Supporting Information *for*

Polystyrene Microplastics Interaction with Oryza sativa: Toxicity and Metabolic Mechanism

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Text S1. Assays of MDA content and SOD, POD activities.

Determination of MDA content:

A 0.1 g fresh leaf sample was immediately frozen in liquid nitrogen, then homogenized in 1.5 mL PBS (50 mM, pH 7.8) with an automatic sample rapid grinder (JXFSTPRP-48, Jingxin, China), until no fibrous residue could be seen. The homogenates were centrifuged at 4 °C for 10 min at 10, 000 g. To 1 mL supernatant 4 mL 20% TCA containing 0.5% TBA were added. The mixture was heated at 95°C for 30 min and then quickly cooled in an ice-bath, using 20% TCA containing 0.5% TBA as the reference standard. After centrifuging at 4 °C for 15 min at 10, 000 g, the absorbance of the supernatant at 450, 532, and 600 nm was read. The concentration of MDA was calculated using the following formula:

$$C_{MDA}(nmol\ g^{-1}FW) = \frac{7.5 \times \left[6.4516(A_{532} - A_{600}) - 0.5590A_{450}\right]}{FW_{(g)}}$$

Preparation of enzyme extracts:

Fresh leaf tissues, 0.1 g, were immediately frozen in liquid nitrogen, then thoroughly ground with an automatic sample rapid grinder (JXFSTPRP-48, Jingxin, China). The grinding medium consisted of PBS (50 mM, pH 7.0) containing 1% (w/v) polyvinyl pyrrolidone (PVP). The homogenates were centrifuged at 15, 000 g for 15 min and the supernatants obtained were collected as crude enzyme extracts for the assays of SOD and POD. All steps in the preparation of the enzyme extracts were carried out in a cold environment.

Enzyme assay-SOD activity:

The reaction mixture was composed of 1.5 mL phosphate buffer (50 mM, pH 7.8), 0.3 mL methionine (130 mM), 0.3 mL nitro blue tetrazolium (NBT) (750 μM), 0.3 mL EDTA (1 mM), 0.3 mL riboflavin (20 μM) and 50 μL enzyme extracts. Riboflavin was added last, and the appropriate volume of distilled water was added to make the final volume of 3 ml. The mixtures in transparent test tubes were shaken and placed 35 cm below eight LED plant tissue culture lights (8 × 4 W). The solutions without enzyme extracts that were illuminated served as controls and not illuminated served as blanks. After 30 min of light, the tubes were covered with aluminum foil to stop the reaction. One enzyme unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT photochemical reduction measured at 560 nm.

Enzyme assay-POD activity:

The reaction mixture (2 mL) consisted of 1.75 mL Tris-buffer (50 mM, pH 7.0), 50 μL enzyme extracts, 0.1 mL 4% guaiacol and 0.1 mL 1% (v/v) H_2O_2. H_2O_2 was added lastly after incubating the mixture for 3 min at 25 °C. The absorbance of the reaction mixture at 470 nm within 1 min was measured immediately and read every 30 s. One enzyme unit of POD activity was defined as an increase of 0.01 absorbance per minute caused by the enzyme aliquot.

Text S2. Metabolomics analysis of rice roots/shoots

Sample preparation:

Fresh rice roots/shoots were immediately frozen in liquid nitrogen to stop all biological activities including metabolites activities, then the roots and shoots were freeze-dried and ground into fine powders by using an automatic sample rapid grinder (JXFSTPRP-48, Jingxin, China). The powders of dried tissues were stored at -80 °C for further analysis.

10 mg accurately weighed sample was mixed with methanol: chloroform: water mixture (2 mL, 5:2:2, v/v/v). The metabolites were extracted by ultrasonication for 40 min at room temperature. The extraction solution was subsequently centrifuged at 10,000 g for 10 min. The first supernatant was transferred to a 5 mL Eppendorf tube. The precipitate was extracted again by the method described above and then the second supernatant was obtained. The two supernatants were collected in the same tube. 1 mL Milli-Q water was added to the total supernatants. Solution stratification occurred after centrifugation at 4000 rpm for 10 min. The upper solution was the methanol-water phase, and the lower solution was the chloroform phase.

The methanol in the methanol-water phase was dried under a stream of nitrogen. The chloroform phase was filtered through a 5 cm silica column and also dried under a stream of nitrogen later. Finally, the treated methanol-water phase and the treated chloroform phase were mixed. The final mixture was filtered through 0.22 μm filters and then freeze-dried.

For derivatization, 50 μL of methoxyamine hydrochloride in pyridine (20 mg/mL) was added to the dried samples in order to reduce the number of reducing sugar derivatives. Then, the sample was vortexed for 1 min and incubated at 37 °C for 90 min. Finally, the sample was silylated by adding 80 μL MSTFA and incubating at 37 °C for 30 min after vortexed for 30 s. The samples were analyzed by GC-MS after at least 2 h at room temperature.

GC-MS conditions:

The derivatised sample was analyzed by gas chromatography-mass spectrometer (GC-MS) (Agilent 7890B-5977A, Agilent, USA). Capillary column HP-5MS (30 m × 0.25 mm × 0.25 μm) was utilized to separate derivatized metabolites. The operating conditions of GC were as follows: the carrier gas (helium) flow rate is maintained at 1 mL/min; the injection volume was 1 μL by split mode (15:1); the injection temperature was 290 °C; the transfer line temperature was 280 °C; the column oven program, 70 °C for 4 min, ramped to 300 °C at a rate of 15 °C/min and finally held at 300 °C for 5 min. The operating conditions of MS were as follows: the ion source temperature was 230 °C; the MS quadrupole temperature was 150 °C; full-scan mode, from 35 to 600 m/z.

Data processing:

The acquired data from GC-MS was analyzed by the Mass Hunter Qualitative Analysis software B.07.00. The metabolites were identified by comparison with the NIST 14 library. Besides, the metabolites with a matching score greater than 75% were considered reliable. The integrated data matrix was imported into MetaboAnalyst 5.0

(https://www.metaboanalyst.ca/) for statistical analysis and pathway analysis. Orthogonal partial least squares discrimination analysis (OPLS-DA) including S-plot and variable importance projection (VIP) was performed using SIMCA-P 14.1.

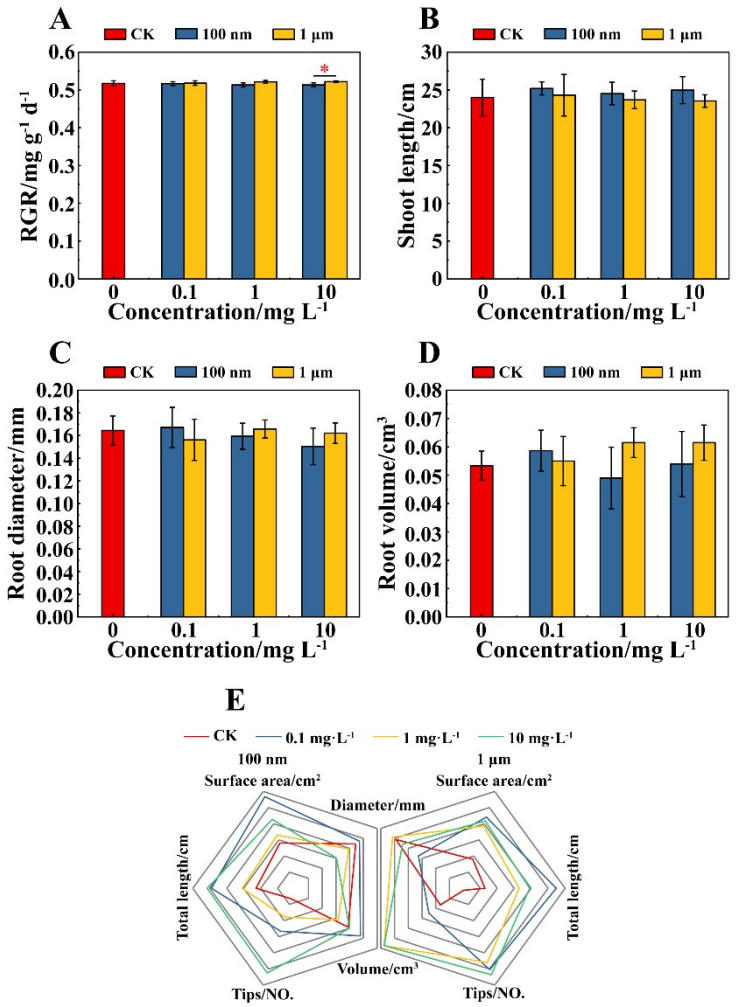

Figure S1. Relative growth rate (A), shoot length (B), root diameter (C), and root volume (D) of rice seedlings after exposure to different concentrations of $PS_{100\ nm}$ and $PS_{1\ \mu m}$ for 2 weeks. the overview of rhizosphere parameters (E). For a given size of PS, significant differences between control and PS suspension (0.1, 1, and 10 mg/L) was marked with "*" ($p < 0.05$) and "**" ($p < 0.01$). For a given concentration, a significant difference among different sizes of PS was marked with "*" ($p < 0.05$) and "**" ($p < 0.01$) above the line segment.

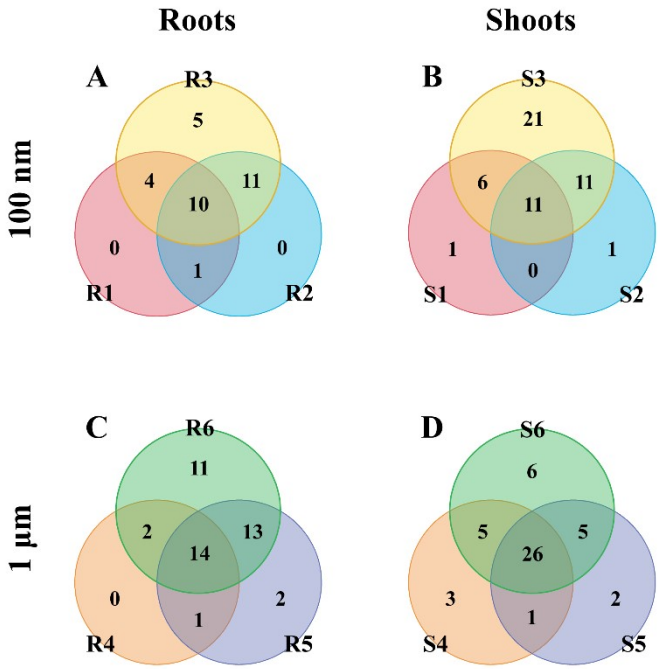

Figure S2. Veen diagram of differentially expressed metabolites (DEMs) of rice roots (A, C) and shoots (B, D) after $PS_{100\ nm}$ (A, B) and $PS_{1\ \mu m}$ (C, D) exposure. Under exposure of $PS_{100\ nm}$, 15 differentially expressed metabolites (DEMs) (6 up-regulated and 9 down-regulated) were detected in roots and 18 DEMs (3 up-regulated and 15 down-regulated) were detected in shoots at 0.1 mg/L; the number of DEMs increased to 22 in roots (1 up-regulated and 21 down-regulated)/23 in shoots (13 up-regulated and 10 down-regulated) at 1 mg/L and 30 in roots (30 down-regulated)/49 (49 down-regulated) in shoots at 10 mg/L. Under exposure of $PS_{1\ \mu m}$, 17 DEMs (3 up-regulated and 14 down-regulated) were detected in roots and 35 DEMs (5 up-regulated and 30 down-regulated) were detected in shoots at 0.1 mg/L; the number of DEMs increased to 30 in roots (4 up-regulated and 26 down-regulated)/34 in shoots (8 up-regulated and 26 down-regulated) at 1 mg/L and 40 in roots (1 up-regulated and 39 down-regulated)/42 (6 up-regulated and 36 down-regulated) in shoots at 10 mg/L.

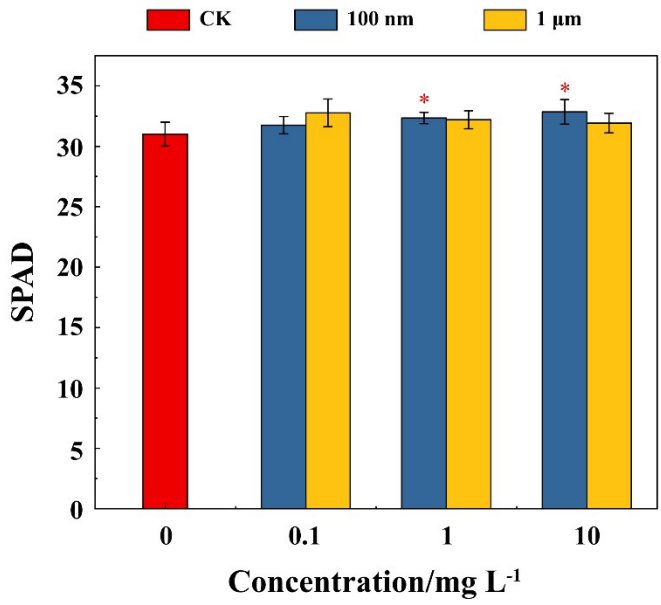

Figure S3. SPAD value of rice leaves after exposure to $PS_{100\ nm}$ and $PS_{1\ \mu m}$ for 2 weeks. For a given size of PS, significant differences between control and PS suspension (0.1, 1, and 10 mg/L) was marked with "*" ($p < 0.05$) and "**" ($p < 0.01$). For a given concentration, a significant difference among different sizes of PS was marked with "*" ($p < 0.05$) and "**" ($p < 0.01$) above the line segment.

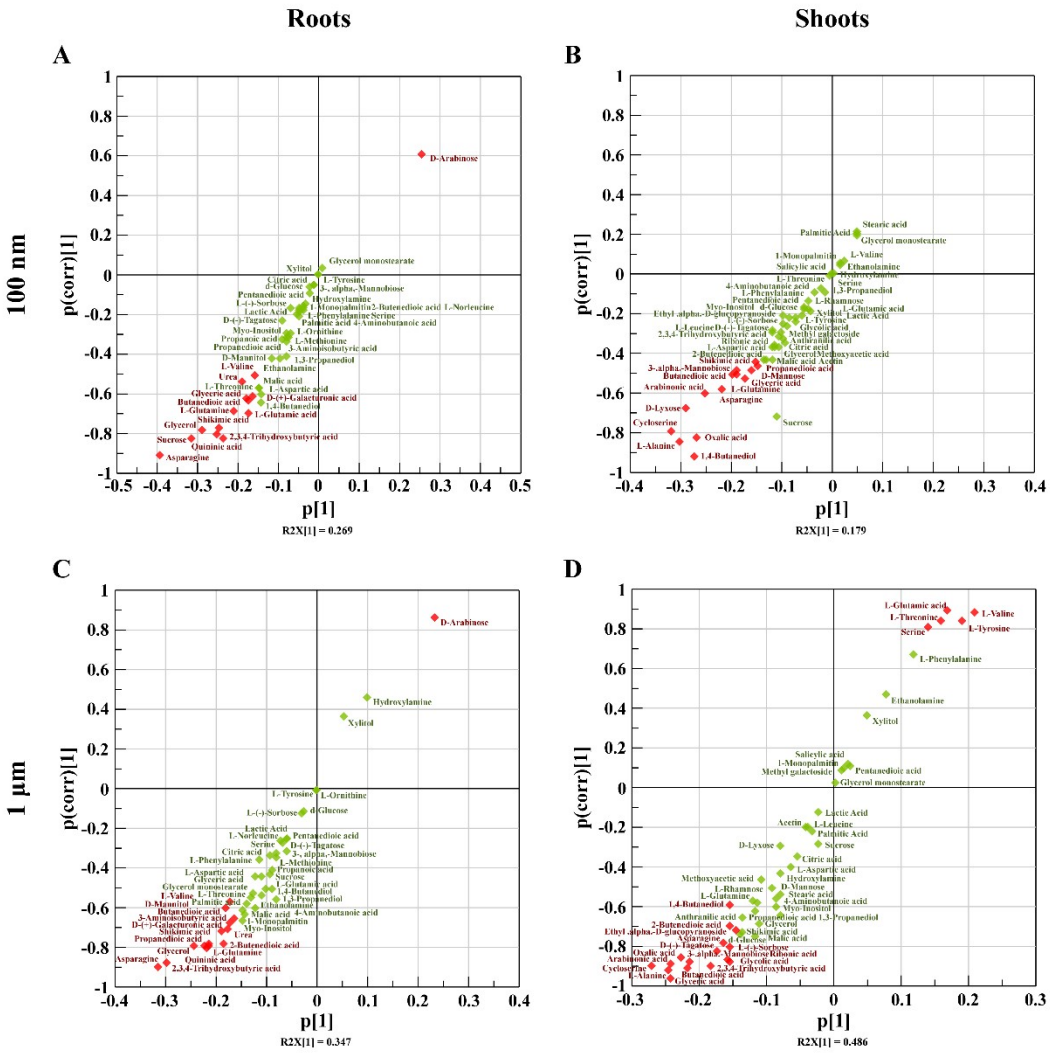

Figure S4. S-plot analysis of rice roots (A, C) and shoots (B, D) after exposure to different concentrations of $PS_{100\ nm}$ (A, B) and $PS_{1\ \mu m}$ (C, D) for 2 weeks. The red point indicates the metabolites with the variable importance projection (VIP) value greater than 1.

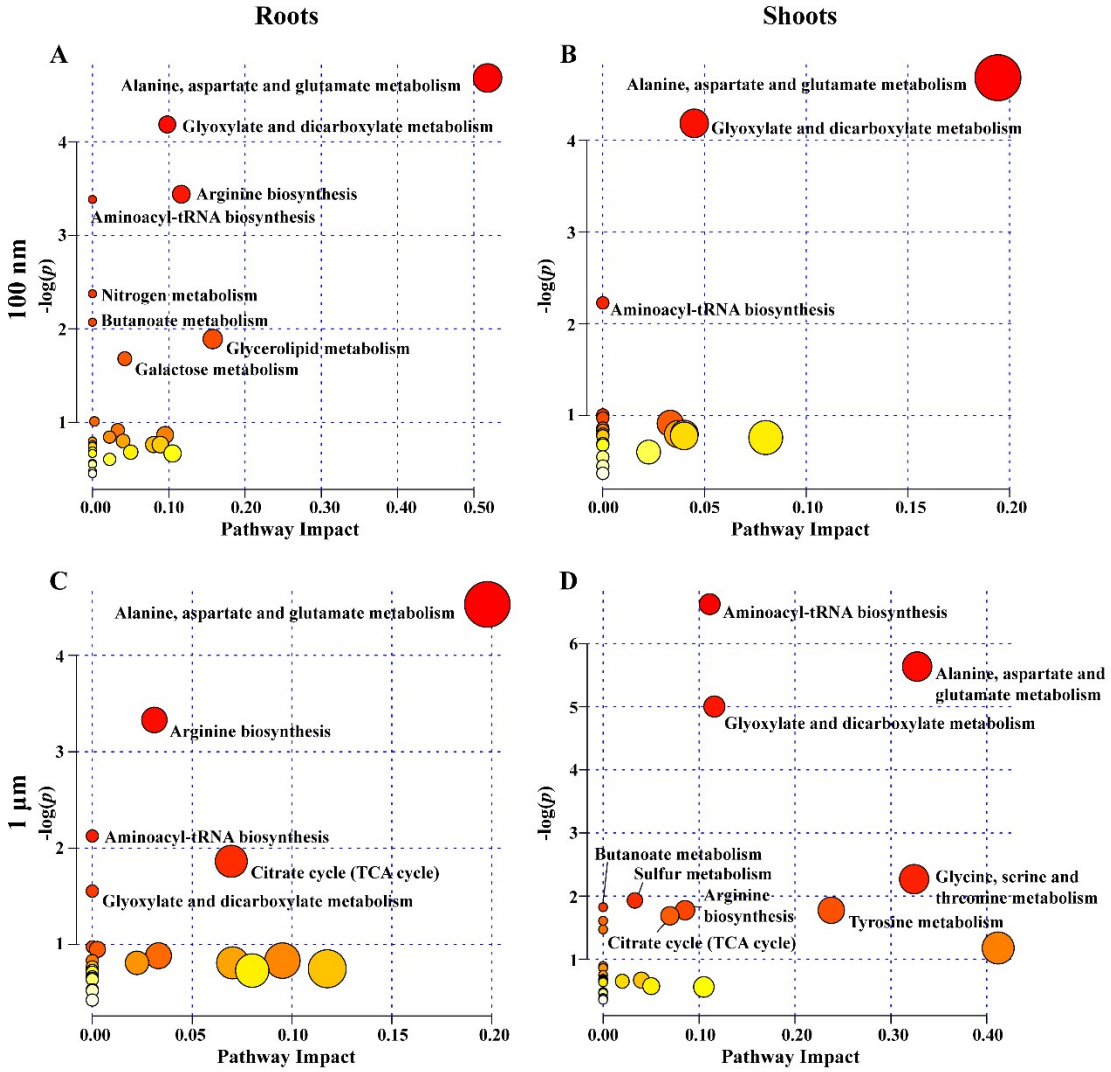

Figure S5. Analysis of metabolic pathways of rice roots (A, C) and shoots (B, D) after exposure to different concentrations of $PS_{100\ nm}$ (A, B) and $PS_{1\ \mu m}$ (C, D) for 2 weeks.

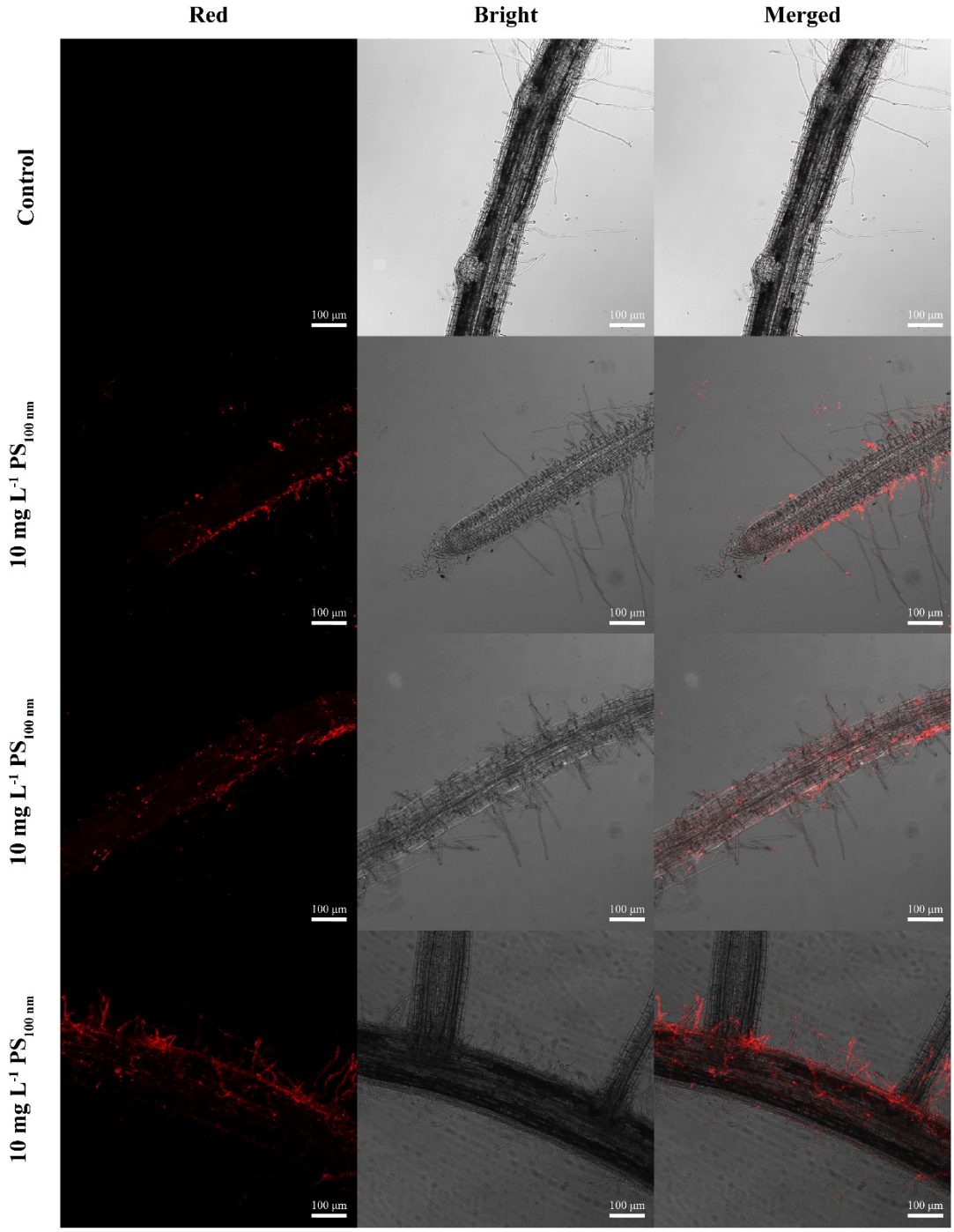

Figure S6. Laser confocal scanning micrographs (LSCM) of rice roots in the control and 10 mg/L $PS_{100\ nm}$ treated groups. Red: red fluorescence. Bright: bright field image. Merged: merged images of bright field and red fluorescence.

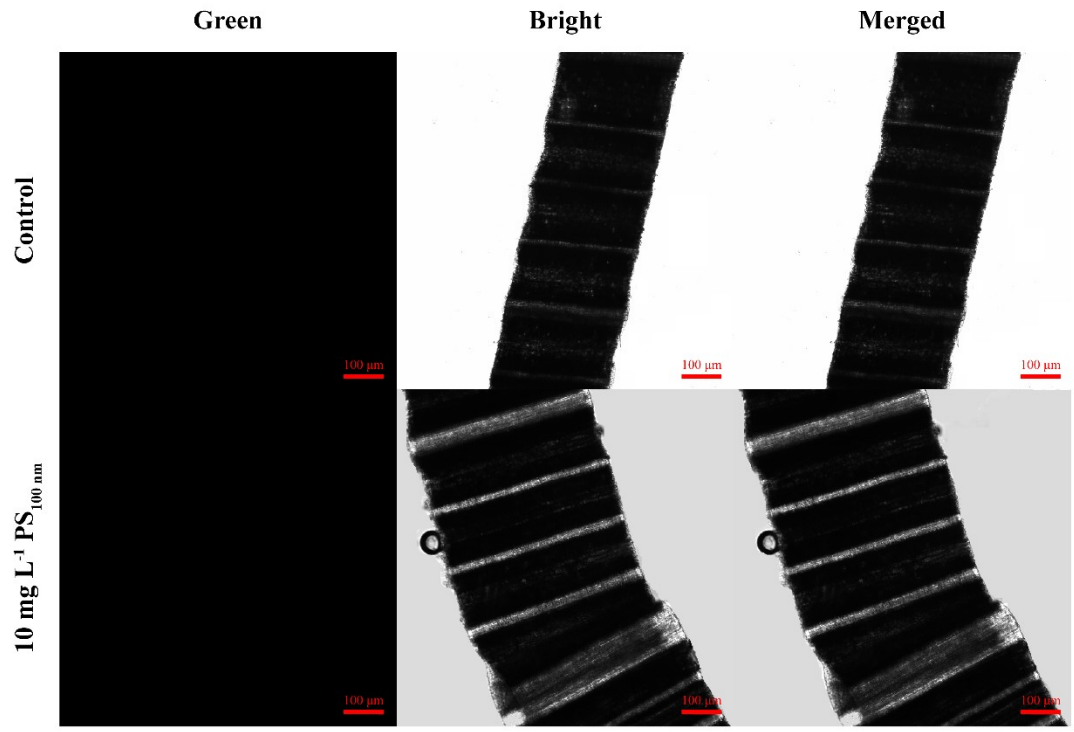

Figure S7. Laser confocal scanning micrographs (LSCM) of rice leaves in the control and 10 mg/L $PS_{100\ nm}$ treated groups. Green: green fluorescence. Bright: bright field image. Merged: merged images of bright field and green fluorescence.

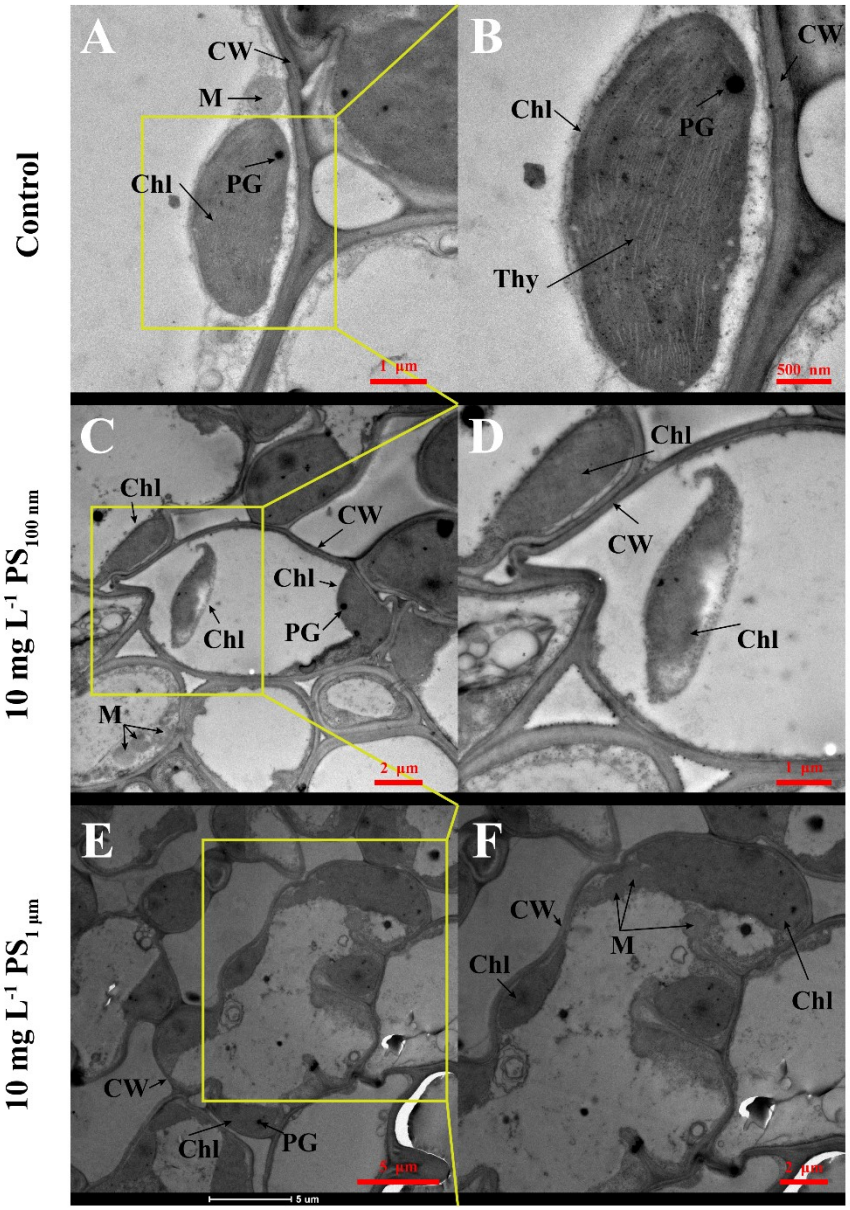

Figure S8. TEM images of rice leaves in the control (A, B), 10 mg/L $PS_{100\ nm}$ (C, D) and 10 mg/L $PS_{1\ \mu m}$ (E, F) treated groups. B, D and F show enlargements of the areas indicated by the yellow squares in the A, C and E, respectively. CW: cell walls; M: mitochondria; Chl: chloroplasts; PG: plastoglobules; Thy: thylakoid.