Silica Nanoparticle Phytotoxicity to *Arabidopsis thaliana*

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Supporting Information

Experimental S1.

Synthesis and Preparation of Silica Nanoparticles. Organosilicasol MT-ST particles (14 nm) were dried under vacuum before use. Tetraethylorthosilicate SiNPs (50 and 200 nm) were synthesized by the Stöber method following conditions reported by Bogush et al.[1] Briefly, 50 nm SiNPs were made by adding TEOS (3.792 mL) to a stirred solution of EtOH (91.378 mL), ammonium hydroxide (3.378 mL), and water (1.452 mL). After 5 h, the particles were collected by adding hexane to the solution in a 2:1 ratio (v/v) and centrifugation (3645*g* for 5 min). The particles were resuspended in EtOH via sonication and collected again by centrifugation (3645*g* for 5 min). This washing procedure was repeated and the particles were then dried under vacuum. The 200 nm SiNPs were made by adding TEOS (3.792 mL) to a stirred solution of EtOH (88.351 mL), ammonium hydroxide (6.757 mL), and water (1.101 mL). These particles were collected, washed, and dried after 2 h using the aforementioned procedure. For select studies, the silica particles were calcined at 1000 °C for 24 h in a muffle furnace.

Reference:

1. Bogush, G. H.; Tracy, M. A.; Zukoski IV, C. F., Preparation of monodisperse silica particles: Control of size and mass fraction. *J. Non-Cryst. Solids* 1988, *104*, 95-106.

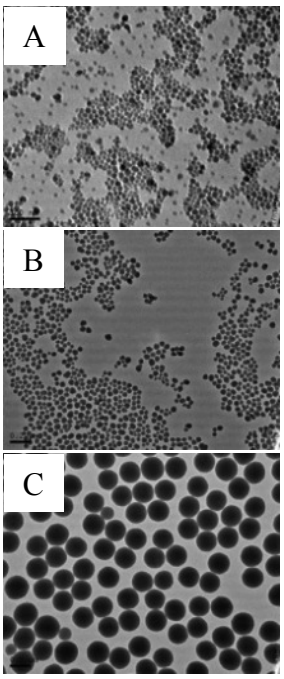

Figure S1. Transmission electron microscopy images of SiNPs before addition to plant nutrient solution: (A) 14 nm; (B) 50 nm; and (C) 200 nm.

Table S1. Carbon and hydrogen weight percents for unmodified and calcined SiNPs as determined by CHN analysis.

	14 nm		**50 nm**		**200 nm**	
	Unmodified	Calcined	Unmodified	Calcined	Unmodified	Calcined
% C	1.18	-0.03	3.46	-0.03	3.08	-0.05
% H	0.40	-0.05	1.28	-0.05	1.45	-0.06

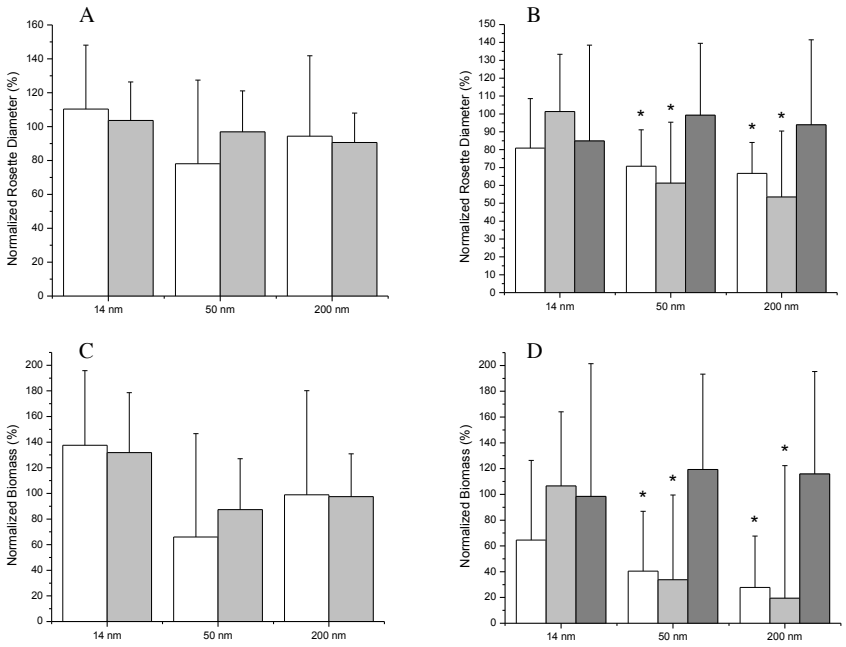

Figure S2. Growth data for plants harvested at 3 weeks with (A, C) pH 5.8 and (B, D) pH unadjusted after exposure to 250 ppm (white), 1000 ppm (light gray), or calcined (dark gray) SiNPs. Values are normalized to plants grown in blank solution. *Significant difference at 95% relative to blank. Of note, stems were not developed by 3 week harvest for measurement.

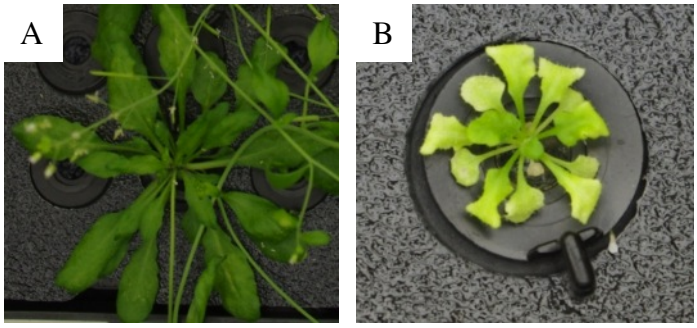

Figure S3. Growth after 6 weeks in (A) blank nutrient solution with no pH adjustment and (B) after exposure to 250 ppm of 200 nm SiNPs with no pH adjustment. Seed holder diameter is 2 cm.

Table S2. Variation in pH for exposure groups where pH was not adjusted over 6 weeks. Blank exposure groups were grown in a solution of Hoagland's #2 Basal Salt Mixture (400 mg L^{-1}).

	Initial	1 Week	3 Weeks	6 Weeks
		250 ppm		
Blank	5.24	5.27	5.38	7.11
14 nm	5.67	5.51	5.26	7.4
50 nm	6.99	6.76	6.70	5.64
200 nm	7.26	7.12	6.98	6.6
		1000 ppm		
Blank	5.24	4.88	5.34	6.49
14 nm	5.98	4.91	5.40	7.05
50 nm	8.31	7.30	7.48	7.27
200 nm	8.59	7.89	7.75	7.25
		Calcined		
Blank	5.39	5.68	5.52	6.81
14 nm	6.78	6.91	6.98	7.15
50 nm	4.69	4.72	6.23	7.03
200 nm	5.19	4.41	5.32	6.38

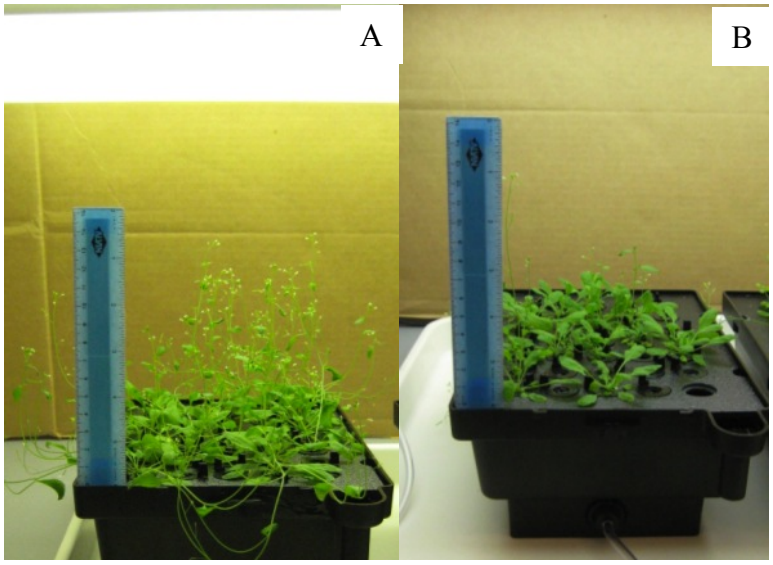

Figure S4. Plants grown for 6 weeks in (A) pH 5.8 nutrient solution and (B) nutrient solution adjusted to pH 8. Scale bar is 15 cm.

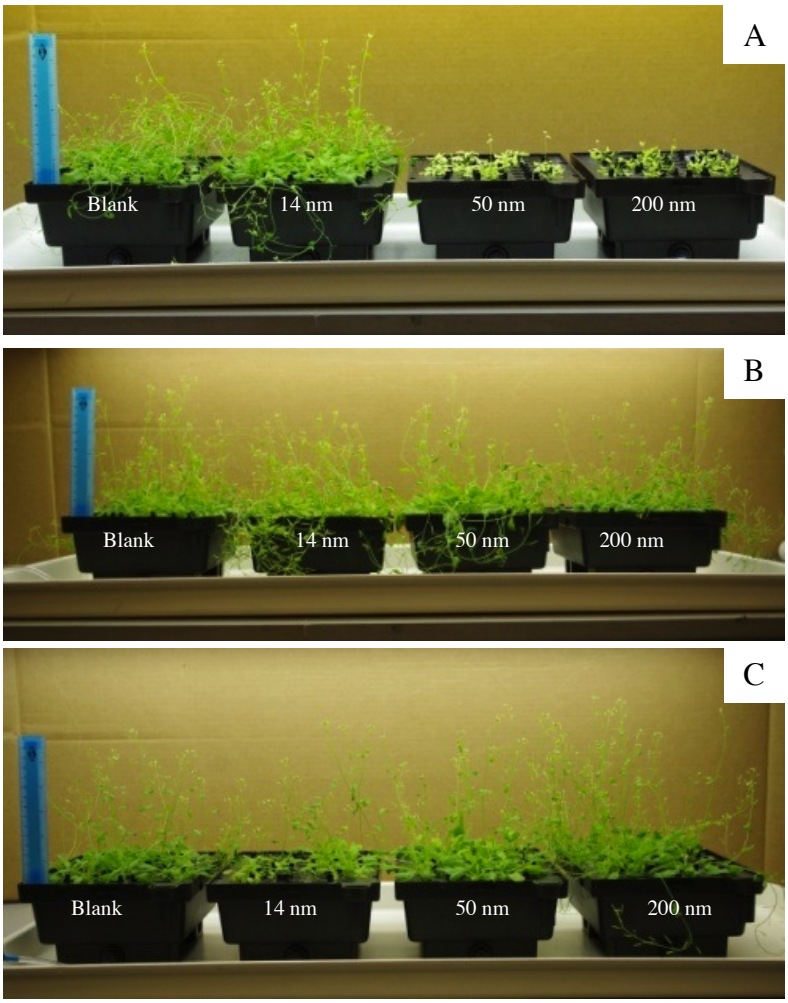

Figure S5. Growth of plants exposed to 1000 ppm SiNPs after 6 weeks showing development with (A) no pH adjustment; (B) pH 5.8; and (C) pH unadjusted with calcined SiNPs. Scale bar is 15 cm.

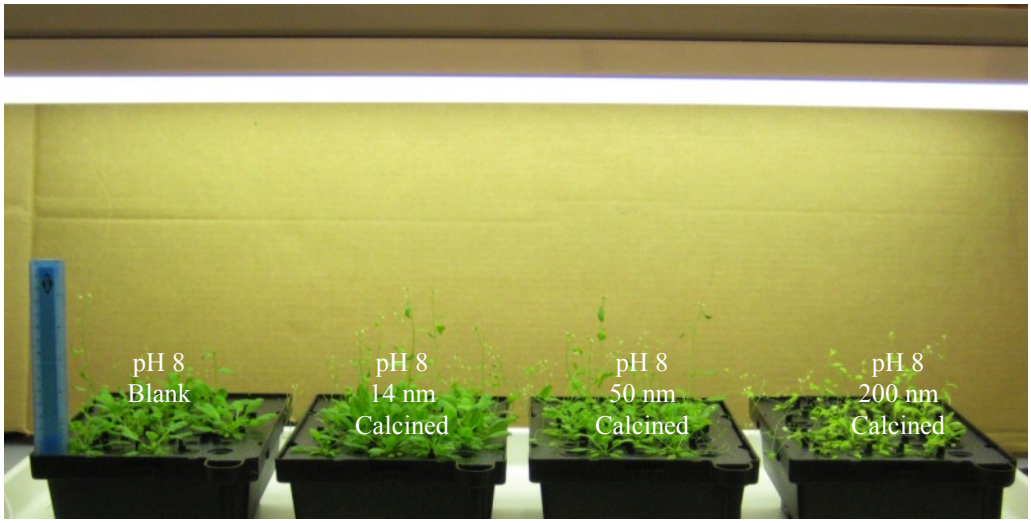

Figure S6. Growth of plants after 6 week exposure to 1000 ppm calcined SiNPs with growth medium maintained at pH 8. Scale bar is 15 cm.

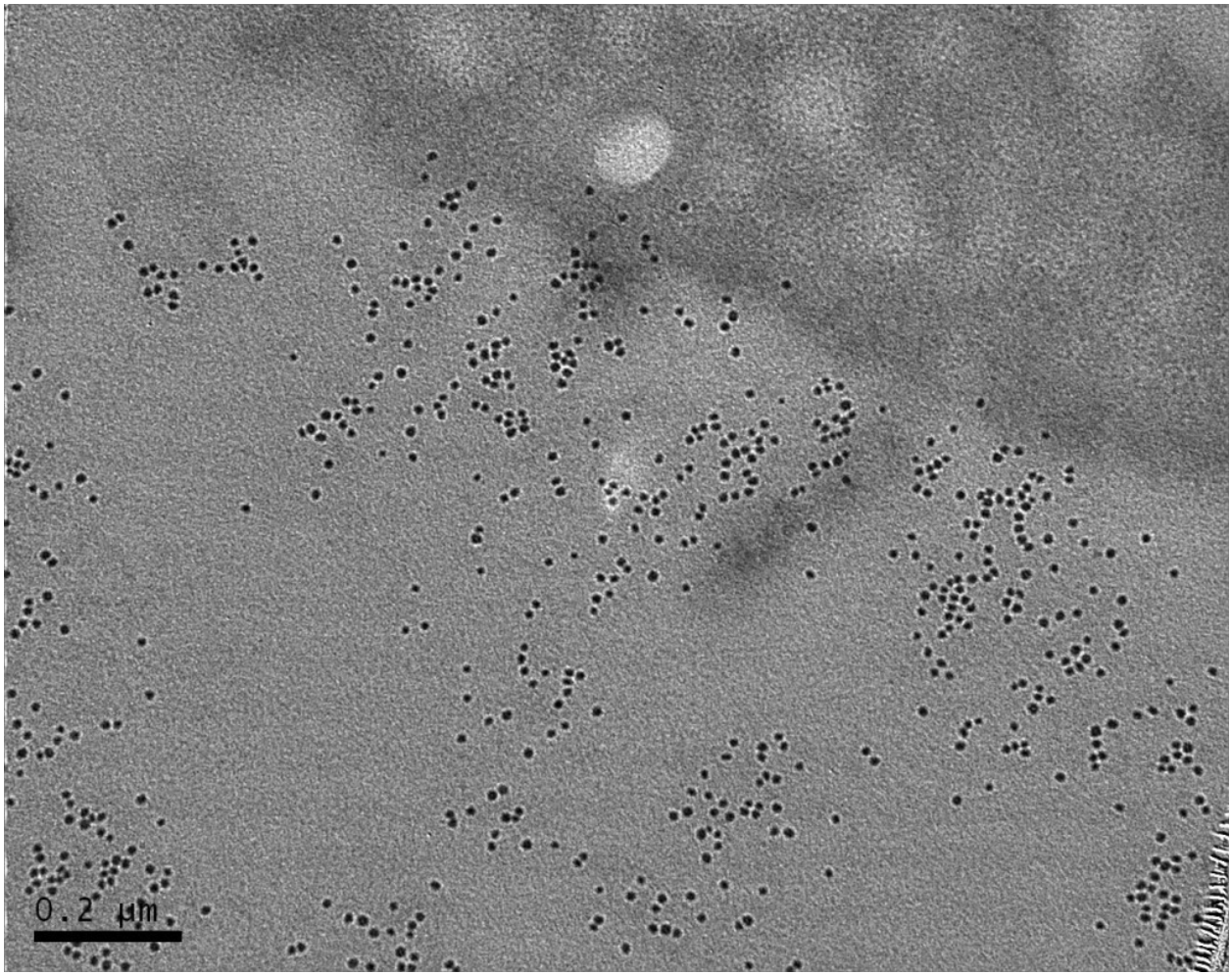

Figure S7. Transmission electron microscopy image of roots from 1000 ppm exposure to 14 nm SiNPs after 6 weeks. Magnification is 72 kx and scale bar is 0.2 µm.

Table S3. Si determination in roots, rosette, and stem for 250 ppm exposure group after 6 weeks using ICP-OES. Results are normalized for the nanoparticle volume.

	14 nm		**50 nm**		**200 nm**	
Sample	pH 5.8	pH unadjusted	pH 5.8	pH unadjusted	pH 5.8	pH unadjusted
Roots (mg Si·kg tissue^{-1}/nm^{3})	25.9 ± 0.6	28.0 ± 0.2	$9.90 \times 10^{-1} \pm 0.002$	$3.70 \times 10^{-2} \pm 7.0 \times 10^{-4}$	$1.20 \times 10^{-2} \pm 1.0 \times 10^{-4}$	$1.40 \times 10^{-2} \pm 1.0 \times 10^{-4}$
Rosette (mg Si·kg tissue^{-1}/nm^{3})	$5.20 \times 10^{-1} \pm 0.01$	$2.4 \times 10^{-1} \pm 0.003$	$1.00 \times 10^{-2} \pm 1.0 \times 10^{-4}$	$2.00 \times 10^{-2} \pm 2.0 \times 10^{-4}$	$3.00 \times 10^{-4} \pm 6.1 \times 10^{-7}$	$6.00 \times 10^{-4} \pm 7.0 \times 10^{-6}$
Stem (mg Si·kg tissue^{-1}/nm^{3})	$7.00 \times 10^{-2} \pm 0.002$	$0.07 \times 10^{-2} \pm 0.001$	$4.00 \times 10^{-3} \pm 3.0 \times 10^{-5}$	$4.00 \times 10^{-3} \pm 3.0 \times 10^{-5}$	$2.00 \times 10^{-5} \pm 4.0 \times 10^{-7}$	$4.00 \times 10^{-4} \pm 1.0 \times 10^{-5}$

Table S4. Si determination in roots, rosette, and stem for 1000 ppm exposure group after 6 weeks using ICP-OES. Results are normalized for the nanoparticle volume.

	14 nm		**50 nm**		**200 nm**	
Sample	pH 5.8	pH unadjusted	pH 5.8	pH unadjusted	pH 5.8	pH unadjusted
Roots (mg Si·kg tissue^{-1}/nm^{3})	32.0 ± 0.3	19.0 ± 0.1	1.85 ± 0.01	1.62 ± 0.003	$7.00 \times 10^{-3} \pm 5.0 \times 10^{-5}$	$1.40 \times 10^{-2} \pm 8.0 \times 10^{-5}$
Rosette (mg Si·kg tissue^{-1}/nm^{3})	$4.20 \times 10^{-1} \pm 0.01$	$7.80 \times 10^{-1} \pm 0.01$	$2.00 \times 10^{-2} \pm 5.0 \times 10^{-4}$	$2.00 \times 10^{-2} \pm 1.0 \times 10^{-4}$	$4.00 \times 10^{-4} \pm 4.0 \times 10^{-6}$	$7.00 \times 10^{-4} \pm 2.0 \times 10^{-5}$
Stem (mg Si·kg tissue^{-1}/nm^{3})	$1.60 \times 10^{-1} \pm 0.004$	$1.10 \times 10^{-1} \pm 5.0 \times 10^{-4}$	$5.00 \times 10^{-3} \pm 1.0 \times 10^{-5}$	$4.00 \times 10^{-2} \pm 7.0 \times 10^{-5}$	$8.00 \times 10^{-5} \pm 6.0 \times 10^{-7}$	$6.00 \times 10^{-4} \pm 1.0 \times 10^{-5}$

Equation S1. Equation for determining theoretical Si content from known SiNP concentration.

$$\text{Theoretical Si Content} = \text{Original SiNP Concentration} \times 0.467^{a} \times \%\,SiO_2{}^{b}$$

[a] Mass % Si in SiO_2
[b] Obtained by subtracting organic content determined with CHN