

# **Silica Nanoparticles Enhance Disease Resistance in *Arabidopsis* Plants – Supplementary Information**

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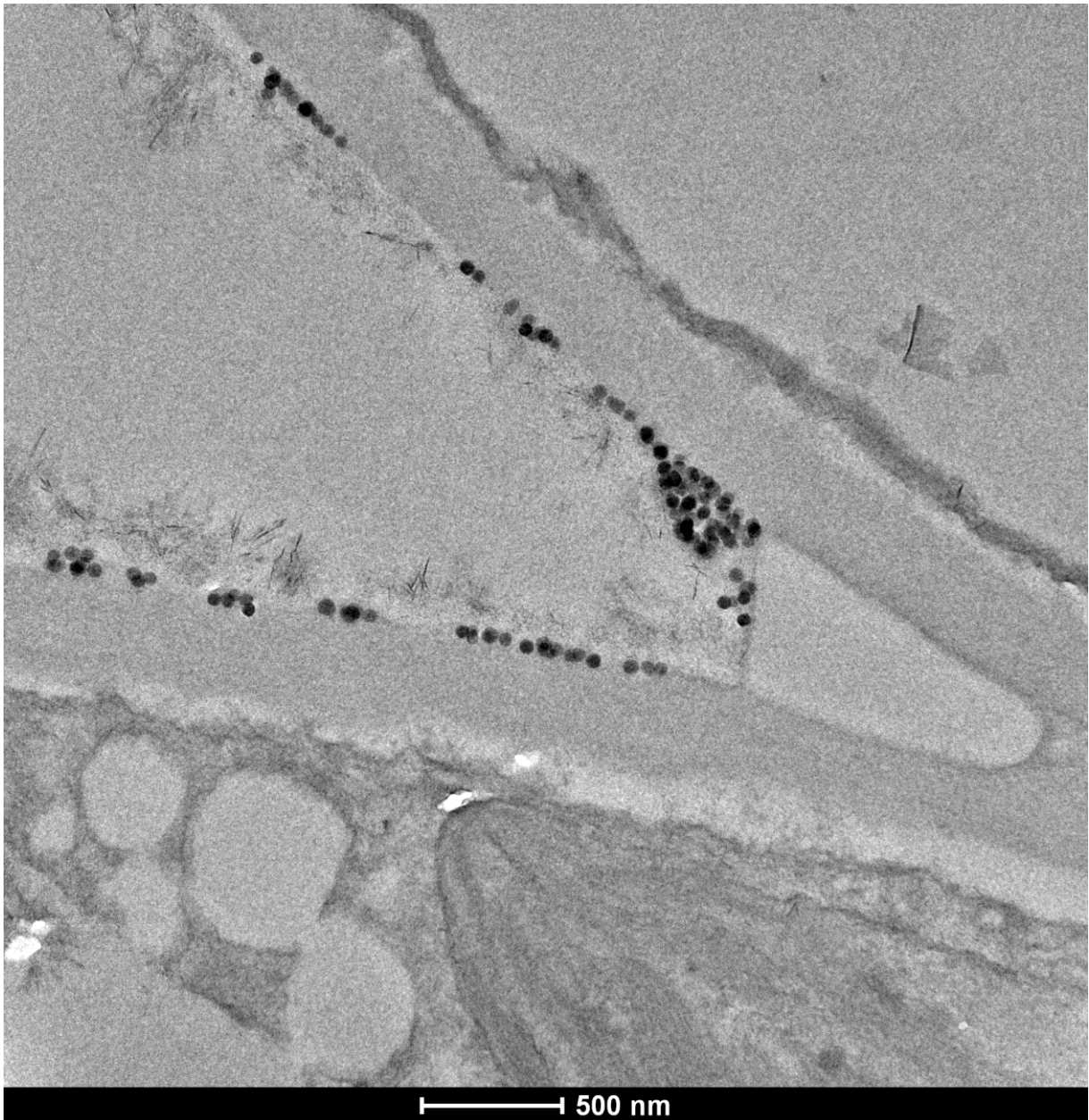
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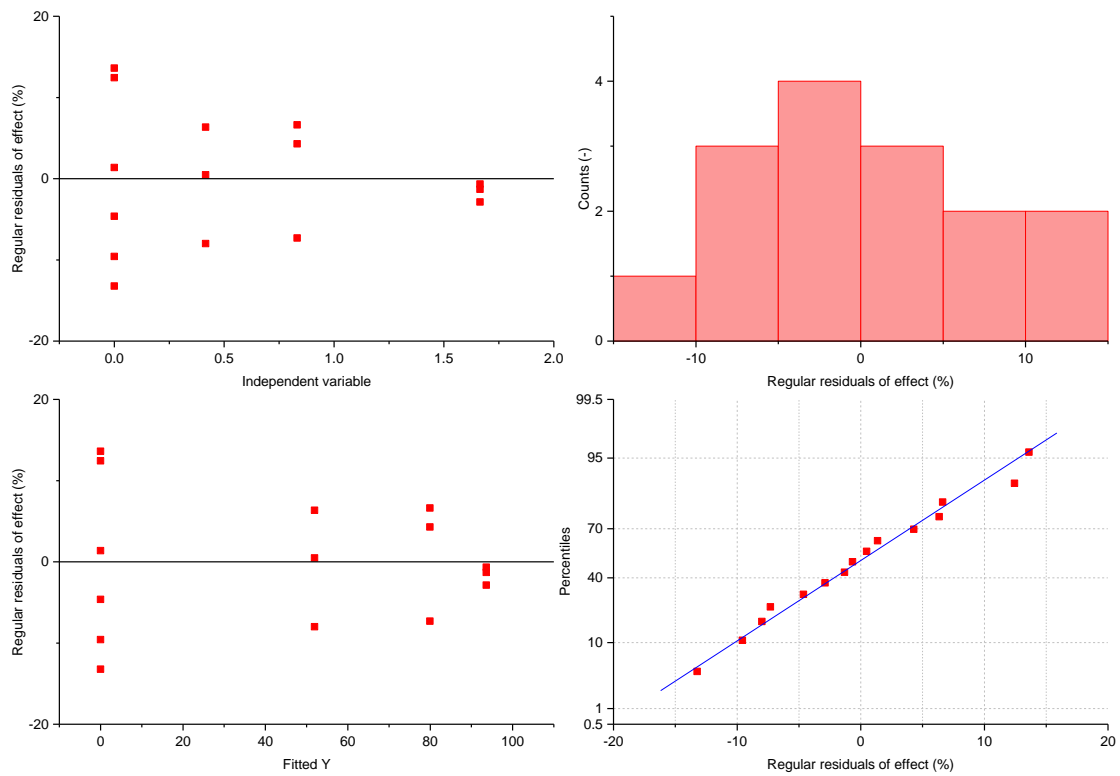
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## **Content**

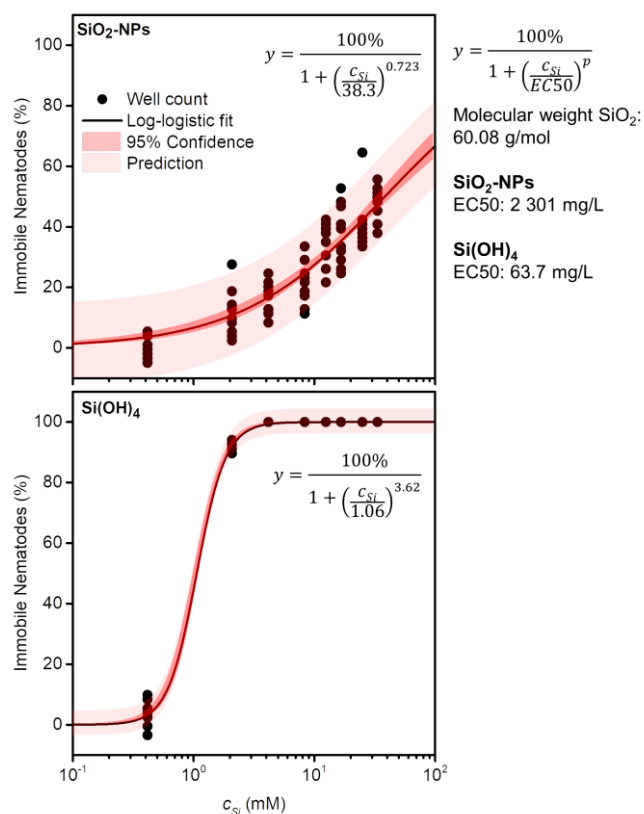
**Supplementary Fig. S1, Supplementary Fig. S2, Supplementary Fig. S3, Supplementary Tab. S1, Supplementary Tab. S2, sections *Details on Si(OH)<sub>4</sub> Analytics, Si Reaction Byproducts, Details on DNA Extraction, Details on RNA Extraction and cDNA Synthesis, Ecotoxicity to Non-target Organisms.***



**Supplementary Figure S 1: High-resolution transmission electron microscopy micrograph of the air space-cell wall interface in *Arabidopsis*.** Leaves infiltrated with 1000 mg L<sup>-1</sup> silica nanoparticles (SiO<sub>2</sub>-NPs) 2 d after exposure. Air space (middle) and two adjacent cells (top right, bottom left). Average size of NPs 49±6 nm. Exclusive adsorption of NPs to the outer cell wall, and absence of internalization into cells.



**Supplementary Figure S 2: Residual analysis of the modelling of the dose-response curve of Fig. 5.** The normal distribution of the fitted and measured residuals suggests that a log-logistic model is appropriate to predict the systemic acquired resistance in *Arabidopsis thaliana* in a concentration range up to 100 mg L<sup>-1</sup> of SiO<sub>2</sub>-NPs. Fitting parameters listed in **Supplementary Tab. S2.**



**Supplementary Figure S 3: Ecotoxicity of silica nanoparticles (SiO<sub>2</sub>-NPs) and orthosilicic acid (Si(OH)<sub>4</sub>) to the nematode *Caenorhabditis elegans*.** The nematode served as a model soil microorganism for an initial assessment of the ecotoxicity of the SiO<sub>2</sub>-NPs and comparison with a conventional liquid Si(OH)<sub>4</sub> preparation. Effective concentration for SiO<sub>2</sub>-NPs that immobilized 50% of the nematodes (EC50): 2 301 mg SiO<sub>2</sub> L<sup>-1</sup>. Compared to SiO<sub>2</sub>-NPs, Si(OH)<sub>4</sub> treatment ~36-fold more toxic with EC50 values of 63.7 mg SiO<sub>2</sub> L<sup>-1</sup>. For more discussion, refer to Supplementary Information section ‘*Ecotoxicity to Non-target Organisms*’.

**Supplementary Table S 1:** Sequences of primers for real-time PCR.  
S: sense primer. A: antisense primer.

<b>Primer</b>	<b>Sequence (5'-3')</b>
<i>oprF</i> (S) (Genebank 878442)	GTGTTTCATCACAAGCGGCAT
<i>oprF</i> (A) (Genebank 878442)	GGGAAGCACCTGGAGTCAAT
<i>HSP17.4CI</i> (S) (AT3G46230)	TCATGAGGAGGTTTCGGTTGC
<i>HSP17.4CI</i> (A) (AT3G46230)	TTAACCAGAGATATCAACGG
<i>PR-1</i> (S) (AT2G14610)	ACTACAACCTACGCTGCGAACAC
<i>PR-1</i> (A) (AT2G14610)	GTTACACCTCACTTTGGCACATC
<i>PR-5</i> (S) (AT1G75040)	GTGTTTCATCACAAGCGGCAT
<i>PR-5</i> (A) (AT1G75040)	GGGAAGCACCTGGAGTCAAT
<i>expG</i> (S) (AT4G26410)	GAGCTGAAGTGGCTTCCATGAC
<i>expG</i> (A) (AT4G26410)	GGTCCGACATACCCATGATCC

**Supplementary Table S 2: Fitting parameters of systemic acquired resistance dose-response curve.** Modelled SiO<sub>2</sub>-NPs, or Si(OH)<sub>4</sub> effective concentrations (*ECX*) causing *X* = 20, 50, and 80% bacterial inhibition 3 d after inoculation of *Arabidopsis thaliana* (Col-1) with *Pseudomonas syringae*. A standard log-logistic dose-response model was numerically fitted (Levenberg-Marquardt iteration algorithm, Origin 2016, build 9.3.2.903, OriginLab Corporation, MA USA). The data of the Si(OH)<sub>4</sub> is given for qualitative comparison purposes only because concentrations below 5 mg L<sup>-1</sup> were not investigated, the fit for this curve was not sufficiently accurate to provide quantitative *ECX* values.

	EC20 (mM)			EC50 (mM)			EC80 (mM)			<i>p</i> (-)			<i>N</i>	<i>R</i> <sup>2</sup>
	av	±	stdev	av	±	stdev	av	±	stdev	av	±	stdev		
SiO <sub>2</sub> -NPs	0.19	±	0.04	0.40	±	0.04	0.83	±	0.11	1.9	±	0.4	15	0.961
Si(OH) <sub>4</sub>	8.6E-05	±	7E-04	0.001	±	0.007	0.02	±	0.05	0.5	±	0.6	15	0.977

### ***Details on Si(OH)<sub>4</sub> Analytics***

Measuring the exact amount of free Si(OH)<sub>4</sub>, dissolved Si(OH)<sub>4</sub> and Si oligomers, and solid SiO<sub>2</sub> species directly *in plantae* is challenging<sup>1-3</sup>. The former polymerizes rapidly to the latter upon small changes of pH, concentration, temperature, ionic strength, and in the presence of certain ligands such as quaternary ammonium ions or silaffins<sup>4</sup>. For example, we observed flocculation (*i.e.* polymerization to SiO<sub>2</sub> species) of Si(OH)<sub>4</sub> when adding Si(OH)<sub>4</sub> stock solution to slightly acidic solutions. Current analytical techniques that can quantify Si under relatively mild conditions in complex plant-based matrices<sup>2</sup> are still too harsh to preserve the speciation of Si in the sample. The few mg kg<sup>-1</sup> Si(OH)<sub>4</sub> expected to be released from the SiO<sub>2</sub>-NPs will thus be indistinguishable using such methods from both the variable natural plant Si content of *Arabidopsis* (in leaves ~72–168 mg Si kg<sup>-1</sup><sup>5</sup>, equivalent to ~154-360 mg SiO<sub>2</sub> kg<sup>-1</sup>, or ~246-576 mg Si(OH)<sub>4</sub> kg<sup>-1</sup>), as well as from Si by intact SiO<sub>2</sub>-NPs dissolved during the sample preparation. Alternative *in situ* measurements of total free Si(OH)<sub>4</sub> have been achieved in xylem exudate of some Si hyperaccumulator plants (wheat shoots) using <sup>29</sup>Si nuclear magnetic resonance spectroscopy (NMR)<sup>1</sup>. However, in *Arabidopsis*, reliable measurements of Si(OH)<sub>4</sub> have not yet been achieved due to the relatively high detection limits of NMR for <sup>29</sup>Si<sup>1</sup> and low concentrations in this plant species. Due to the small size of *Arabidopsis*, the collection of sufficient xylem exudate even for a limited amount of treatments and repetitions of our experimental design would have required growing several thousand *Arabidopsis* plants. Finally, due to the high reactivity of Si(OH)<sub>4</sub>, it is unknown to which degree the destructive collection of the xylem exudate could trigger its polymerization to other dissolved Si species or SiO<sub>2</sub>.

### ***Si Reaction Byproducts***

Due to the rigorous washing of the SiO<sub>2</sub>-NPs by at least five dialysis steps, it can be excluded that Si-containing reaction byproducts were responsible for the observed effects in the plants, albeit a small contribution due to unavoidable traces of (re-)dissolved Si in the concentrated SiO<sub>2</sub>-NP stock suspension at the solubility limit of Si(OH)<sub>4</sub> of ~1.5 mM, or +30% more in case of supersaturation, remains a possibility. Verified by inductively coupled plasma – optical emission spectrometry (ICP-OES) measurements in the background solution of fridge-stored concentrated dialyzed SiO<sub>2</sub>-NP stock solutions (~12.3 mg dissolved SiO<sub>2</sub> species L<sup>-1</sup>, all concentrations given in SiO<sub>2</sub> L<sup>-1</sup> for the sake of comparability), we calculated a maximal possible background concentration of dissolved Si left in the test suspensions of ~4.2-8.2 mg SiO<sub>2</sub> L<sup>-1</sup> in the 1600 mg SiO<sub>2</sub> L<sup>-1</sup> nanoparticle treatments and 0.065-0.13 mg SiO<sub>2</sub> L<sup>-1</sup> in the lowest treatment at a concentration of 25 mg SiO<sub>2</sub> L<sup>-1</sup>. As discussed in the main text and apparent from **Fig. 4**, Si(OH)<sub>4</sub> concentrations of 5 mg SiO<sub>2</sub> L<sup>-1</sup> are not sufficient to cause a full protective effect anymore.

### ***Details on DNA Extraction***

Plant leaf samples were ground using a ceramic mortar and pestle. Total DNA was extracted with a Plant DNA mini Kit (peqlab, VWR, Germany). According to the kit instructions, 400  $\mu\text{L}$  of lysis buffer (PL1) and 15  $\mu\text{L}$  RNase A (20 mg/mL) were added to each sample tube. After 10 s of vortexing, the tubes were incubated at 65°C in a thermo-shaker for 30 min. Then, 100  $\mu\text{L}$  of lysis buffer (PL2) were added to each tube and vortexed for 5 s. After that, the tubes were incubated on ice for 5 min. and centrifuged at 10 000  $\times g$  for 5 min. The supernatant was transferred to a new 2.0 mL collection tube containing a microfilter. The collection tubes were centrifuged for 1 min. at 10 000  $\times g$ . Next, DNA binding buffer (0.5 volumes) was mixed with the filtrate, the mixture was transferred to a 2.0 mL collection tube containing a DNA binding column, and centrifuged at 10 000  $\times g$  for 1 min. Subsequently, the DNA binding columns were placed into new tubes and washed twice with 650  $\mu\text{L}$  of the DNA wash buffer. Next, the tubes were centrifuged for 2 min. at 10 000  $\times g$  to dry. After drying, 100  $\mu\text{L}$  of elution buffer were added to the DNA binding column, which was placed into a new 1.5 mL centrifuge tube. Last, the tubes were centrifuged at 6 000  $\times g$  for 1 min. to elute the DNA.

### ***Details on RNA Extraction and cDNA Synthesis***

Plant leaf samples (10 leaf discs taken from different infiltrated plant leaves/sample) were flash-frozen in liquid nitrogen. The frozen tissues were ground in 1.5 mL tubes (Eppendorf, Hamburg, Germany) containing three 3 mm glass beads using a mixer mill (Retsch® MM400, Retsch Technology GmbH, Haan, Germany) at 30 Hz for 3 min. The total RNA of the plant leaf samples was extracted using the Spectrum Plant Total RNA Kit (Sigma Life Science, USA). Following the kit instructions, the ground tissues in each tube were homogenized with 500  $\mu\text{L}$  lysis buffer and 5  $\mu\text{L}$  2-mercaptoethanol. Then, all tubes were vortexed for 30 s, incubated at 56 °C for 5 min., centrifuged for 3 min., and the supernatant was transferred into a filtration column seated in a new 2 mL tube, which was centrifuged for 1 min. Next, 250  $\mu\text{L}$  of binding solution were added to the filtrate, mixed thoroughly, added to a binding column in a new 2 mL collection tube, and the tubes were centrifuged for 1 min. Afterwards, the binding column was washed twice with 500  $\mu\text{L}$  and 300  $\mu\text{L}$  wash solution No. 1. Subsequently, 80  $\mu\text{L}$  of a mixture containing 70  $\mu\text{L}$  DNase digestion buffer (Catalog No. D1566) and 10  $\mu\text{L}$  DNase 1 (Catalog No. D2816), were added to each binding column and incubated for 15 min. at room temperature. Then, the column was washed 3 $\times$  with 500  $\mu\text{L}$  wash solution No. 2 and centrifuged for 1 min. after the first wash and 30 s after the next two washes. Next, the column was centrifuged for 1 min. to dry. Afterwards, 50  $\mu\text{L}$  elution buffer were added to the column, which was transferred to a new collection tube. The tubes were left at room temperature for 5 min and then centrifuged for 1 min. at 10 000  $\times g$  to elute the RNA. The RNA concentration was determined with the Thermo Scientific NanoDrop™ 1000 Spectrophotometer (Thermo Fisher Scientific, USA) using 1  $\mu\text{L}$  of each sample. One microgram of total RNA was used for cDNA synthesis using an Omniscript Reverse Transcription Kit (No. 205113, Qiagen, Germany). The template RNA (1  $\mu\text{g}$ ) was added to a tube containing the master mix in a total reaction volume of 20  $\mu\text{L}$ . The master mix components were 2  $\mu\text{L}$  10 $\times$  reverse transcriptase (RT) buffer, 2  $\mu\text{L}$  deoxyribonucleotide triphosphate mix (5 mM each deoxyribonucleotide triphosphate), 2  $\mu\text{L}$  oligo deoxythymidine (dT) primer (10  $\mu\text{M}$ ), 1  $\mu\text{L}$  Omniscript reverse transcriptase, and a variable amount of RNase-free water. The reaction mixture was incubated at 37 °C for 1 h. The samples were stored at -20°C.



### ***Ecotoxicity to Non-target Organisms***

To compare the effects of the SiO<sub>2</sub>-NPs and Si(OH)<sub>4</sub> on a model soil microorganism in case of future agricultural application, we performed an ecotoxicity assay on *Caenorhabditis elegans* nematode larvae (**Supplementary Fig. S3**). The effective concentration for SiO<sub>2</sub>-NPs that immobilized 50% of the nematodes (EC50) was 2 301 mg SiO<sub>2</sub> L<sup>-1</sup>. This corresponds to concentrations more than 95-fold above the EC50 that induced defence in *Arabidopsis* (**Fig. 5a**). Compared to the nanoparticles, the Si(OH)<sub>4</sub> treatment was ~36-fold more toxic with EC50 values of 63.7 mg SiO<sub>2</sub> L<sup>-1</sup> (**Supplementary Fig. S3**). A dose of 125 mg SiO<sub>2</sub> L<sup>-1</sup> Si(OH)<sub>4</sub> immobilized 92±% of the nematode larvae. These results suggest that SiO<sub>2</sub>-NPs have little to no adverse effects on the tested non-target organism under investigation in the concentration range that was beneficial for plants, and are less toxic to nematodes compared to conventionally used dissolved Si(OH)<sub>4</sub>.

### ***References***

- 1 Casey, W., Kinrade, S., Knight, C., Rains, D. & Epstein, E. Aqueous silicate complexes in wheat, *Triticum aestivum* L. *Plant, Cell Environ.* **27**, 51-54 (2004).
- 2 Bossert, D. *et al.* A hydrofluoric acid-free method to dissolve and quantify silica nanoparticles in aqueous and solid matrices. *Sci. Rep.* **9**, 1-12 (2019).
- 3 Choppin, G. R., Pathak, P. & Thakur, P. Polymerization and complexation behavior of silicic acid: A review. *Main Group Met. Chem.* **31**, 53 (2008).
- 4 Sumper, M. & Kröger, N. Silica formation in diatoms: the function of long-chain polyamines and silaffins. *J. Mater. Chem.* **14**, 2059-2065 (2004).
- 5 Slomberg, D. L. & Schoenfisch, M. H. Silica nanoparticle phytotoxicity to *Arabidopsis thaliana*. *Environ. Sci. Technol.* **46**, 10247-10254 (2012).