Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supporting information

Transcriptomics and Metabolomics Reveal the Mechanisms of Enhanced Constitutive Resistance in Rice (*Oryza Sativa* L.) by Silica Nanomaterials

Jing Wang[ab], Xuesong Cao[ab], Chuanxi Wang[ab], Zhenggao Xiao[ab], Feiran Chen[ab], Yan Feng[ab], Le Yue*[ab], Zhenyu Wang[ab], Baoshan Xing[c]

[a] *Institute of Environmental Processes and Pollution Control and School of Environment and Civil Engineering, Jiangnan University, Wuxi 214122, China.*

[b] *Jiangsu Engineering Laboratory for Biomass Energy and Carbon Reduction Technology, Wuxi 214122, China.*

[c] *Stockbridge School of Agriculture, University of Massachusetts, Amherst, Massachusetts 01003, United States.*

*Corresponding author:

Tel.: +86 0510 85911911

E-mail address: leyue@jiangnan.edu.cn (Dr. Le Yue)

Text S1. Confocal imaging of SiO_2 NMs-FITC in rice plants

One month-old hydroponic rice seedlings were transferred to 50 mg L^{-1} SiO_2 NMs-FITC and Na_2SiO_3 solution. As the control, the seedlings were grown in DI water and 10 mM FITC solution without Si amendment. These plants were transferred to a green house at 25 ℃ with the humidity of 60% for seven days. Then leaf discs were cut from leaves and imaged with a confocal microscope under the laser excitation of 488 nm (Nikon A1+ Confocal Superresolution Imaging System, Japan).

Text S2. The operating parameters of LC-MS/MS

LC-MS/MS includes a Vanquish UPLC system (Thermo Fisher, USA) and Q Exactive Orbitrap mass spectrometer (Thermo Fisher, USA). 10 μL samples were injected onto a Acquity UPLC HSS T3 (2.1×100 mm, 1.8μm) using an 18-min linear gradient at a flow rate of 0.35 mL/min. The eluents in negative/positive mode were eluent A (0.1% v/v formic acid in water) and eluent B (0.1% formic acid in acetonitrile). The solvent gradient was set as follows: 5% B, 0 min; 5% B, 1.5 min; 100% B, 14.0 min; 100% B, 15.5 min; 5% B, 16 min; 5% B, 18 min. The column temperature was 35℃. The method was operated in the positive/negative electrospray ionization switching mode and the ionization parameters setup as follows: spray voltage 3.5 kV(positive), 3 kV(negative), sheath gas flow rate: 35 bar; aux gas flow rate: 15 bar; capillary temperature: 320℃; aux gas heater temperature: 350℃.

A nontarget metabolic profiling analysis was performed in Full/dd-MS^2. Data were acquired using the following settings: the ion scanning range was 70–1050 m/z; the full MS resolution was 70,000; AGC target value was 1 e^6 and the maximum ion time was 100 ms. The MS/MS resolution was 17,500; AGC target value was 5 e^4; the maximum ion time was 50 ms; isolation window was1.5 m/z and the collision energy was 20/40/60 in NCE mode. The mix sample was performed in the Full MS/dd-MS^2 mode by Q Exactive Orbitrap LC-MS/MS using Compound Discoverer 3.3 software to analyze the raw data.

Text S3. Determination of lignin, proline, and total phenolics

Lignin contents were determined using the method of Ke *et al*[1]. The fresh rice roots, stems and leaves were immersed by 1.5 mL 95% ethanol and centrifuged for 10 min for three times, and then oven-dried at 80 ℃. A total of 10 mg of dried samples were collected to a test tube, with subsequent adding 1 mL 25% acetyl bromide glacial acetic acid. Then the test tube was heated at 70℃ for 30 min. A mixed solution containing 0.9 mL NaOH (2 M), 5 mL glacial acetic acid, 0.1 mL hydroxylamine hydrochloride (7.5 M) and 3 mL glacial acetic acid were added to the test tube Then the mixed solution was centrifuged at 1000 g for 5 min, and the absorbance of the supernatant was determined at 280 nm to determine the lignin content.

The proline in rice leaves and stems were determined according to Wang *et al*[2]. A total of 500 mg fresh rice leaves and stems were ground in liquid nitrogen with a pestle and mortar and transferred to a glass tube. 5 mL 3% sulfosalicylic acid solution were added into the glass tube, the glass tube was then placed in boiling water bath for 10 minutes. The filtrate is the extract of proline. A volume of 2 mL of the extract was transferred to another clean test tube, and then 2 mL glacial acetic acid and 2 mL acidic ninhydrin reagent were added into the test tube. After shaking, the test tube was heated in a boiling water bath for 30 min. 4 mL toluene was added into the solution after cooling. Then the mixture solution was centrifuged at 3000 g for 5min. The absorbance of the supernatant was determined at 520 nm to determine the proline content.

Total phenolics was determined according to Ainsworth and Gillespie[3]. with some modifications. A total of 20 mg fresh rice leaves and stems were homogenized in an ice-cold mortar and pestle, and 2 mL of ice-cold 95% (v/v) methanol was added to each sample. The samples were incubated at room temperature for 48 h in the dark. Then the samples were centrifuged at 13,000 g for 5 min at room temperature and the supernatant

was collected in a fresh 2 mL microtube. After shaking thoroughly, the assay tubes were incubated at room temperature for 2 h. Finally, 200 μL sample was transferred from the assay tube to a clear 96-well microplate and the absorbance of each well was measured at 765 nm. Gallic acid was selected as the standards and 95% (v/v) methanol as the blank.

References:

1. Ke, S.; Luan, X.; Liang, J.; Hung, Y. H.; Hsieh, T. F.; Zhang, X. Q., Rice OsPEX1, an extensin-like protein, affects lignin biosynthesis and plant growth. *Plant Mol Biol* **2019,** *100*, (1-2), 151-161.
2. Wang, Y.; Wang, L.; Ma, C.; Wang, K.; Hao, Y.; Chen, Q.; Mo, Y.; Rui, Y., Effects of cerium oxide on rice seedlings as affected by co-exposure of cadmium and salt. *Environ Pollut* **2019,** *252*, (Pt B), 1087-1096.
3. Ainsworth, E. A.; Gillespie, K. M., Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc* **2007,** *2*, (4), 875-877.

Text S4. The number of the obtained data and PCA analysis in transcriptome

A total of 73.18 Gb of clean data were obtained from the 6 leaf and 6 stem samples, and each of these samples contained ≥5.43 Gb of data with Q30 quality scores ≥92.80% (Table S3). The gene expression levels were used to conduct a PCA for each of the biological replicates. The samples from leaves upon NMs were clustered far from each other and the control groups, which indicated that NMs induced significant changes in leaf gene expression (Fig S8a). The Q value≤0.05 and Log_2-fold change (Log_2FC) ≥ 2 or ≤ − 2 were set as thresholds for differentially expressed genes (DEGs). A total of 4637 DEGs were identified in rice leaves and stems. Briefly, compared to control, 2648 genes (1905 up-regulated and 743 down-regulated) were differentially expressed in rice leaves after SiO_2 NMs exposure, and 1989 genes (1332 upregulated and 657 down-regulated) were differentially expressed in rice stem (Fig S8b). The distribution of up- and downregulated genes was calculated for leaves and stems are presented in a Venn diagram (Fig S8c-d). 270 and 106 genes showed increased and decreased expression at both leaves and roots in response to NMs.

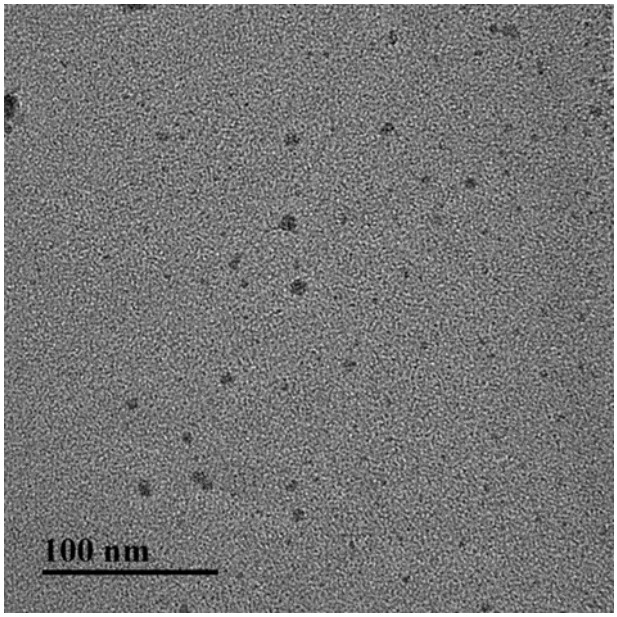

Fig S1. TEM image of SiO_2 NMs.

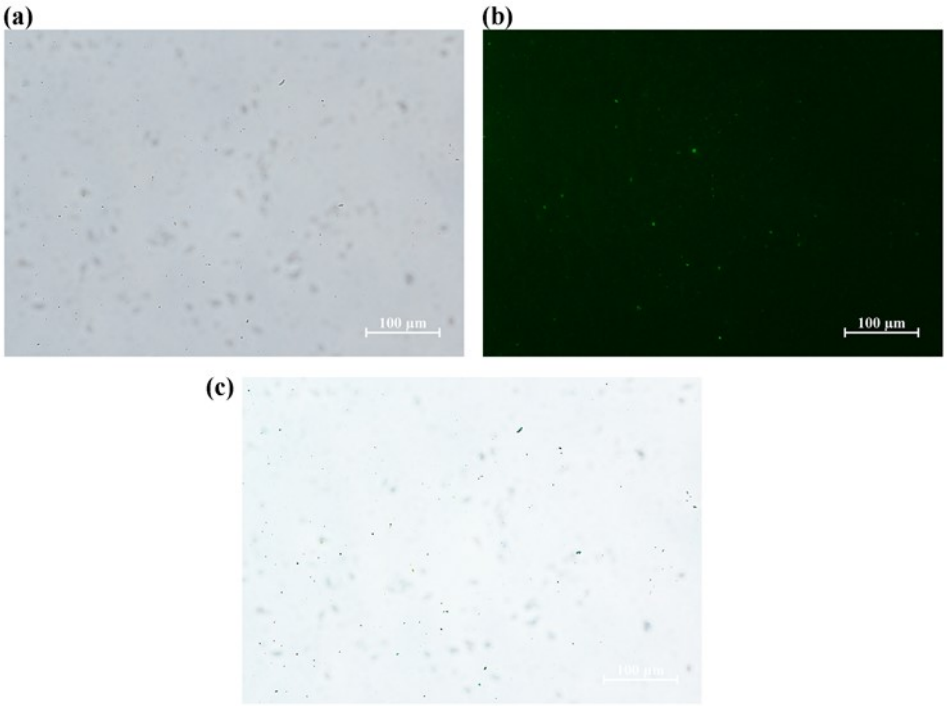

Fig S2. Fluorescence microscope images of SiO_2 NMs-FITC suspended in deionized water. (a) Bright field; (b) green channel; (c) the overlapping images of bright-field and green channel.

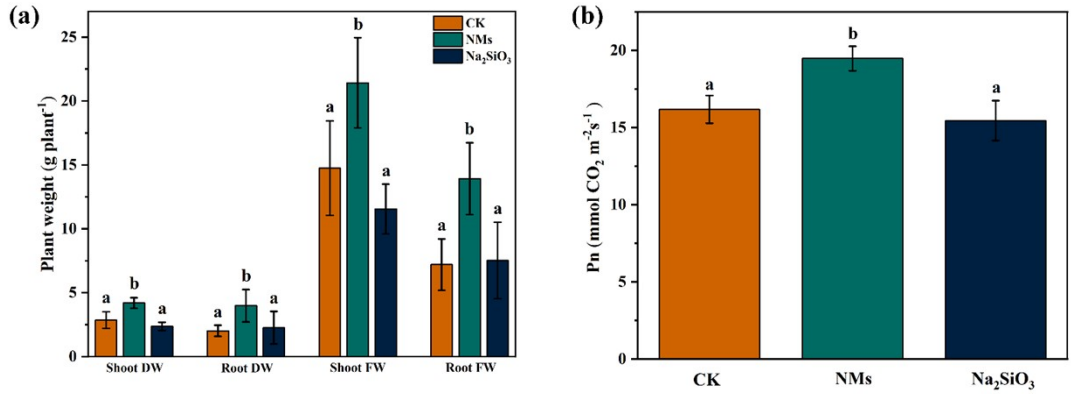

Fig S3. Effects of SiO_2 NMs on rice growth. **(a)** Fresh and dry weight of the rice shoot and root in the presence of SiO_2 NMs and Na_2SiO_3. **(b)** Net photosynthetic rate (Pn) of rice subjected to 50 mg kg^{-1} SiO_2 NMs and Na_2SiO_3. The values are given as mean±SD (standard deviation). The significance difference is marked with "a-b" (p<0.05, n=5).

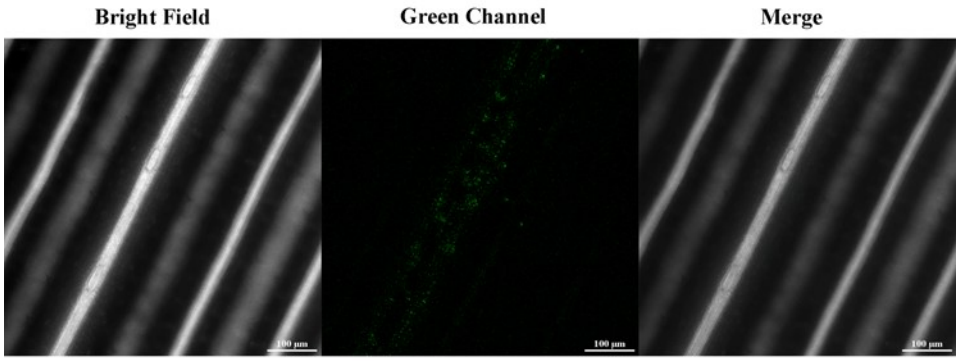

Fig S4. Confocal images of rice leaf after treated with 10 mM FITC solution. Scale bar = 100 μm.

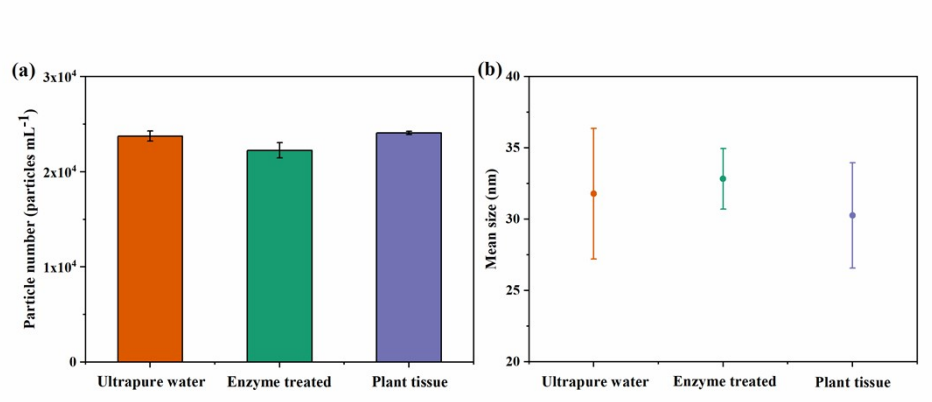

Fig S5. Particle number (a) and mean size (b) of Au NPs after spiking into ultrapure water, macerozyme R-10 enzyme treated and plant tissue. The values are given as mean±SD (standard deviation).

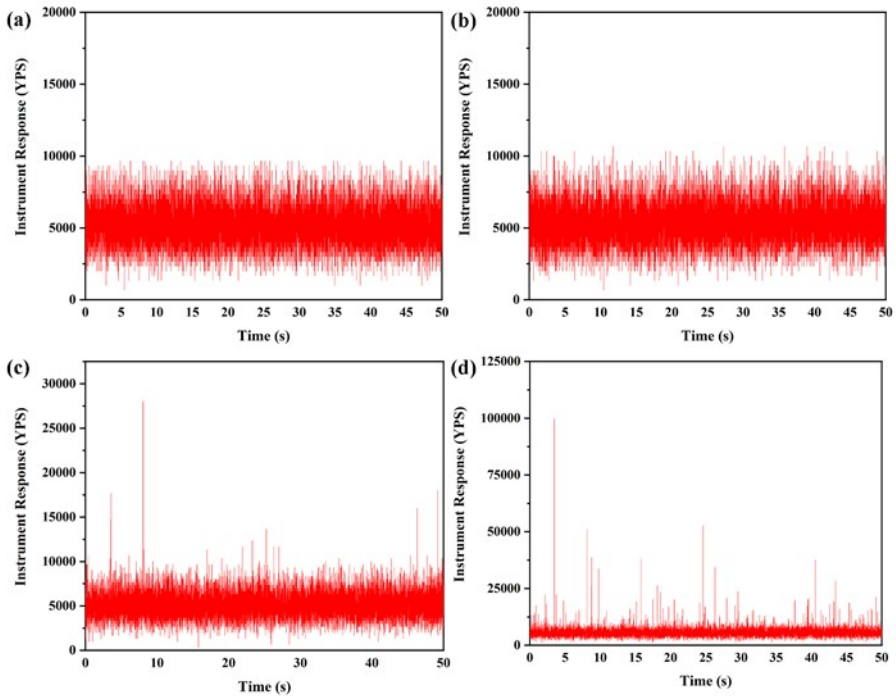

Fig S6. Raw peak data corresponding to Si-containing NPs detected in old leaves **(a)**, new leaves **(b)**, stems **(c)** and roots **(d)** of the control rice plant.

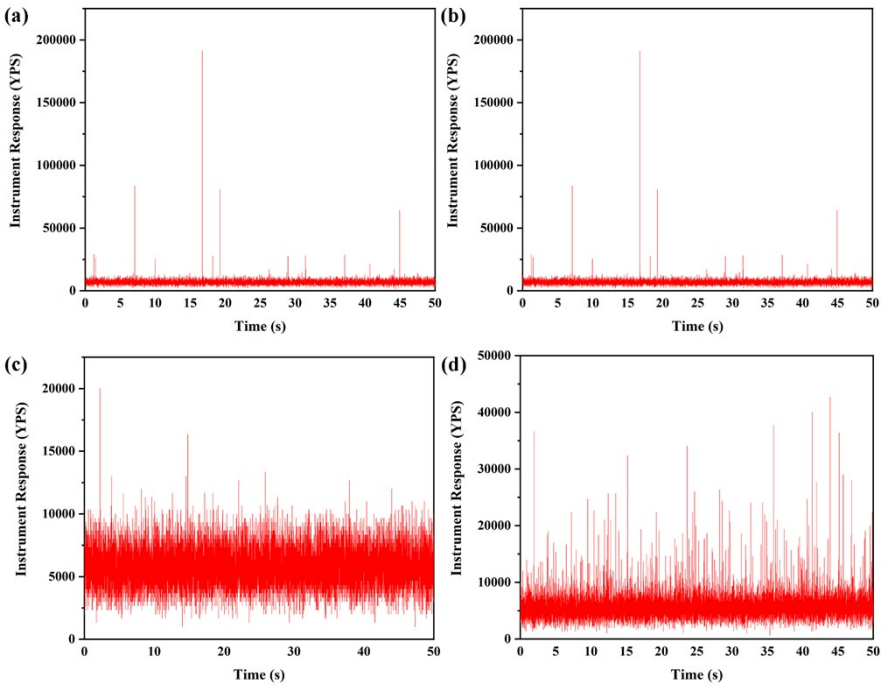

Fig S7. Raw peak data corresponding to Si-containing NPs detected in old leaves **(a)**, new leaves **(b)**, stems **(c)** and roots **(d)** of rice plant treated with SiO_2 NMs.

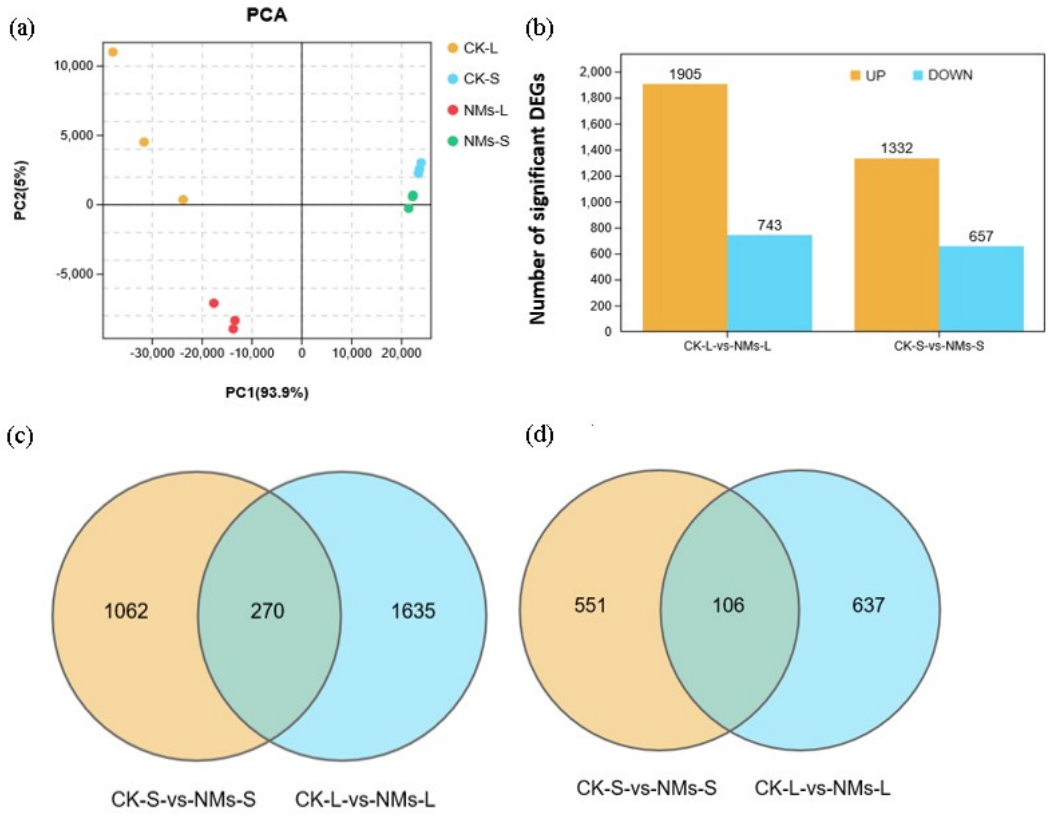

Fig S8. Overview of differential gene expression under SiO_2 NMs exposure. **(a)** PCA plot of global transcriptome profiles. **(b)** Numbers of differentially up- and downregulated genes between each group **(c, d)**: Venn diagram illustrating the number of genes up- or downregulated. $Q < 0.05$ and $|\text{Log2 FC}| \geq 1$.

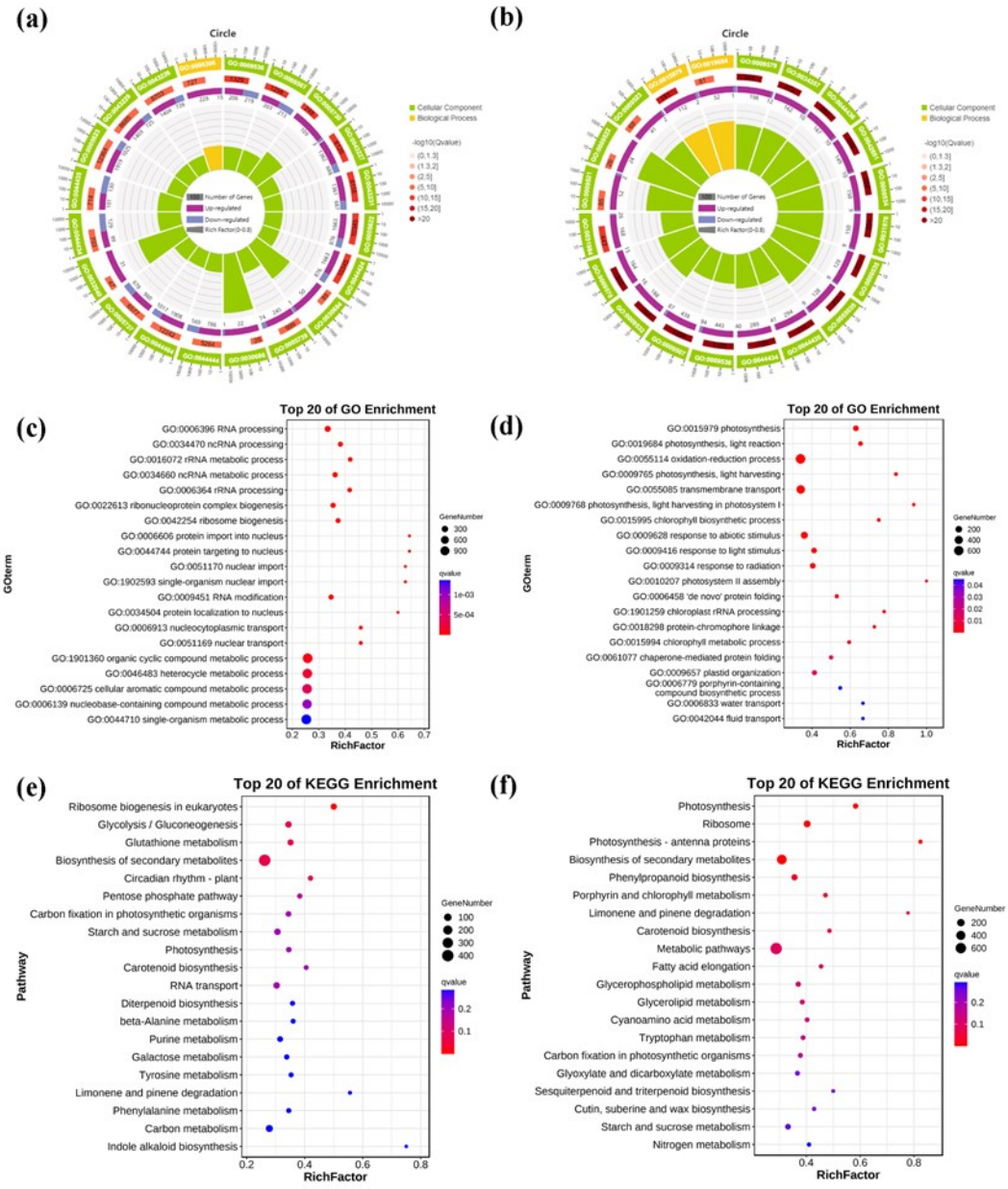

Fig S9. Transcriptomic analysis in rice leaves and stems. Gene ontology analysis of rice leaves **(a)** and stems **(b)** under Na_2SiO_3 exposure. The results are categorized into three: biological process, molecular function and cellular component. GO enrichment analysis for Na_2SiO_3 vs. CK in rice leaves **(c)** and stems **(d)**. KEGG enrichment analysis for Na_2SiO_3 vs. CK in rice leaves **(e)** and stems **(f)**. The color and size of the dots indicate the *q* value and number of enriched DEGs, respectively.

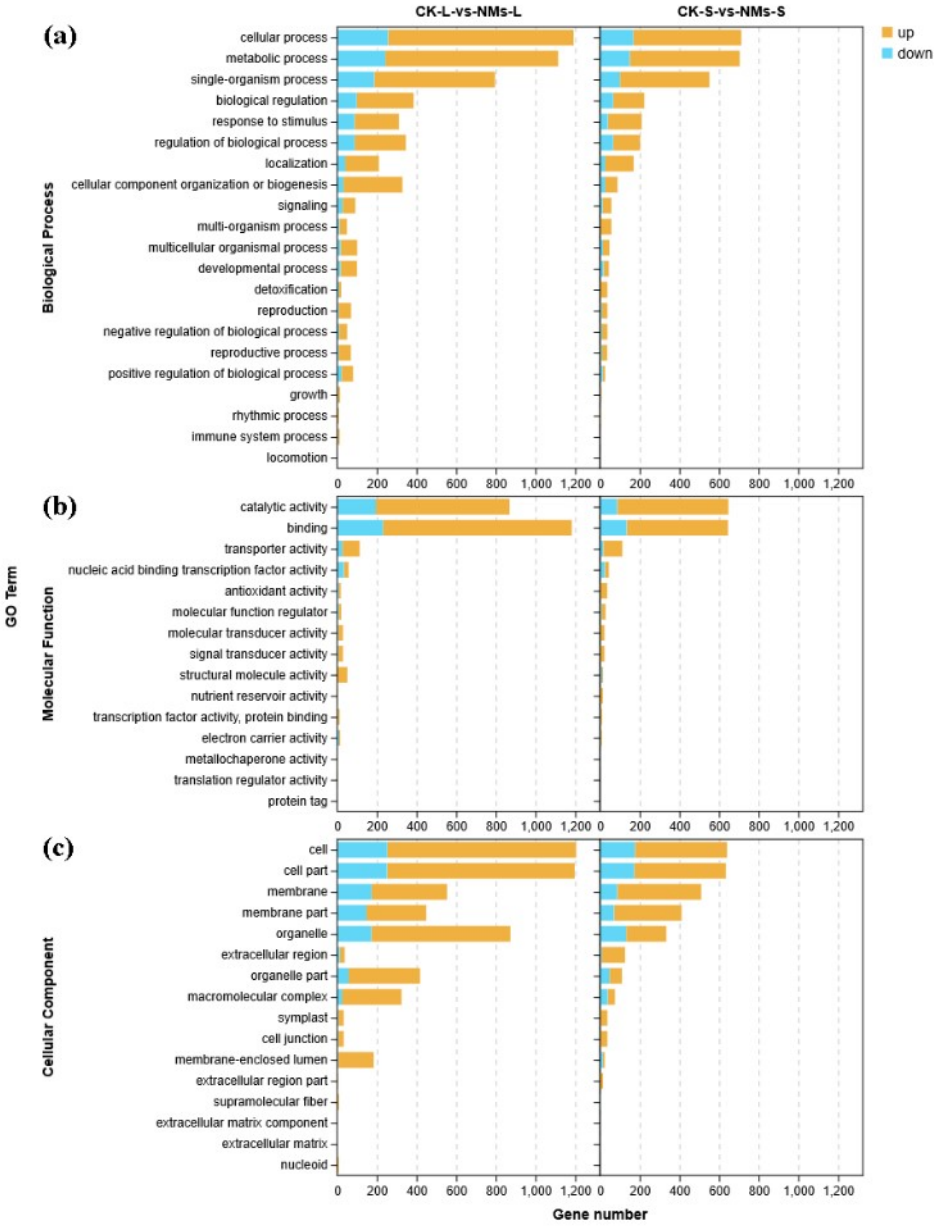

Figure S10. GO enrichment analysis of upregulated and downregulated DEGs in rice leaves and stems in response to SiO_2 NMs exposure.

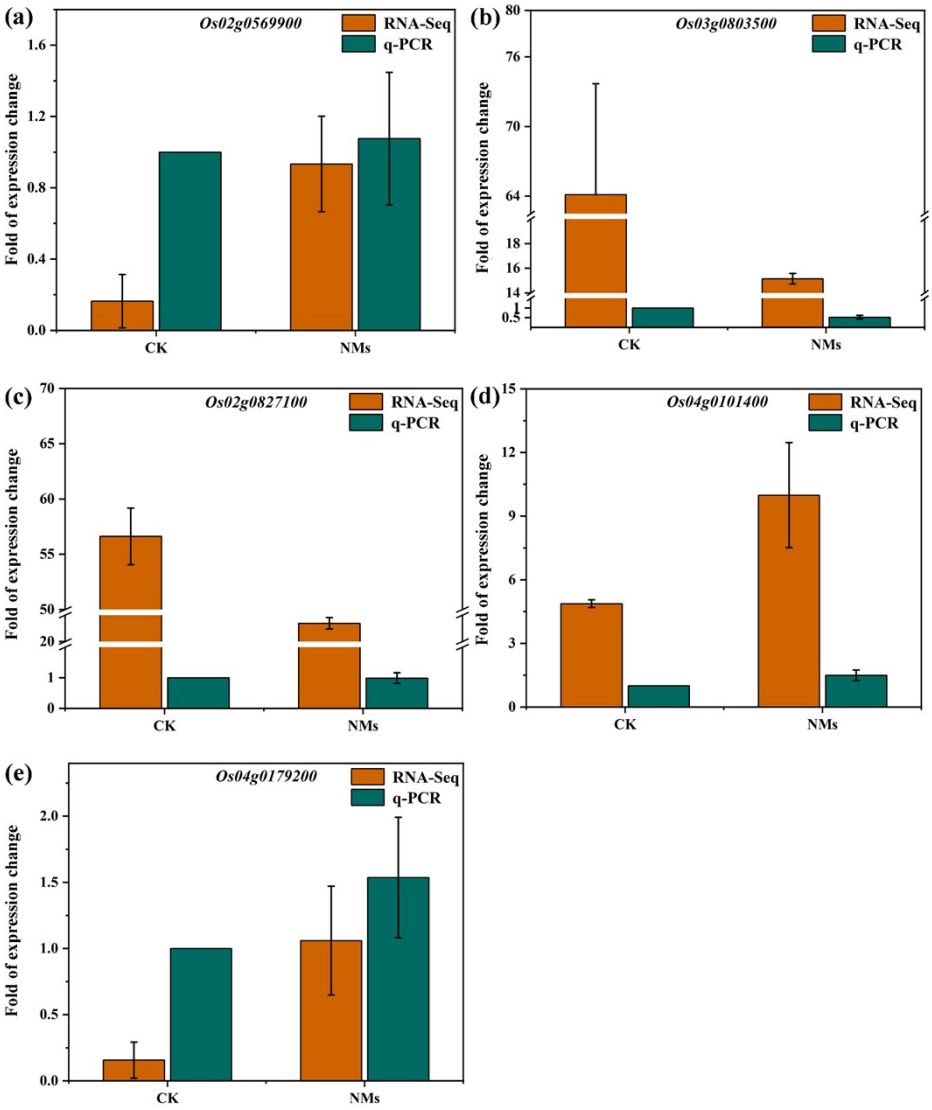

Fig S11. Verification of RNA-Seq expression by qRT-PCR. The orange bar column means the data in transcriptome analysis, and green indicates the data of q-PCR. The bars represent standard deviation.

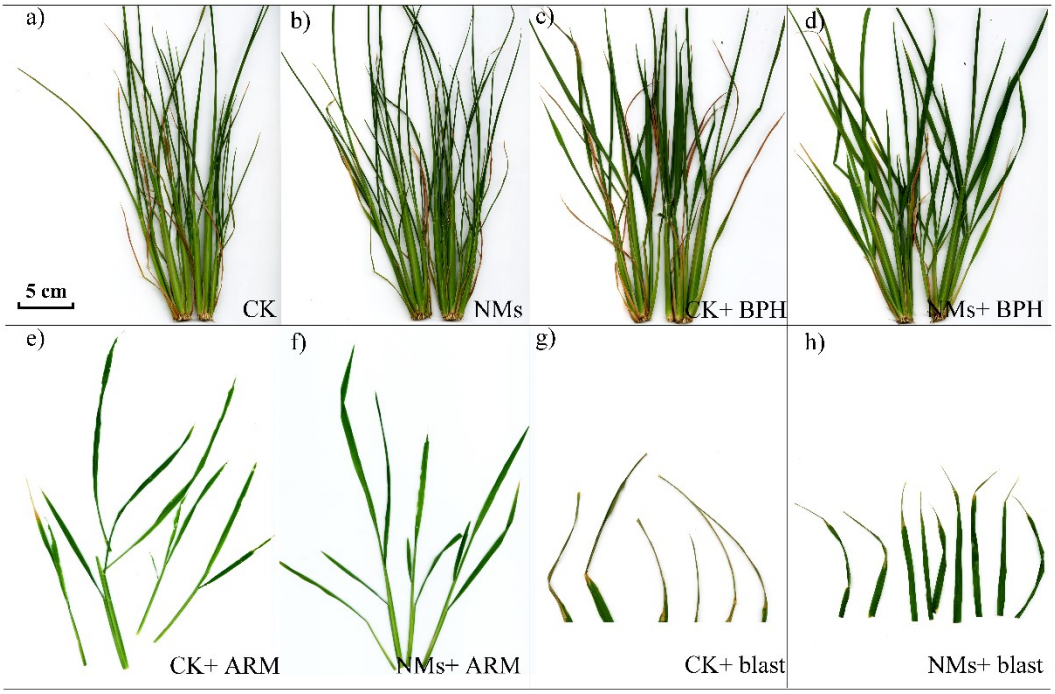

Fig S12. The increased constitutive resistance against insects and fungi by SiO_2 NMs. **(a-b)** Photos of rice grown without infestation by insects or fungi. **(c-d)** In the presence of BPH. **(e-f)** In the presence of ARM. **(g-h)** Infected with rice blast.

Table S1. Characteristics of particles used in this study

Particle	Hydrodynamic diameter (nm)	Zeta potential (mV)
SiO_2 NMs	284.9±40.9	22.2±1.3

Table S2. The specific primer sequences of five selected DEGs

Names (gene ID)	Forward sequence (5'-3')	Reverse sequence (3'-5')
Os02g0569900	TACACTGGCCTCCTCAACCT	AGGAACGGGTAGAAGTCGGA
Os03g0803500	AGCACGAGGACGACATTGTT	AAGACTGGCTGGATCCGTTG
Os02g0827100	CCAGATGCCACTCTAGACCC	TCACGGACATGCATCCACTT
Os04g0101400	GTGTTCATCAACGTGTGGGC	CCATGCACTGCCAATCGAAG
Os04g0179200	GAGGCAATCGAGGCGATCAT	CCAAAGCTGCTGTTGACGAC

Table S3. Summary for the transcriptome of rice leaves and stems in response to SiO_2 NMs at one month using Illumina RNA-seq.

Sample	Raw reads	Clean reads	Clean bases	Q20(%)	Q30(%)	GC content (%)
CK-L-1	40398280	40180936(99.46%)	6.03G	97.74%	93.65%	51.65%
CK-L-2	45528110	45311566(99.52%)	6.80G	97.96%	94.05%	52.01%
CK-L-3	39665868	39480110(99.53%)	5.92G	97.95%	94.07%	51.87%
CK-S-1	42494770	42303030(99.55%)	6.35G	97.80%	93.72%	48.95%
CK-S-2	41710280	41512284(99.53%)	6.23G	97.82%	93.71%	49.08%
CK-S-3	45980348	45745130(99.49%)	6.86G	97.85%	93.85%	49.10%
NMs-L-1	40284200	40077224(99.49%)	6.01G	97.63%	93.45%	51.13%
NMs-L-2	40701914	40436834(99.35%)	6.07G	97.57%	93.30%	51.43%
NMs-L-3	37340844	37140708(99.46%)	5.57G	97.35%	92.80%	51.31%
NMs-S-1	39456504	39262460(99.51%)	5.89G	97.71%	93.53%	50.24%
NMs-S-2	34601078	36214220(99.49%)	5.43G	97.68%	93.51%	50.48%
NMs-S-3	40309100	40112512(99.51%)	6.02G	97.65%	93.41%	50.48%