Supporting information

Phyto-Stimulation of Poplars and Arabidopsis Exposed to Silver Nanoparticles and Ag^+ at Sub-Lethal Concentrations

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Materials and methods

Arabidopsis growth and treatment on agar. Arabidopsis thaliana, ecotype Col-0, seeds were germinated on 0.8% agar (1.01614, EMD Millipore chemicals, Darmstadt, Germany) with ¼-strength Hoagland solution (pH 6.8).[1] All Arabidopsis plant growth was at 22ºC under 16-hour photoperiods, with 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For analysis of seedlings on solid media, germinated seedlings were gently transferred to fresh plates containing no additional supplements, AgNPs or $AgNO_3$, as indicated in text.

Dissolution test of AgNPs. 0.1 mg/L of 25-nm, 10-nm and 5-nm AgNPs solutions were prepared as the plant exposure medium and kept in the plant growth room. Each treatment was prepared in triplicate. Solution samples were taken on day 0, 1, 2, 5, and 11. To separate released Ag^+ from AgNPs, each sample was centrifuged in Amicon ultra centrifugal filter (2~5-nm pore size, molecular weight cutoff 10,000, Millipore, MA) at 7000 rpm for 7 min. The filtrate containing only Ag^+ was collected and analyzed with ICP-MS after HNO_3 (1%) dilution.

Chlorophyll measurements. Arabidopsis chlorophyll was extracted and quantified following the method described previously.[2] Leaf disks of one centimeter in diameter were incubated with 200

μL of 100% of methanol overnight at 4 ºC. After centrifugation at 14000 g for 5 minutes, 100 μL supernatant was transferred to 96-well plates (3590, Costar, Corning, NY). Absorbance at 652 and 665 nm was read using a Tecan Infinite M1000 plate reader (Tecan, Research Triangle Park, NC). Chlorophyll content was determined using the following equation: Chlorophyll (μg/mL) = $[22.12(A_{652}) + 2.71(A_{665})]$. Values were normalized to fresh weight.

Anthocyanin measurements. Arabidopsis anthocyanin was quantified using a method previously described [3] with the following modifications. Leaf tissue (20 mg to 100 mg) was ground in liquid nitrogen and extracted with TRI reagent (TR118, MRC Inc., Cincinnati, OH). Chlorophyll was eliminated by extracting with chloroform and the collected water phase was analyzed at A_{530} and A_{657}. Anthocyanin content was calculated by following equation. Anthocyanin = $[A_{530} - 0.25(A_{657})]$.

Supporting Figures and Results

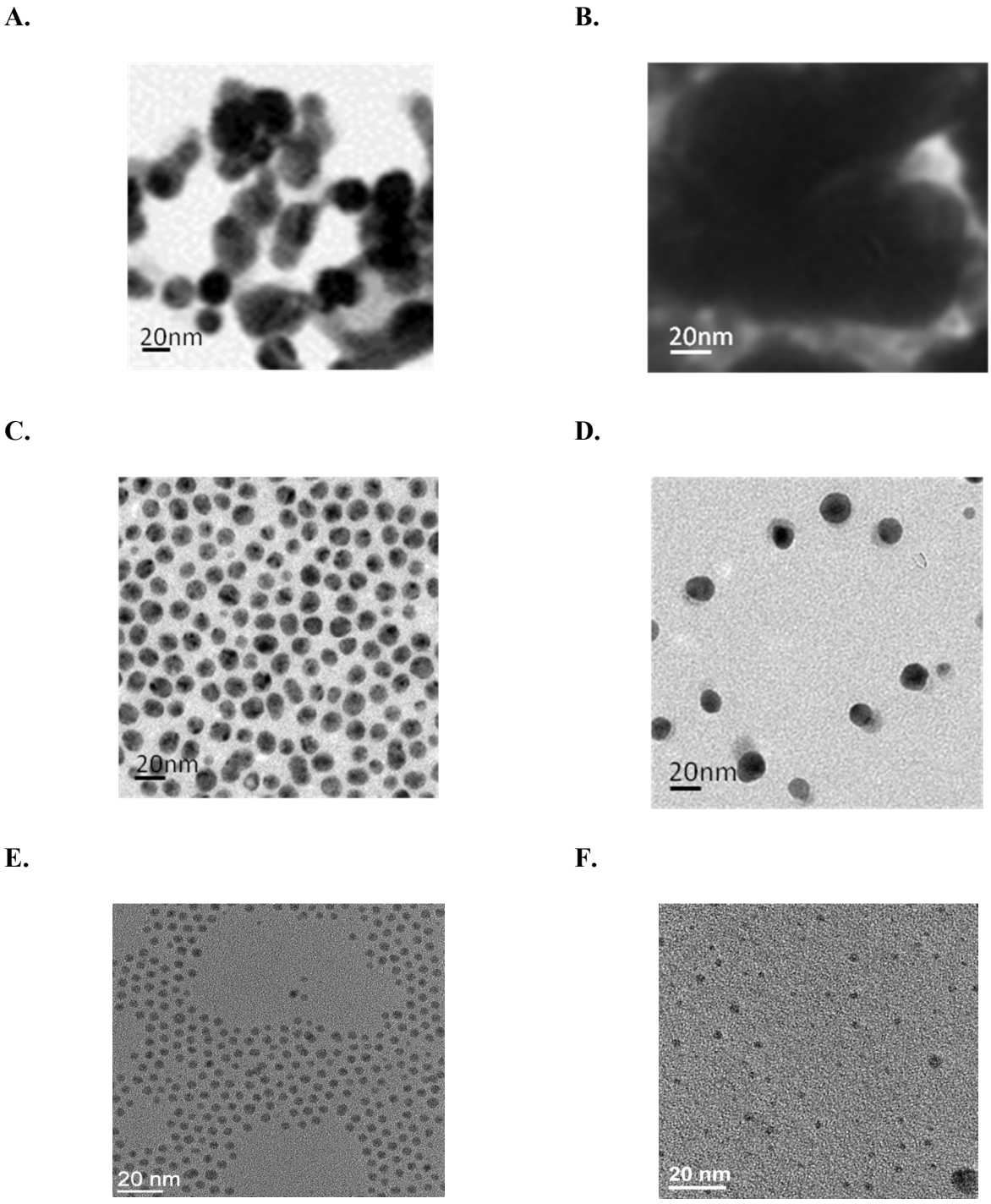

Figure S1. TEM image of 25-nm AgNPs before (A) and after (B) 15-day incubation in ¼-strength Hoagland solution, 10-nm AgNPs before (C) and after (D) 1-month incubation in ¼-strength Hoagland solution, and 5-nm AgNPs before (E) and after (F) 22-day incubation in ¼-strength Hoagland solution. Scale bar = 20 nm.

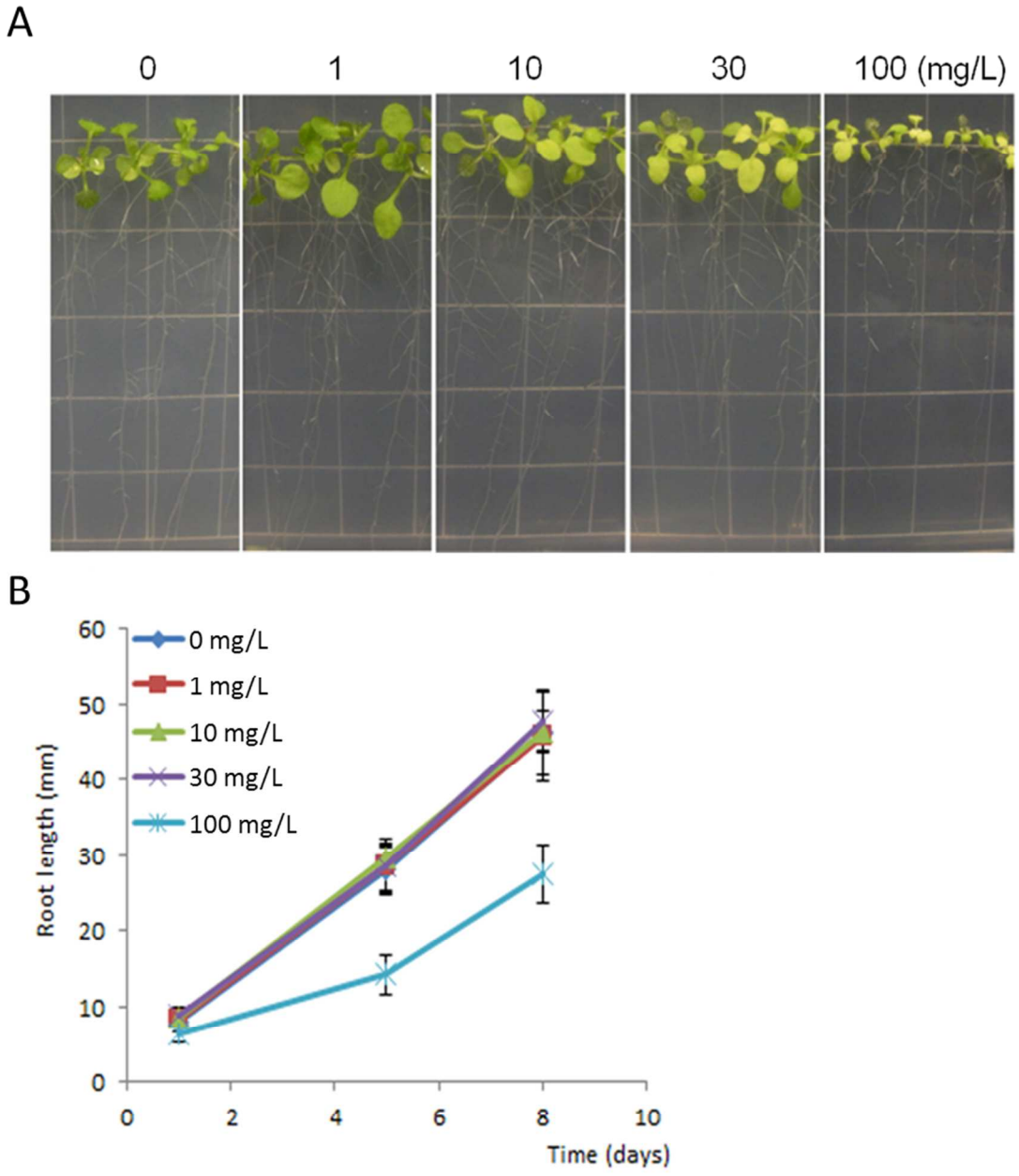

Figure S2. Effects of 25-nm AgNPs on *Arabidopsis*. Five-day-old *Arabidopsis* plants were transferred to medium harboring various concentrations of AgNPs. Representative *Arabidopsis* plants were photographed after 3 weeks exposure to AgNPs (A), and root length measured at 1st, 5th and 8th days after exposure (B). Error bars represent ± one standard deviation from the mean of 10 replicates.

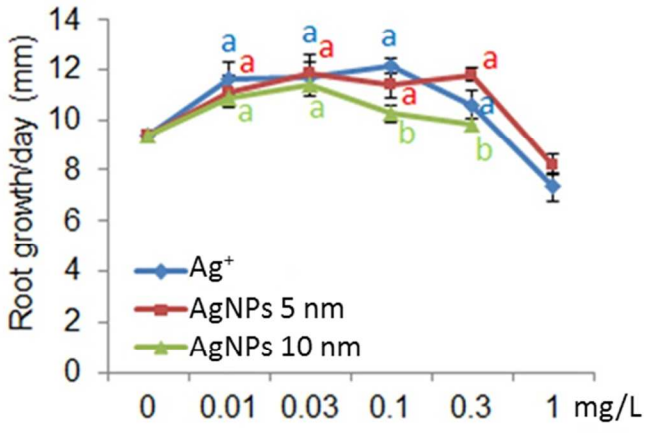

Figure S3. Effects on root growth of 5- or 10-nm AgNPs and Ag^{+} on 5-day-old *Arabidopsis* after 3 day exposure in agar medium. Letters denote statistically significant ($p < 0.05$) phytostimulation compared to untreated controls. Error bars represent ± one standard deviation from the mean 4 replicates.

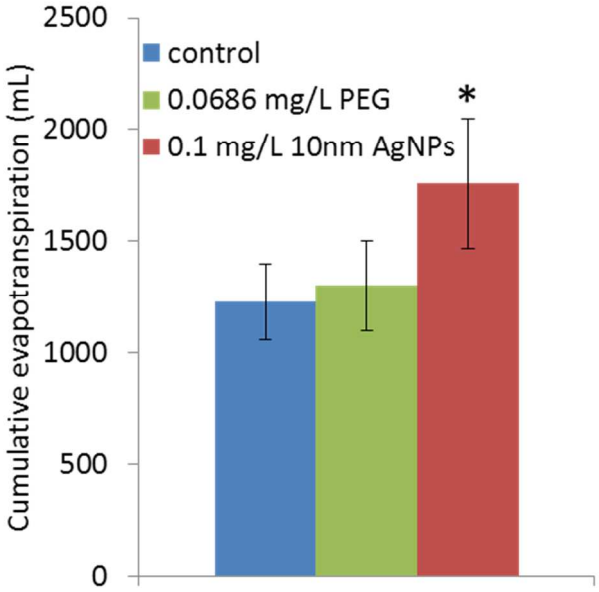

Figure S4. Effects of 0.0686 mg/L PEG coating and 0.1 mg/L 10 nm AgNPs coated with PEG on poplar cumulative evapotranspiration. The PEG coating concentration in the 0.1 mg/L 10-nm AgNPs was 0.0686 mg/L. Asterisks (*) shows significant differences (t-test, $p < 0.05$) from unexposed controls. The enhancing effect of 0.1 mg/L 10 nm AgNPs was due to the AgNPs, not the PEG coating.

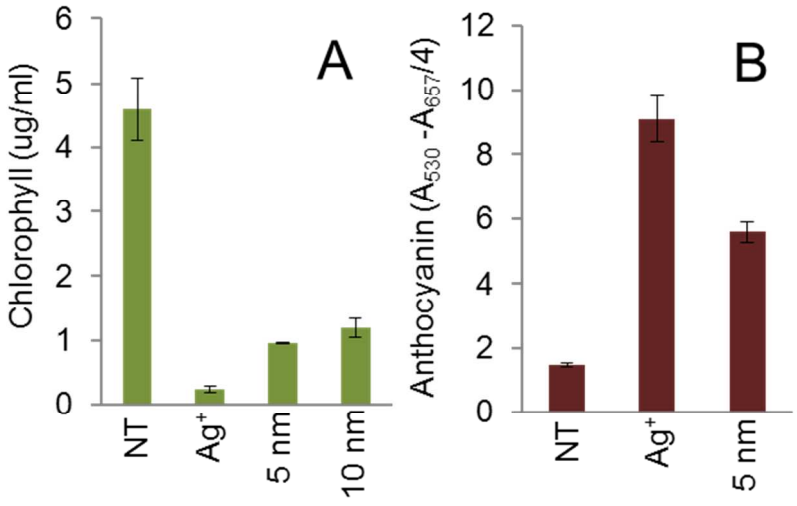

Figure S5. Effects of Ag^+ and AgNPs (5 and 10 nm) on 2-week-old *Arabidopsis* leaf chlorophyll (A) and anthocyanin (B) content after 1-week hydroponic exposure with Ag^+ or AgNPs. Error bars represent ± one standard deviation from the mean of 3 replicates.

Figure S6. Root color of hydroponically grown 4-week-old *Arabidopsis* were exposed to an additional week of hydroponic growth in the absence of treatment (NT) or in the presence of 1 mg/L 5-nm AgNPs (AgNP).

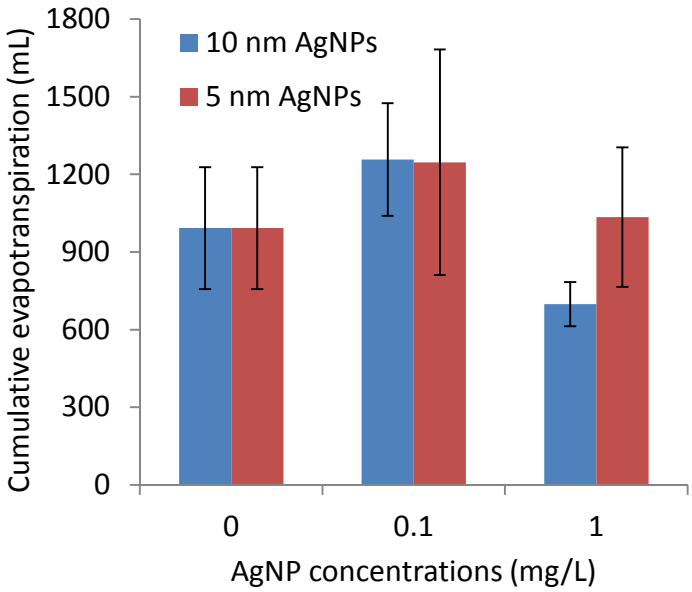

Figure S7. Cumulative evapotranspiration (7 day) of poplars exposed to 5- and 10-nm AgNPs at 0.1 and 1 mg/L. At both concentrations tested, the evapotranspiration of 5-nm AgNP treated poplars was similar ($p > 0.05$) to that of 10-nm AgNP treated ones.

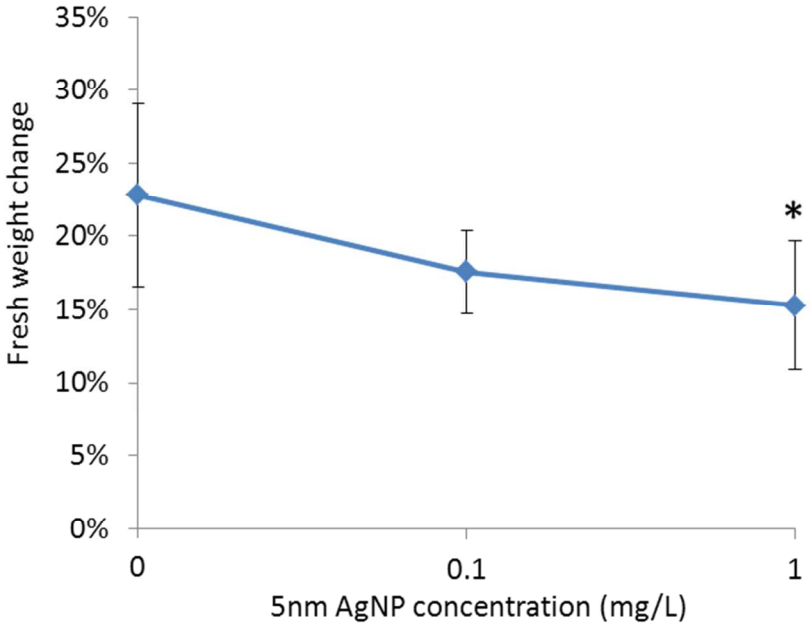

Figure S8. Fresh weight change percentage of poplar during the 7-day exposure to 5-nm AgNPs. Asterisk (*) shows statistically significant ($p < 0.05$, t-test) difference from the change at 0 mg/L. Error bars represent +/- one standard deviation from the mean of 4 replicates.

Tables

Table S1. Equilibrium speciation of Ag and their relative abundance in ¼-strength Hoagland solution modeled by Visual MINTEQ.

Medium	Total Ag concentration (mg/L)	Relative abundance of Ag species (%)					
		Ag^+	AgCl (aq)	$AgSO_4^-$	$AgNH_3^+$	$Ag(NH_3)^{2+}$	$AgNO_3$ (aq)
0.25 Hoagland solution	0.01	94.1	3.8	0.3	0.8	0.03	0.9
	0.1	94.1	3.8	0.3	0.8	0.03	0.9
	1	94.1	3.8	0.3	0.8	0.03	0.9

Table S2. Concentration (μg/L) of Ag^+ released from AgNPs after 11 day incubation in ¼-strength Hoagland solution without plants.

AgNP size	5nm	10 nm	25 nm
0.1ppm	6.5±0.9	1.6±0.6	0.5±0.1
1 ppm	121.1±13.9	17.1±2.3	11.0±0.8
10 ppm			189.0±26.4
100 ppm			738.8±27.2

Table S3. Mass of Ag assimilated by poplar leaves in the AgNP treatments compared with the potential Ag mass assimilated as Ag^+.

Ag mass (μg)	25 nm AgNPs		5 nm AgNPs	
	0.1 mg/L	1 mg/L	0.1 mg/L	1 mg/L
Total Ag assimilated by poplar leaves	1.4	6.4	4.8	2.0
Released Ag^+ in hydroponic solution	0.2	4.4	2.6	48.5
Potential Ag assimilated as Ag^+	0.1	2.5	1.0	17.6

(1) Total Ag mass assimilated by poplar leaves (μg) = Ag concentration in leaves (μg/g) * Total leaf dry weight (g),

Where, Total leaf dry weight (g) = Total leaf fresh weight (g) * (1-0.8)

The typical water content of poplar leaves in our experiment is 80%.

For example, in 0.1 mg/L 25 nm AgNP treatment,

Total leaf dry weight (g) = 13.5 (g) * (1-0.8) = 2.7 (g)

Total Ag mass assimilated by poplar leaves (μg) = 0.51 (μg/g) * 2.7 (g) = 1.4 (μg).

(2) Released Ag^+ mass in hydroponic solution (μg) = Released Ag^+ concentration in hydroponic solution (μg/L) * Total volume of hydroponic solution (L),

Where, Total volume of hydroponic solution = 0.4 L.

For example, in 0.1 mg/L 25-nm AgNP treatment,

Released Ag^+ mass in hydroponic solution (μg) = 0.5 (μg/L) * 0.4 (L) = 0.2 (μg).

(3) Potential Ag mass assimilated as Ag^+ (μg) = Daily uptake (μg/day) * 7 (day)

Daily uptake (μg/day) = TSCF × Released Ag^+ concentration in hydroponic solution (μg/L) * Daily evapotranspiration (L/day) (Eqn. S-1)

TSCF is the transpiration stream concentration factor. It is calculated from the uptake data of 0.1 mg/L Ag^+ treatment using Eqn. S-1. TSCF = 0.14.

For example, in 0.1 mg/L 25 nm AgNP treatment,

Daily uptake (μg/day) = 0.14 * 0.5 (μg/L) * 0.1555 (L/day) = 0.01 (μg/day)

Potential Ag mass assimilated as Ag^+ (μg) = 0.01 (μg/day) * 7 (day) = 0.1 (μg)

Table S3 shows that total Ag mass assimilated by poplar leaves was much higher than the potential Ag mass assimilated as Ag^+ within the sub-inhibitory concentration range of AgNPs. This indicates the AgNP uptake by poplar leaves. However, the inhibitory effects of AgNPs on poplar affected this uptake process as in the case of 5 nm AgNPs at 1 mg/L.

Table S4. Silver accumulation detected in hydroponically grown *Arabidopsis* after 3 days of exposure to 1 mg/L of Ag^+ or 5-nm AgNP.

	Ag quantity (ng)		
	Ag^+	AgNP (5-nm)	t-test
Media	1303 ± 197	3010 ± 375	p<0.01
Root	2993 ± 208	956 ± 80	p<0.01
Aerial	71 ± 15	36 ± 6	p<0.05
Total	4367 ± 260	4001 ± 329	

References

1. Hoagland, D. R.; Arnon, D. I., The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station* **1950,** *347*, (2nd edit).
2. Porra, R. J., The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b. *Photosynth Res.* **2002,** *73*, (1-3), 149-156.
3. Teng, S.; Keurentjes, J.; Bentsink, L.; Koornneef, M.; Smeekens, S., Sucrose-specific induction of anthocyanin biosynthesis in Arabidopsis requires the MYB75/PAP1 gene. *Plant Physiol* **2005,** *139*, (4), 1840-52.