Electronic Supplementary Information for

Self-assembly of 1-triacontanol onto Layered Doubled Hydroxide Nano-carrier toward Sustainable Growth Regulation of Maize

Chong Wang,^{‡a} Fei Gao,^{‡a} Changjiao Sun,^a Yue Shen,^a Shenshan Zhan,^a Xingye Li,^a Haixin Cui,^a Liusheng Duan,^{*b} Ye Wang, ^{*C} and Yan Wang^{*a}

^aInstitute of Environment and Sustainable Development in Agriculture, Chinese Academy of Agricultural Sciences, Beijing, 100081, China.

^bState Key Laboratory of Plant Physiology and Biochemistry, Engineering Research Center of Plant Growth Regulators, Ministry of Education, College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China.

^cCollege of Plant Science and Technology, Beijing University of Agriculture, Beijing 102206, China.

‡ Chong Wang and Fei Gao contributed equally to this work

Reagent and characterization

All the chemicals were analytical reagent grade. Deionized water was used throughout the experimental process. Powder X-ray diffraction patterns of all the samples were operated on a Shimadzu XRD-6000 diffractometer with a scan step of 10°/min and a scan range from 3 to 70 degree. The FT-IR spectra were recorded on a Nicolet iS50, FT-IR Spectrometer, Thermo Fisher Scientific. The samples were scanned against the KBr background with wavenumbers ranging from 4000 to 500 cm⁻¹. The morphologies of samples were investigated using a scanning electron microscope, Zeiss SUPRA 55.

Preparation of rhodamine 6G tagged tria-LDH fluorescent tracer

Rhodamine B tagged tria-LDH fluorescent nanosheets were fabricated with the same method as that for LDH nano-carriers with some adjustments. Briefly, solution A was prepared as follows: $Mg(NO_3)_2 \cdot 6H_2O$ (0.2 M), $Al(NO_3)_3 \cdot 9H_2O$ (0.1 M) were dissolved in deionized water (500 ml); solution B was prepared as follows: sodium dodecyl sulfonate (SDS) (0.002 M) rhodamine B (0.00001 M) and NaOH (0.6 M) were dissolved in deionized water (500 ml). Then the above two solutions were quickly mixed with vigorous stirring of 3000 rpm for 3 min. The product was collected by centrifugation at 6000 rpm for 10 minutes and washed with ethanol and water several times until no fluorescence was observed in the supernatant. After the above operation, the fluorescent tracer was obtained.

Measurement of 1-triacontanol

Tria-LDH (1 mL) was dissolved in HCl (18 M, 1 mL), to remove the LDH nano-carrier. After dissolved absolutely into the solution, dichloromethane (10 mL) was added into the solution to extract triacontanol. Then the sample was set on shaking table with 28 °C temperature (THZ-98C, Shanghai, China) for 12 h. The accurate content of triacontanol was measured with GC-MS method on Agilent 7890A (gas chromatography, with DB-5MS column (30 m \times 0.25 mm \times 0.25 µm) and 5975C (mass spectrometry).

Physiological indexes of maize

Days after treatment (day)	Water (cm)	LDH (cm)	Tria-ME (cm)	Tria-LDH (cm)
0	27.67±1.34	27.98 ± 1.43	24.13 ± 0.96	25.77±1.41
3	35.78±0.545	36.01±0.42	35.32±1.75	36.00 ± 0.98
5	35.84±0.72	36.23 ± 0.98	37.10±0.99	37.82±1.29
10	36.04±1.35	36.35±1.82	38.26±1.57	39.01±1.67

Table S1. The seedling height data of maize samples with different treatments under drought stress.

Table S2. The stem diameter data of maize samples with different treatments under drought stress.

Days after	Water	LDH	Tria-ME	Tria-LDH
treatment	(mm)	(mm)	(mm)	(mm)
(day)				
0	2.78 ± 0.05	2.82±0.12	2.60±0.12	3.08±0.25
3	3.02±0.21	3.01±0.23	3.20 ± 0.09	3.75±0.22
5	3.00±0.13	2.99±0.18	3.16±0.09	3.23 ± 0.07
10	2.13±0.20	2.23±0.12	2.21±0.35	2.86±0.24

Table S3. The leaf area data of maize samples with different treatments under drought stress.

Days after	Water	LDH	Tria-ME	Tria-LDH
treatment	(cm^2)	(cm^2)	(cm^2)	(cm^2)
(day)				
0	47.95±4.45	48.01±4.54	46.40 ± 4.84	57.79±5.96
3	67.66±1.37	66.21±1.77	62.18±3.92	72.05±2.74
5	53.48±3.45	54.15±3.59	60.75 ± 3.66	67.96±3.19
10	29.71±2.12	28.47 ± 2.09	32.18±5.19	38.22 ± 4.80

As the weight of a single seedling is too light to ensure the accuracy, the weight data were the average value of 3 plant in the same treated group measured together.

Table S4	1. The abovegro	und part dry v	veight of sample	es with different	treatments under	drought stress.

Days after	Water	LDH	Tria-ME	Tria-LDH
treatment	(g)	(g)	(g)	(g)
(day)				
0	0.11 ± 0.01	0.12 ± 0.01	$0.14{\pm}0.02$	0.13±0.02
3	0.37 ± 0.05	0.37 ± 0.06	0.41 ± 0.01	0.45 ± 0.04
5	0.35 ± 0.06	0.34 ± 0.06	0.37 ± 0.01	0.38 ± 0.04
10	0.25 ± 0.05	0.26 ± 0.04	0.33 ± 0.03	$0.36{\pm}0.03$



Figure S1. The digital pictures of maize seedlings with different treatments during days of drought stress for (A) 0 day. (B) 3 days. (C) 5 days and (D) 10 days. The samples are treated with water, tria-ME and tria-LDH from left to right, respectively.

Biochemical indexes of maize

Days after treatment (day)	Water (nmol/g)	LDH (nmol/g)	Tria-ME (nmol/g)	Tria-LDH (nmol/g)
3	50.95±2.85	52.76±0.74	29.21±0.95	31.43±1.92
5	72.75 ± 0.50	70.14 ± 0.97	70.97 ± 0.65	41.17±0.95
10	85.22±1.47	85.13±0.46	42.64 ± 0.64	34.95±1.76

Table S5. The MDA content data of maize samples with different treatments under drought stress.

Table S6. The SOD activity data of maize samples with different treatments under drought stress.

Days after treatment (day)	Water (U/g)	LDH (U/g)	Tria-ME (U/g)	Tria-LDH (U/g)
3	309.75±10.56	297.05±11.71	271.78±13.45	262.27±24.76
5	427.42 ± 4.78	434.12 ± 7.69	444.52±3.10	452.98±3.84
10	360.96±12.74	372.71± 9.86	390.14±3.63	432.24±14.77

Biochemical index	Days after treatment (day)	Water (g)	LDH (g)	Tria-ME (g)	Tria-LDH (g)
Saadling	0	23.45±0.97	23.05±0.76	22.80±0.51	23.30±0.42
beight	3	34.53 ± 0.79	34.32 ± 0.60	35.05±1.32	33.95±0.69
(em)	5	45.98 ± 0.60	46.01±0.33	46.20±1.09	46.98 ± 0.55
(em)	10	55.78±0.79	56.02 ± 0.18	56.78±1.23	59.15±0.59
Stom	0	2.70 ± 0.09	2.66 ± 0.07	$2.64{\pm}0.08$	$2.54{\pm}0.04$
diamatar	3	3.00 ± 0.06	2.99 ± 0.03	3.33 ± 0.14	3.51 ± 0.27
(mm)	5	3.30 ± 0.14	3.30 ± 0.32	3.38 ± 0.02	3.59 ± 0.02
(11111)	10	3.72 ± 0.08	3.71 ± 0.07	4.33 ± 0.02	4.21±0.14
	0	49.28±4.50	48.01 ± 5.10	49.04 ± 4.84	51.79±5.96
Leaf area	3	54.22±1.37	53.72±1.77	58.18±3.92	58.21±2.74
(cm^2)	5	63.35±3.46	64.16±3.28	69.75±3.66	72.96±3.19
	10	74.71±2.10	75.11±2.25	79.65±5.19	83.22 ± 4.80
Aboveground	0	0.51 ± 0.03	0.51 ± 0.03	$0.50{\pm}0.06$	0.48 ± 0.05
part dry	3	$0.79{\pm}0.02$	0.87 ± 0.05	$0.79{\pm}0.05$	0.87 ± 0.05
weight	5	1.02 ± 0.03	1.01 ± 0.02	1.17 ± 0.04	1.23 ± 0.03
(g)	10	1.55 ± 0.20	1.52 ± 0.06	1.98 ± 0.11	2.10 ± 0.21
	3	51.95 ± 2.05	51.46 ± 1.00	30.10±2.94	32.41±1.05
(nmol/g)	5	64.75±1.53	65.22±2.45	68.65 ± 0.75	41.35±0.22
	10	84.70±2.46	84.95 ± 1.46	39.95±1.22	30.95 ± 1.35
SOD (U/g)	3	308.05±17.16	312.68±7.17	267.81±21.06	258.52±17.62
	5	423.12±20.17	419.55±9.06	$448.92{\pm}14.57$	454.08±13.59
	10	364.71±6.94	366.12±14.17	389.06 ± 9.56	430.86±7.17

Table S7. The biochemical index of samples with different treatments under normal circumstance.