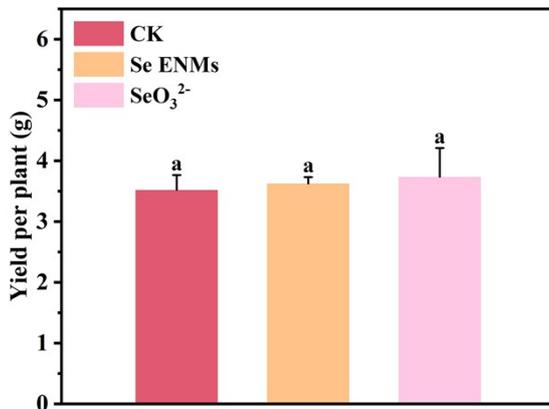


Fig. S8 Grain yield per soybean plant after treated with Se ENMs ($0.1 \text{ mg}\cdot\text{L}^{-1}$) and SeO_3^{2-} ($0.1 \text{ mg}\cdot\text{L}^{-1}$).