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

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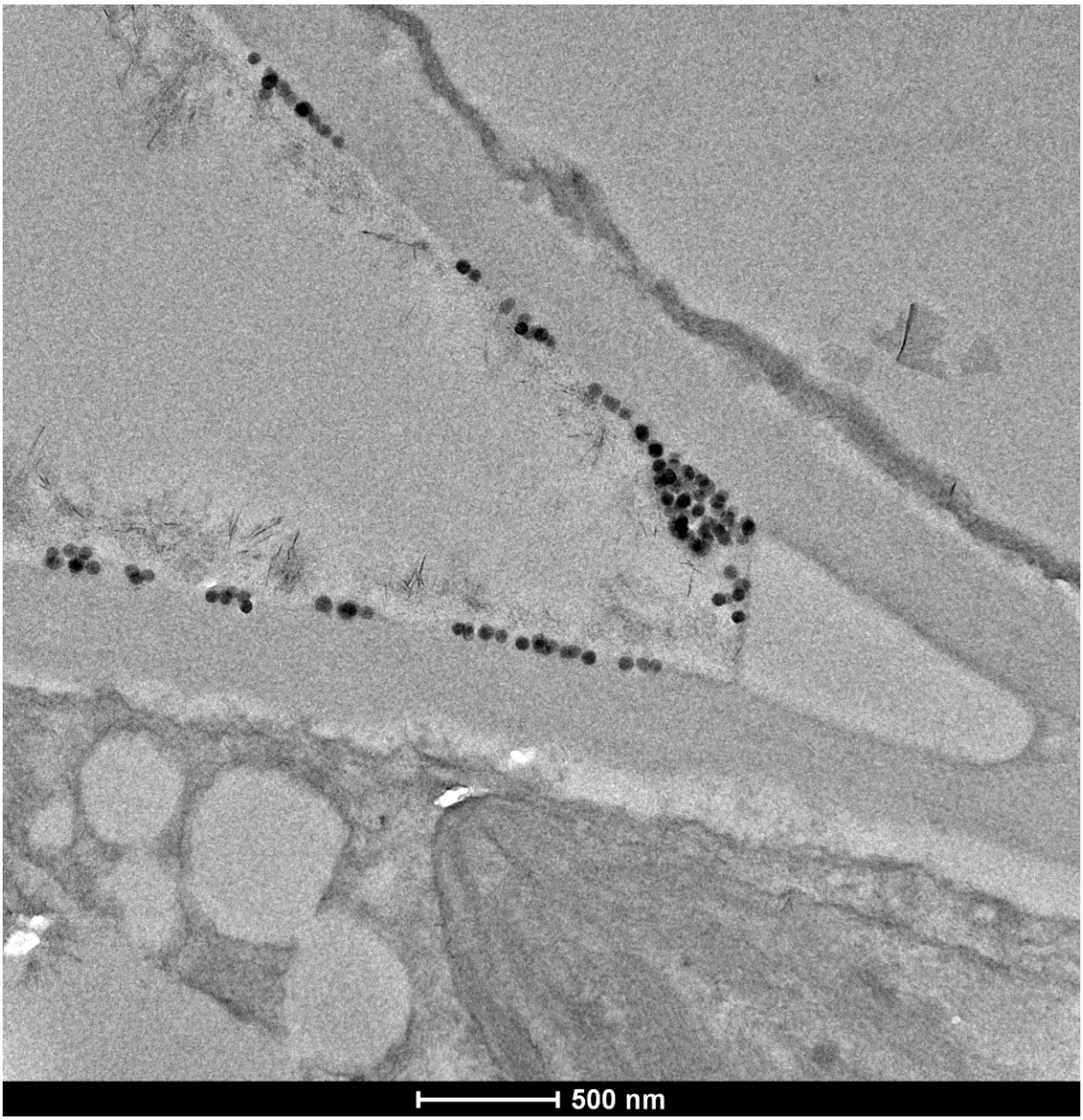
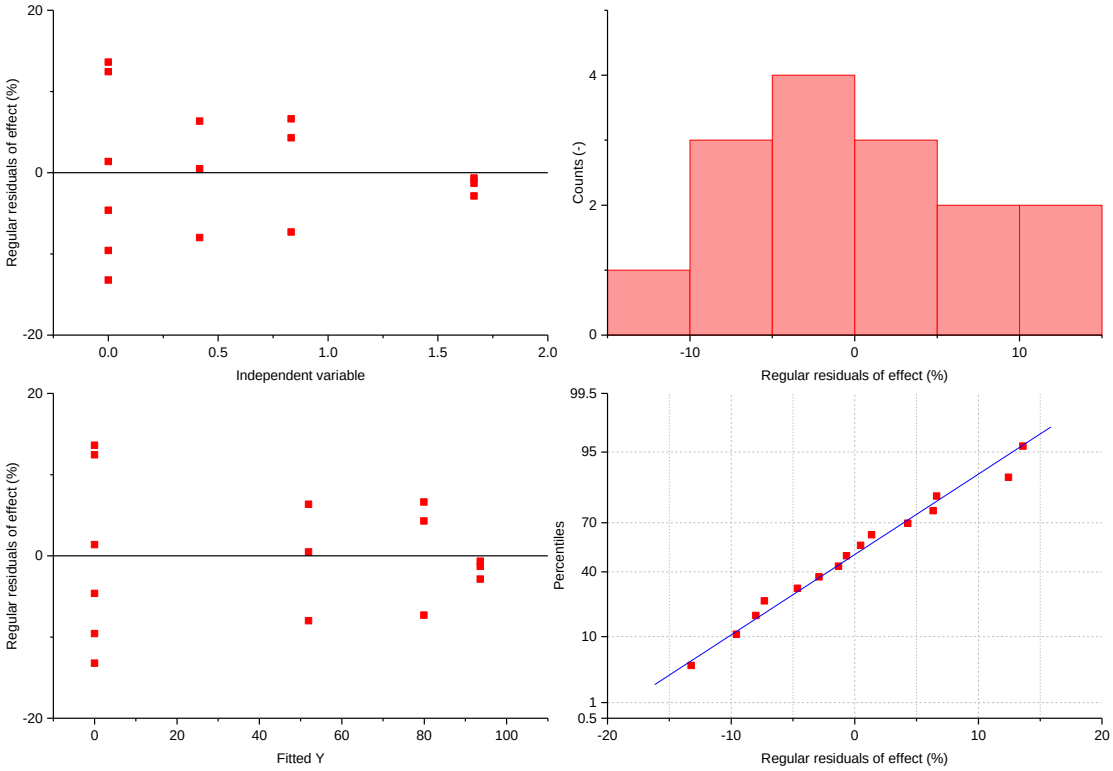
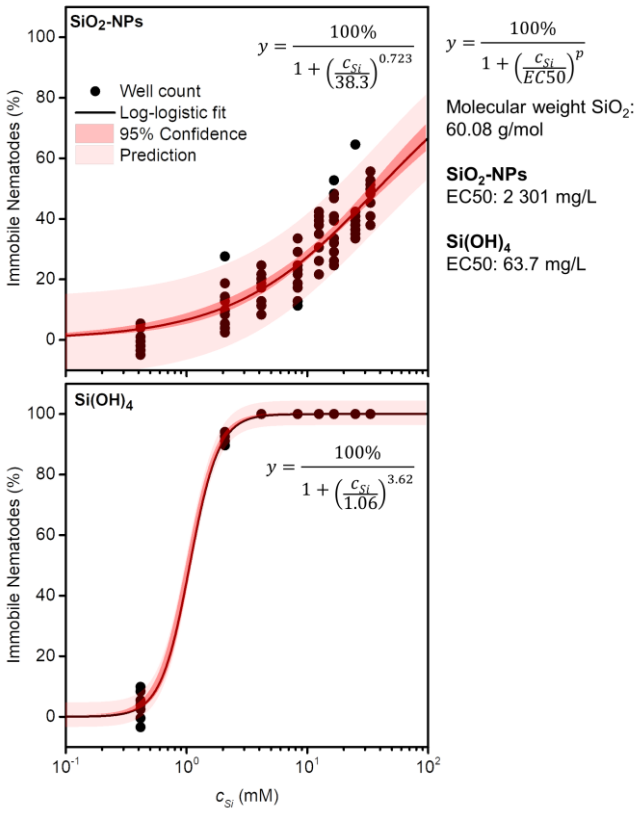
Supplementary Fig. S1, **Supplementary Fig. S2**, **Supplementary Fig. S3, Supplementary Tab. S1**, **Supplementary Tab. S2**, sections ***Details on $Si(OH)_4$ Analytics***, ***Si Reaction Byproducts***, ***Details on DNA Extraction***, ***Details on RNA Extraction and cDNA Synthesis,*** ***Ecotoxicity 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^{-1} silica nanoparticles (SiO_2-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^{-1} of SiO_2-NPs. Fitting parameters listed in **Supplementary Tab. S2**.



Supplementary Figure S 3: Ecotoxicity of silica nanoparticles (SiO_2-NPs) and orthosilicic acid ($Si(OH)_4$) to the nematode *Caenorhabditis elegans*. The nematode served as a model soil microorganism for an initial assessment of the ecotoxicity of the SiO_2-NPs and comparison with a conventional liquid $Si(OH)_4$ preparation. Effective concentration for SiO_2-NPs that immobilized 50% of the nematodes (EC50): 2 301 mg SiO_2 L^{-1}. Compared to SiO_2-NPs, $Si(OH)_4$ treatment ~36-fold more toxic with EC50 values of 63.7 mg SiO_2 L^{-1}. 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.

Primer	**Sequence (5'-3')**
oprF (S) (Genebank 878442)	GTGTTCATCACAAGCGGCAT
oprF (A) (Genebank 878442)	GGGAAGCACCTGGAGTCAAT
HSP17.4CI (S) (AT3G46230)	TCATGAGGAGGTTTCGGTTGC
HSP17.4CI (A) (AT3G46230)	TTAACCAGAGATATCAACGG
PR-1 (S) (AT2G14610)	ACTACAACTACGCTGCGAACAC
PR-1 (A) (AT2G14610)	GTTACACCTCACTTTGGCACATC
PR-5 (S) (AT1G75040)	GTGTTCATCACAAGCGGCAT
PR-5 (A) (AT1G75040)	GGGAAGCACCTGGAGTCAAT
expG (S) (AT4G26410)	GAGCTGAAGTGGCTTCCATGAC
expG (A) (AT4G26410)	GGTCCGACATACCCATGATCC

Supplementary Table S 2: Fitting parameters of systemic acquired resistance dose-response curve. Modelled SiO_2-NPs, or $Si(OH)_4$ 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 $Si(OH)_4$ is given for qualitative comparison purposes only because concentrations below 5 mg L^{-1} were not investigated, the fit for this curve was not sufficiently accurate to provide quantitative *ECX* values.

	EC20 (mM)			EC50 (mM)			EC80 (mM)			*p*(-)			*N*	R^2
	av	±	stdev	av	±	stdev	av	±	stdev	av	±	stdev		
SiO_2-NPs	0.19	±	0.04	0.40	±	0.04	0.83	±	0.11	1.9	±	0.4	15	0.961
$Si(OH)_4$	8.6E-05	±	7E-04	0.001	±	0.007	0.02	±	0.05	0.5	±	0.6	15	0.977

Details on $Si(OH)_4$ Analytics

Measuring the exact amount of free $Si(OH)_4$, dissolved $Si(OH)_4$ and Si oligomers, and solid SiO_2 species directly *in plantae* is challenging[1-3]. 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[4]. For example, we observed flocculation (*i.e.* polymerization to SiO_2 species) of $Si(OH)_4$ when adding $Si(OH)_4$ stock solution to slightly acidic solutions. Current analytical techniques that can quantify Si under relatively mild conditions in complex plant-based matrices[2] are still too harsh to preserve the speciation of Si in the sample. The few mg kg^{-1} $Si(OH)_4$ expected to be released from the SiO_2-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^{-1} [5], equivalent to ~154-360 mg SiO_2 kg^{-1}, or ~246-576 mg $Si(OH)_4$ kg^{-1}), as well as from Si by intact SiO_2-NPs dissolved during the sample preparation. Alternative *in situ* measurements of total free $Si(OH)_4$ have been achieved in xylem exudate of some Si hyperaccumulator plants (wheat shoots) using ^{29}Si nuclear magnetic resonance spectroscopy (NMR)[1]. However, in *Arabidopsis*, reliable measurements of $Si(OH)_4$ have not yet been achieved due to the relatively high detection limits of NMR for ^{29}Si[1] 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 $Si(OH)_4$, it is unknown to which degree the destructive collection of the xylem exudate could trigger its polymerization to other dissolved Si species or SiO_2.

Si Reaction Byproducts

Due to the rigorous washing of the SiO_2-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_2-NP stock suspension at the solubility limit of $Si(OH)_4$ 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_2-NP stock solutions (~12.3 mg dissolved SiO_2 species L^{-1}, all concentrations given in SiO_2 L^{-1} 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_2 L^{-1} in the 1600 mg SiO_2 L^{-1} nanoparticle treatments and 0.065-0.13 mg SiO_2 L^{-1} in the lowest treatment at a concentration of 25 mg SiO_2 L^{-1}. As discussed in the main text and apparent from **Fig. 4**, $Si(OH)_4$ concentrations of 5 mg SiO_2 L^{-1} 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 µL of lysis buffer (PL1) and 15 µL 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 µL 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 ×*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 ×*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 ×*g* for 1 min. Subsequently, the DNA binding columns were placed into new tubes and washed twice with 650 µL of the DNA wash buffer. Next, the tubes were centrifuged for 2 min. at 10 000 ×*g* to dry. After drying, 100 µL 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 ×*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 µL lysis buffer and 5 µL 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 µL 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 µL and 300 µL wash solution No. 1. Subsequently, 80 µL of a mixture containing 70 µL DNase digestion buffer (Catalog No. D1566) and 10 µl 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× with 500 µL 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 µL 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 ×*g* to elute the RNA. The RNA concentration was determined with the Thermo Scientific NanoDropTM 1000 Spectrophotometer (Thermo Fisher Scientific, USA) using 1 µL 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 µg) was added to a tube containing the master mix in a total reaction volume of 20 µL. The master mix components were 2 µL 10× reverse transcriptase (RT) buffer, 2 µL deoxyribonucleotide triphosphate mix (5 mM each deoxyribonucleotide triphosphate), 2 µL oligo deoxythymidine (dT) primer (10 µM), 1 µL 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_2-NPs and $Si(OH)_4$ 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_2-NPs that immobilized 50% of the nematodes (EC50) was 2 301 mg SiO_2 L^{-1}. This corresponds to concentrations more than 95-fold above the EC50 that induced defence in *Arabidopsis* (**Fig. 5a**). Compared to the nanoparticles, the $Si(OH)_4$ treatment was ~36-fold more toxic with EC50 values of 63.7 mg SiO_2 L^{-1} (**Supplementary Fig. S3**). A dose of 125 mg SiO_2 L^{-1} $Si(OH)_4$ immobilized 92±% of the nematode larvae. These results suggest that SiO_2-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 $Si(OH)_4$.

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